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Inbreeding and brood stock management

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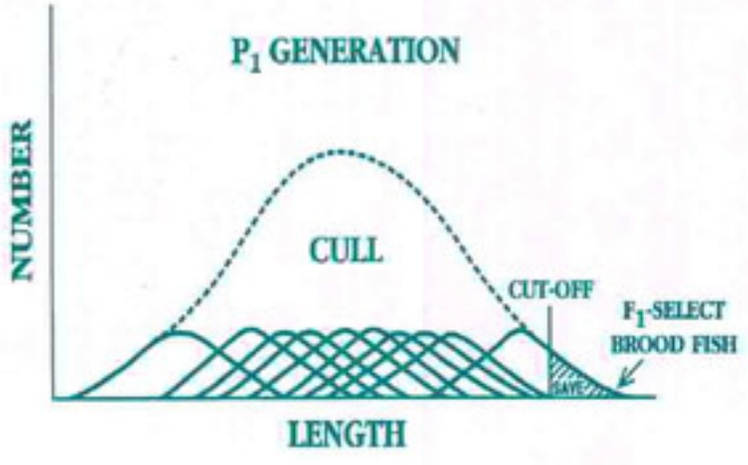

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Inbreeding and brood stock management

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PREPARATION OF THIS DOCUMENT

This document has been prepared within the framework of the Regular Programme activities of the Inland Water Resources and Aquaculture Service of the Fishery Resources Division. The primar objective of this document is to outline how inbreeding can be avoided or minimized and thus prevent genetic problems in cultured populations of fish at fish farms and fish culture stations. A secondary objective is to outline how inbreeding can be used to improve cultured populations of food fish. This document is directed to extension personnel, aquaculturists, and those who plan natural resource management programmes.

The original manuscript was prepared by Douglas Tave of Urania Unlimited, Coos Bay, Oregon, USA. It was reviewed by Gary Thorgaard, Daryl Kuhlers, Devin M. Bartley, and Katherine Bruner Tave. The figures were prepared by Sally Rader under the supervision of Douglas Tave.

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ABSTRACT

This manual, written for extension workers, aquaculturists, and those who work with natural resource management programmes, primarily deals with the problems caused by unwanted inbreeding in cultured fish populations and describes management techniques that can be used to prevent or minimize inbreeding. The manual also describes how inbreeding can be used to improve captive populations of fish. The manual contains chapters on: basic genetics and the genetics of inbreeding; how to determine individual inbreeding values when pedigrees are known; how to determine the average inbreeding value in a population when pedigrees are not known; genetic drift, which is random changes in gene frequency; how inbreeding programmes can be used to improve cultured populations of food fish; how to prevent inbreeding depression and loss of genetic variance in farmed populations; and recommendations on how to manage cultured populations of fish to prevent unwanted inbreeding and genetic drift from depressing productivity, profits, and survival. One of the most important aspects of managing a closed population of fish at a fish farm or fish culture station is the management of the population's effective breeding number, because inbreeding is inversely related to the effective breeding number. Techniques to determine and manage the effective breeding number are described, and recommended minimum effective breeding numbers are provided for a variety of farm sizes and fish culture goals. A number of culture techniques can affect inbreeding, and ways to modify them so there is minimal impact on inbreeding are discussed. Finally, ways to minimize inbreeding during selective breeding programmes are described.

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PREFACE

I first became interested in the problems of inbreeding (the mating of relatives) in hatchery populations when I was at Auburn University working on my dissertation: *a* study on heritability for growth in Nile tilapia. The results were disappointing because the heritabilities were small, so I studied the population's history to see if I could explain why the heritabilities were smaller than expected. What I found surprised me. The population had been transferred across three continents, and each time the size of the population had been severely reduced. I surmised that these reductions in population size produced inbreeding and genetic drift (random changes in gene frequency, resulting in the loss of genetic variance), and that this combination ruined the population genetically. Subsequent studies by other students found that the population could not be improved by selection and that there was no heterozygosity at loci that were examined electrophoretically, which confirmed my conclusion the population's gene pool had been severely damaged by improper brood stock management.

At the same time, I discovered "saddleback," a bizarre deformity in the Auburn population of blue tilapia; I also saw orders for foundation brood stock that were being filled by collecting and shipping offspring produced by a single mating. This led me to the conclusion that inbreeding and genetic drift caused by small hatchery populations could explain a variety of problems.

Ironically, even though the Auburn University population of blue tilapia is highly inbred because it was founded by a single female and three males, the saddleback phenotype had nothing to do with inbreeding. But research on this phenotype and others has led me on a quixotic 19-year adventure to discover effective ways to explain inbreeding and genetic drift to farmers, hatchery managers, and fisheries administrators and to try and show them how to manage hatchery populations so that inbreeding and genetic drift do not cause inbreeding depression or rob their populations of valuable alleles and genetic variance. This manual is the culmination of those efforts.

This manual is a full-sib companion to Selective Breeding Programmes for Medium-sized Fish Farms (FAO Fisheries Technical Paper 352). As was the case in that manual, this one arbitrarily defines medium-sized farms as those with 2 ha of ponds; small and large farms are obviously those on the lesser and greater side of 2 ha. Additionally, a fourth category was created for this manual: public hatcheries that produce fish for stocking lakes and rivers.

The goal of this manual is two-fold: The first part is to provide easy-to-understand and easy-to-follow recommendations about how to avoid the accumulation of inbreeding and genetic drift so these twin problems do not reduce growth rate, viability, fecundity, etc. This manual was written to educate extension agents and university-trained aquaculturists so that they would be able to understand what inbreeding and genetic drift are and to illustrate how uncontrolled accumulations of both can decrease growth rate, fecundity, viability, and survival; increase the percentage of deformed fish; and make future selective breeding programmes ineffective.

Often, what is considered to be "normal" hatchery management is, in reality, the type of brood stock mismanagement that causes inbreeding and genetic drift to accumulate to levels that cause problems. The biggest culprit is the genetic size of the breeding population, a concept called the "effective breeding number." When no selection is occurring, managing a population's effective breeding number is the most important aspect of brood stock management, because it is inversely related to both inbreeding and genetic drift. Techniques that can be used to manage a population's effective breeding number are described, along with recommended minimum effective breeding numbers that can easily be incorporated into yearly work plans.

Unfortunately, there is an appalling lack of information about how specific levels of inbreeding affect growth, viability, disease resistance, fecundity, survival, and other production phenotypes for important cultured species of fish. What information does exist has been generated by a handful of studies that, for the most, part simply skip across the surface of the sea of knowledge. The prime reason for this lack of information is short-sighted bureaucrats and administrators who are unwilling to fund long-term studies that will generate the kind of data that are readily available to other animal breeders. This is somewhat understandable, because short-term projects that provide immediate benefits are needed. However, breeding programmes are long-term

investments, and they should be funded before they are necessary, so that information will be waiting and available when it will be needed in the future.

The second part of the goal of this manual is to illustrate how inbreeding can be used to improve a population. Inbreeding is a powerful breeding tool that has been used to improve livestock and grains, but most fish farmers and hatchery managers do not know how it can be used; they only want to avoid it. Although most fish farmers and hatchery managers will probably never use inbreeding in their breeding programmes, if they know how it can be used, they will have a better appreciation of how it can be prevented or controlled.

This manual is not a genetics textbook; it is written for aquaculturists who do not have a good background in genetics but who are interested in applying genetics and breeding principles to brood stock management. It is written in a simple, straightforward manner. I have tried to use as little jargon as possible, but when it was unavoidable, I defined each term when it was first used. In addition, there is a glossary that defines many of the terms.

Many of the ideas and recommendations in this manual have to be developed mathematically. In all cases, the math that was used is simple elementary-school arithmetic. No calculus was used. All math can be solved with pencil and paper or can be done with inexpensive hand-held calculators; every example in this manual and the values that appear in every table were derived using a $12 hand-held calculator. It is not necessary to do the math or to understand it in order to understand how inbreeding works and to comprehend how it can be avoided. The math was provided for those who wanted to see how the recommendations were determined and to enable others to create their own recommendations, since they are site-specific and are based on species, hatchery size, budget, goals, manpower, etc.

The manual was written so that each chapter is self-contained. Those who already know a subject or those who are not interested in a particular topic can read only those chapters that interest them.

Chapter 1 is an introduction that explains what the manual is about and gives a brief explanation of what inbreeding is, in relation to other breeding programmes.

Chapter 2 is a totally abridged discussion about selected concepts in genetics. The only topics that are covered are those needed to help explain what inbreeding is and to show how it works.

Chapter 3 explains how individual inbreeding values are calculated. Individual inbreeding values can be determined if fish can be marked and if family pedigrees can be determined. Two methods are discussed: path analysis and covariance analysis.

Chapter 4 explains how the average inbreeding value in a population is determined. This approach must be used when individuals cannot be identified. This chapter introduces the concept of effective breeding number. When managing a hatchery population, managing its effective breeding number may be the most important aspect of brood stock management, because inbreeding is inversely related to effective breeding number.

Chapter 5 is a brief explanation of genetic drift. Like inbreeding, it too is inversely related to effective breeding number. Genetic drift is random changes in gene frequency due to sampling error. In aquaculture, this is caused by the transfer of fish from one hatchery to another; choice of which brood fish will be allowed to spawn; or sudden decreases in population size because of disease, dissolved oxygen depletion, etc.

The major thrust of this manual is to discuss inbreeding, explain what it can do to a population when it inadvertently reaches high levels, and to prescribe palatable management plans that can be used to prevent inbreeding depression (decreased growth rate, etc.). Consequently, some may feel that genetic drift is simply a sideline topic and not understand why it needs to be included. Its inclusion is necessary because inbreeding and genetic drift are inextricably linked by effective breeding number. Additionally, if you manage only to prevent unwanted inbreeding, unwanted genetic drift can cause similar problems. Consequently, a prudent farmer or hatchery manager will try and manage both, especially since managing both requires little extra effort.

Chapter 6 explains how inbreeding can be used to improve a population. Inbreeding is one of the three major types of breeding programmes. Although it is not as appreciated as selection and crossbreeding, it has been used to produce outstanding animals and plants, and the crops and livestock it produces help feed a hungry world. Inbreeding is extremely important in breed or strain development. Inbreeding can be used to improve response to selection when heritability is small. And inbreeding is the classic way to improve the results of crossbreeding programmes. Consequently, any farmer or hatchery manager who wants to manage the genetics of his population should know how inbreeding works and what it can do. In order to use inbreeding to improve a population, a farmer or hatchery manager must know how to design and maintain regular systems of inbreeding. Several regular systems of inbreeding are illustrated, along with the levels of inbreeding that they produce.

Chapters 7 and 8 are the heart and soul of the manual and are the chapters that will interest most farmers and hatchery managers. Chapter 7 discusses ways to prevent inbreeding in hatchery populations and shows the methods that can be used to determine the effective breeding numbers that are needed to prevent inbreeding from accumulating to levels that cause inbreeding depression and that will prevent genetic drift from robbing the population of needed genetic variance.

The single most important aspect of brood stock management under these circumstances is to prevent bottlenecks. A bottleneck is a drastic reduction in effective breeding number. A single bottleneck can cause permanent genetic damage to the population by producing high levels of inbreeding and by producing high levels of genetic drift. A single bottleneck can ruin years of excellent brood stock management.

In addition, several spawning and breeding techniques that can be used to increase effective breeding number are presented.

Chapter 8 presents a series of recommendations that farmers and hatchery managers can use to prevent inbreeding depression and the loss of genetic variance via genetic drift. This is a key aspect of brood stock management when no selective breeding programme will be used to improve growth rate or other phenotypes. For most farmers and hatchery managers this means managing effective breeding number at a pre-determined size. Recommended effective breeding numbers are made for small, medium, and large farms and for public hatcheries. Several recommendations are made for all four situations, based on a farmer's or hatchery manager's goals, the type of fish culture that is being done, and the level of genetic risk that is acceptable. The process by which a farmer or hatchery manager can determine the effective breeding number that he needs for a customized set of goals is also presented.

Recommendations are also made about mating protocols that can be used to minimize inbreeding during a selective breeding programme. Inbreeding is inevitable when a farmer conducts a selective breeding programme. Selection produces inbreeding because each act of selection creates a bottleneck; additionally, when only the best are allowed to mate, the mating of relatives is inevitable. Inbreeding is of secondary importance during a selective breeding programme, because the major genetic goal is to alter the genetics of the population in order to improve productivity. However, inbreeding needs to be monitored and it should be minimized, or selection will be used only to counteract inbreeding depression.

Genetic drift will also occur during a selective breeding programme. In fact, genetic drift can accumulate at a faster rate during a selective breeding programme because each act of selection creates a bottleneck and the number of families is often reduced. The major effect of genetic drift under these circumstances will be on genes that are not affected by the selective breeding programme. Genetic drift will minimally effect the genes which control phenotypes that are undergoing selection or that are undergoing indirect selection. The problems caused by genetic drift can generally be ignored during a selective breeding programme, because selection and conservation of genetic variance are diametrically opposed breeding programmes.

I did not include any citations in the text or tables. This was not done to slight the contributions by others; it was done to make the manual uncluttered and more readable. I have included a list of recommended reading at the end of the manual for those who wish to pursue one of the topics discussed in this manual in greater detail.

I thank Gary Thorgaard, Daryl Kuhlers, and Katherine Bruner Tave for critical review of the manuscript. Once again, Sally Rader has turned my preliminary sketches into works of art. I would also like to thank Devin Bartley for giving me the opportunity to synthesize my thoughts in this area.

This book is for Katherine and Kai, the Sancho and Panza of my life, who never fail to put me back on my Rozinante when the windmills get too fierce.

Douglas Tave
July, 1998

TABLE OF CONTENTS

CHAPTER 1.

INTRODUCTION

Inbreeding is a genetic concept that most aquaculturists have heard about, but few understand what it is or know how it can be used or abused. Inbreeding is one of the few natural biological processes that evokes deep- seated prejudices and invokes legal sanctions; as a result, it is probably the least understood and least appreciated aspect of animal and plant breeding. Most have heard of inbreeding because of legal and moral laws against certain consanguineous ("blood-related") marriages and because extension agents and others have repeatedly preached about the evils of inbreeding. However, inbreeding is no different from any other piece of technology. If used properly, it can improve things; if used improperly, it will make them worse.

Inbreeding has been a concept that has plagued and intrigued humanity for thousands of years. The Torah, Bible, and Koran all contain proscriptions against incest. Most societies have laws pre-dating constitutional rights that prohibit marriages between certain relatives, although feudal rulers routinely ignored these laws, because it was a prudent way of consolidating money, land, and power.

The major reason why inbreeding was considered to be immoral was that no one knew anything about genetics, and consequently no one knew how inbreeding worked. It was easy to observe that when close relatives mated, some offspring were deformed or were weak and died. Since genetics was unknown, people associated the deformities, etc. with the act of incest itself and thus concluded it was evil and the deformed offspring were some form of divine punishment.

Our aversion to consanguineous marriages and abhorrence of incest is probably rooted in our evolutionary past and is controlled by our genes. Studies with several species of animals have shown that various behaviours, such as dispersal patterns-either forced or voluntary-of juveniles who are about to become sexually mature prevent parent-offspring, brother-sister, or first cousin matings which produce high levels of inbreeding. Since inbreeding produces some subviable or inviable offspring, genetically controlled behaviour which prevents such matings will be favored by natural selection.

Prohibitions and prejudices against inbreeding were so deeply rooted in Europe that as recently as 200 years ago European animal breeders were told that they should not use inbreeding in their brood stock management plans because it was against the Laws of God and Nature. However, animal breeders ignored these injunctions when they discovered that inbreeding was a breeding technique that could be used to develop new breeds, improve herds, and produce outstanding animals.

Once the science of genetics was established, geneticists were finally able to explain why some inbred offspring were deformed and were able to show that this was a problem of heredity, not of morality. They were also able to explain how inbreeding produced better animals and plants. An understanding of the genetics of inbreeding enabled geneticists to devise regular systems of inbreeding so animal and plant breeders could use inbreeding to improve growth rate and other phenotypes in a reliable and predictable manner.

Inbreeding is the mating of relatives, or the mating of fish more closely related than the population average (which is another way of defining relatives). Some geneticists want to restrict the definition of inbreeding and say it is the mating of "close relatives," and that means the mating of two individuals more closely related than second cousins. The restricted definition might be more practical as a breeding definition, because it is based on the amount of inbreeding in the offspring that are produced by a mating. However, the mating of any relatives, whether they are closely related or distantly related, is inbreeding, and it will produce inbred offspring.

Inbreeding is, for all practical purposes, the opposite of crossbreeding. While inbreeding is the mating of individuals more closely related than the population average (between relatives), crossbreeding is the mating of individuals less closely related than the population average (between individuals from two populations).

Inbreeding, along with selection and crossbreeding (hybridization), is one of the three major breeding programmes that have been traditionally used to improve livestock and plants. While selection and

crossbreeding are more widely known and appreciated, inbreeding is a powerful breeding technique that has been used to: establish new breeds and varieties; improve the results of selection; create better brood stock; and improve the results of crossbreeding programmes.

There is a wealth of information about inbreeding in livestock and laboratory animals and an even greater treasure trove for plants. However, comparatively little information exists for farmed fish; most of the information that does exist is spotty or consists of warnings about why inbreeding should be avoided and explanations about how it can be prevented.

While inbreeding is an important breeding programme that can be used to improve a population when it is planned and directed, unplanned and uncontrolled inbreeding can ruin a population through something called "inbreeding depression," which is a decrease in growth and viability coupled with an increase in abnormalities. Genetic aspects of fish stock management are still in their infancy. Many farmers are still raising stocks that are either wild or only a few generations removed from the wild. Most farmers worry only about the environmental aspects of management-nutrition, water quality management, disease control and prevention-and ignore the genetic aspects. Since the modern science of aquaculture is still in its infancy, these management decisions make sense, because the best way to raise a crop has often not been quantified. Vast and immediate improvements can be obtained by refinements in fertilization regimens, feeding practices, or water quality management. These changes are often very cost effective because the changes are inexpensive.

The genetic aspects of fish fanning have received far less attention because they require sophisticated managers, are long-term forms of management, require extra facilities, and add additional financial burdens; furthermore, the results are often unpredictable. While great improvements are possible, they are usually more costly than environmental improvements, and the return is not immediate.

Those who are interested in incorporating genetic improvements into yearly work plans usually want to conduct selective breeding or crossbreeding programmes; inbreeding is ignored. There are two probable reasons for this: The first is the deep-rooted prejudice against inbreeding. The second is the warnings against inbreeding that come from fish geneticists and aquaculture extension agents.

These warnings have been sounded because fish farming is a form of animal husbandry where inbreeding can be a more serious problem than it is with livestock. For the most part, fish farmers work with small populations. When new stock is acquired, the number of fish acquired is usually small because: shipping fish is a lot of trouble; it is costly, especially since water is heavy; and it is difficult to ship fish great distances. Consequently, many shipments contain only a handful of fish. Often, the fish that are acquired come from one or two matings, which means the genetic size of the population is quite small. Finally, fish are highly fecund compared to traditional livestock, which are capable of producing only one to a dozen offspring each year. Because of this, there is often a great temptation to spawn as few fish as possible in order to save money and labour and to lower the cost per fingerling produced. The fecundity of some fish is so great and the size of many farms is so small that a single spawn will often produce enough fish to stock many farms.

A number of warnings against inbreeding have been published because aquaculture is a unique form of animal husbandry, in that it produces fish for the sea as well as fish for the table. Many fish culture programmes are conducted not to raise food, but to raise fish that will be stocked in lakes, rivers, and oceans to help sustain or re-establish fisheries. These programmes are successful only if survival of the stocked fish is large enough to improve the creel or if the stocked fish spawn and increase the size of the next year-class. Unfortunately, many stocking programmes have not been successful, and a major reason for this is improper brood stock management at the hatchery. Brood stock management plans that make fish farming economically successful are plans that make stocking programmes unsuccessful. Much of the blame can be attributed to the small numbers of fish that are spawned every year which lead to unwanted levels of inbreeding that will, in turn, lower survival.

Even when fish are raised for food, the inbreeding produced by small populations can cause problems. If unwanted inbreeding occurs, it can reach levels that cause growth rate or other production phenotypes to decrease. This means yields will decline unless management is intensified; in other words, the environmental aspects of management must be increased to counteract genetic mismanagement.

Furthermore, small population sizes produce genetic drift, which is random changes in gene frequency. The ultimate effect of genetic drift, particularly in small populations, is the loss of alleles. The loss of genetic variation can make future selective breeding programmes ineffective. Selection improves productivity by exploiting a population's genetic variance; genetic drift decreases a population's genetic variance.

Inbreeding can occur when a farmer conducts a selective breeding programme; in fact, it is inevitable. Selection is a breeding programme that improves a population by allowing only superior animals to produce offspring. When this happens, the size of the breeding population is reduced. When only the best are allowed to mate and the only criterion for mating is a certain phenotype, relatives are often mated. This causes unwanted inbreeding. While inbreeding will be produced during selection and while it can cause some problems, it is of secondary importance. The major genetic goal during this type of breeding programme is to improve the population by selection. However, inbreeding should be moderated when conducting a selective breeding programme, because there is no point in conducting selection simply to counteract inbreeding depression.

Consequently, the loudest and most persistent message that comes from geneticists and extension agents is: avoid inbreeding and do what it takes to prevent it from occurring. And that is the major thrust of this manual. Most of the manual describes what inbreeding is, what causes inbreeding, how it works genetically, how it is measured, and what can be done to prevent it from occurring. These subjects are developed in order to compile a list of recommendations that farmers and hatchery managers can incorporate into yearly work plans to better manage their populations and prevent inbreeding from ruining the population genetically. Since the problems associated with genetic drift and inbreeding are linked, part of this manual is devoted to this topic and to management techniques that can be used to minimize the effects of genetic drift.

A manual simply stating that inbreeding is bad and should be avoided would be only partially useful. Since inbreeding is an important breeding programme, one that has been used to produce superior animals and plants, there is no reason why aquaculturists cannot use inbreeding to produce superior stocks of fish. Consequently, one chapter is devoted to the ways inbreeding can be used to improve growth and other phenotypes and to the development of regular inbreeding programmes that can be used to produce inbred lines which can be used in selective breeding and crossbreeding programmes.

Few farmers will want to or be able to use inbreeding programmes. The decision to conduct any breeding programme is one that must be made for each farmer or each fry/fingerling production center on a case-by-case basis. Breeding programmes are expensive and time consuming. They require a certain level of sophistication, because good record keeping is a requirement. This is especially true for inbreeding programmes. Inbreeding is the mating of relatives, and you cannot mate relatives if you cannot follow pedigrees. Also, these programmes require facilities. Ponds that are being used for inbreeding programmes are ponds that cannot be used to raise food. Finally, breeding programmes usually do not produce immediate improvements. Improvements are usually not seen for at least one growing season; when inbreeding is used, there is often a 2- to 3-generation lag before improvements are noted. Consequently, a farmer must be able to incorporate long-term planning into his farm management programme, and he must be patient. As a result, within a region, only a small percentage of farmers or fingerling production centers should or will ever conduct inbreeding programmes.

But even if few farmers or hatchery managers will conduct inbreeding programmes, an understanding of how inbreeding can be used to improve productivity will enable the rest to better understand what inbreeding is; how it occurs; what is does; and most importantly, how it can be controlled.

CHAPTER 2.

GENETICS

This chapter is not an all-encompassing review of genetics. It is simply an overview of selected topics that will help those who do not have a good background in genetics to understand the genetics of inbreeding and thus understand how inbreeding affects production phenotypes, such as weight and fecundity. This background information will also help explain how inbreeding can be used to improve a population and how a population can be managed to minimize the effects of unwanted inbreeding.

Those who are not interested in this background information or those who already understand it can skip this chapter and go to: Chapters 3 and 4, which explain how inbreeding values are calculated; Chapter 5, which explains genetic drift; Chapter 6, which describes how inbreeding can be used to improve a population; or Chapters 7 and 8, which discuss ways and techniques that can be used to manage a population in order to prevent unwanted inbreeding from causing inbreeding depression and to prevent genetic drift from decreasing genetic variance.

GENES AND CHROMOSOMES

Genes are located on structures called "chromosomes," which are located in the nucleus of every cell. Although there are some exceptions, chromosomes typically occur as pairs in animals, and for practical fish culture management, fish can be considered to be diploids (chromosomes occur in pairs). Some rare species of fish are triploids (chromosomes occur in groups of three), but none are aquacultured species. Some species of fish are tetraploids (chromosomes occur in sets of four), notably the salmonids and catostomids; however, for practical breeding work these species behave as if they were diploids, so they can be considered to be diploids.

Because chromosomes occur in pairs, each gene also occurs as a pair. There are some exceptions in fish with morphologically distinct sex chromosomes, but few fish have such chromosomes; consequently, for practical fish breeding work, genes can be considered to occur in pairs. One chromosome of each pair comes from a fish's mother, while the other comes from its father; this means one gene from each pair comes from the mother, while the other comes from the father. The two chromosomes that form a pair are called "homologues."

MEIOSIS

Meiosis is the process during which the primary gametocytes develop into eggs or sperm. A number of important cellular changes occur during meiosis, but for a geneticist, the important ones are those that affect the genes and chromosomes.

Meiosis consists of two cell divisions. The first meiotic division is called the "reduction division," and the second is called the "equational division." Prior to the reduction division, the homologues that form each chromosome pair replicate. The replicated homologues do not separate; they are joined together at the centromere, and the joined replicated homologues are called "sister chromatids" (Figure 1).

The replication of the chromosomes is usually uneventful in that they replicate themselves perfectly. However, mistakes in the replication process occur. These mistakes are called "mutations." The mutation rate for each gene is low, ranging from one in 10,000 replications to one in 100,000 replications.

When a mutation occurs, the erroneous copy of the gene is often capable of producing an alternate version of the original phenotype (phenotypes are described in the next section). Most mutations are harmful, but some mutations create new versions of genes, and these new variants produce new or improved phenotypes that increase survival; this can lead to the evolution of new species. If replication were perfect, life would not have evolved beyond the single-celled state. Mutations also help explain some of the consequences of inbreeding.

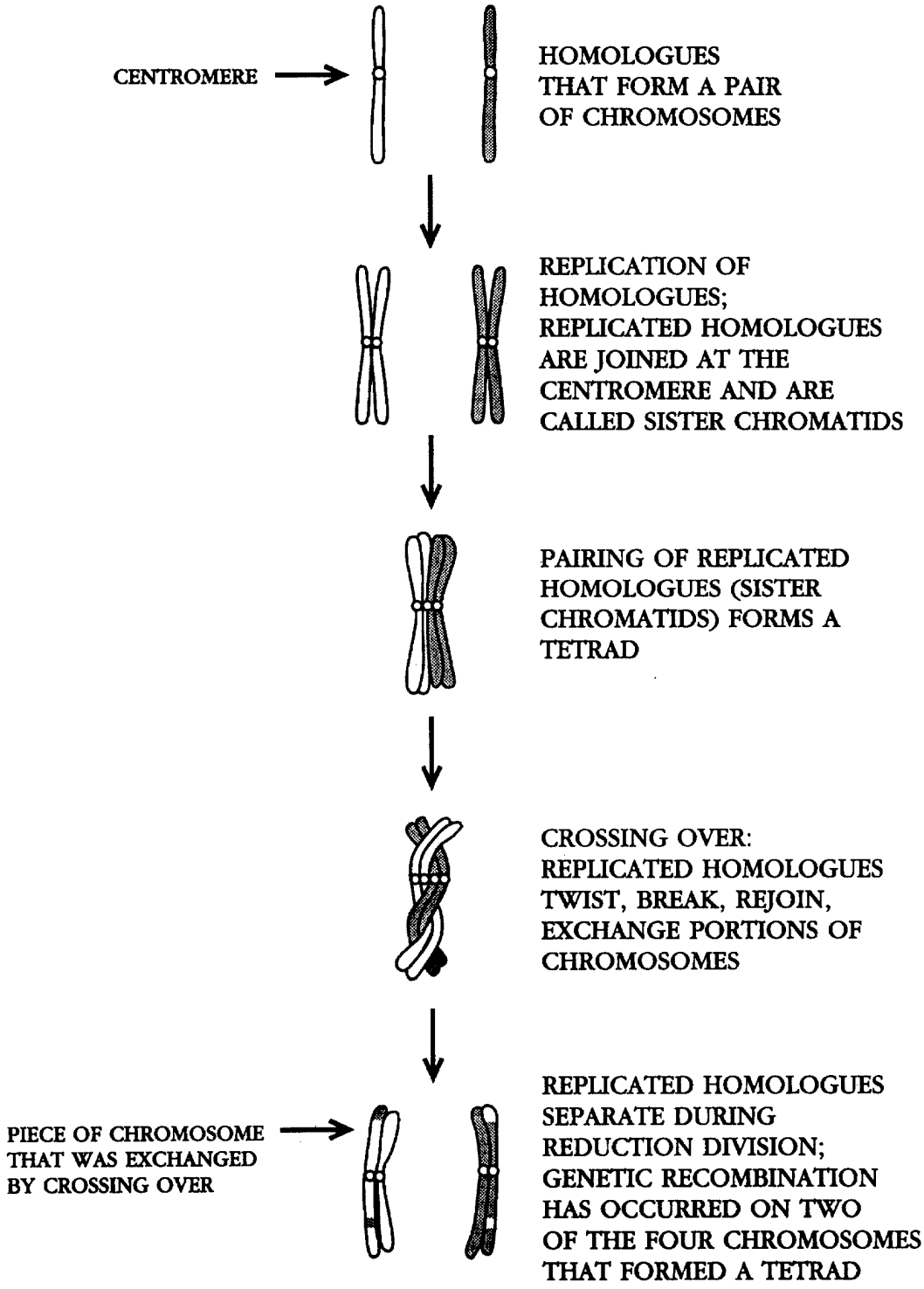


Figure 1. Schematic diagram of crossing over, which occurs in the initial phase of the reduction division during meiosis. Prior to the reduction division, the homologues that form each pair of chromosomes are replicated. This is when mutations (mistakes in replication) occur. In this figure, the homologue that came from the mother is white, while the homologue that came from the father is gray. The replicated homologues are joined at the centromere and are called sister chromatids. During the initial phase of the reduction division, the replicated homologues (sister chromatids) pair and form a bundle of four chromosomes called a tetrad. The chromosomes that form a tetrad typically twist around each other, break, and rejoin. Pieces of chromosome (and genes) can be exchanged between maternal and paternal homologues when this occurs. This process is called crossing over.

During the early phase of the reduction division, the replicated homologues that form each chromosome pair come together. Because each homologue is composed of a pair of sister chromatids, each bundle is composed of four chromosomes called a "tetrad."

The chromosomes that form a tetrad typically twist around each other and break. When the chromosomes rejoin, pieces can be exchanged between homologues. When this happens, pieces of chromosomes, along with the genes that are located along those pieces, are transferred from one homologue to the other. This process is called "crossing over" (Figure 1). Crossing over is one of the most important biological processes, because it greatly increases genetic variability.

In inbreeding, crossing over is important because it can affect the amount of inbreeding produced during one form of chromosomal manipulation. Crossing over tends to occur at particular locations along each chromosome. Each gene has a given cross over frequency. Genes located near the centromere cross over less frequently than those further away. Gene that are located near each other on a chromosome cross over as a unit and are called "linked"; the closer they are, the more tightly linked they are. Consequently, crossing over causes actual levels of inbreeding to deviate from predicted levels during meiotic gynogenesis (this will be discussed in Chapter 6).

During reduction division, the chromosome number is reduced from the diploid (paired) state to the haploid (unpaired) state. The homologues that form each chromosome pair separate and are parceled into the two daughter cells (for males, the two secondary spermatocytes; for females, the secondary oocyte and first polar body). When the chromosomes divide, they are not parceled into maternal and paternal sets. The direction in which each replicated homologue (sister chromatids) of each chromosome pair goes is random and independent of that for all other chromosomes. This process is called "independent assortment." Although each secondary gametocyte contains a haploid set of chromosomes, each chromosome is duplicated.

The second meiotic division is called the "equational division." During the equational division, the replicated homologues (sister chromatids) in each secondary gametocyte separate and are parcelled into either two sperm cells or into the egg and second polar body. This produces haploid gametes; each gamete contains a single chromosome from each pair, which means that it contains a single copy of each gene.

PHENOTYPE AND GENOTYPE

Each gene contains the chemical blueprint for the production of a protein. This protein either forms or helps produce a specific phenotype (also called "trait" or "character"), such as body colour or weight. A phenotype is the physical or chemical expression of what a gene produces; it is either observed and described (qualitative phenotype) or it is measured (quantitative phenotype).

A gene can occur in one or more forms. Alternate forms of a gene are called "alleles." In a population, a gene can have one to perhaps a dozen alleles. If there is only one allele in the population, the gene is said to be "monomorphic." If there are two or more alleles at a locus (locus = gene; plural is loci), the gene is said to be "polymorphic." Monomorphic genes are not very interesting, because there is no genetic or phenotypic variance associated with that locus. On the other hand, polymorphic genes are of great interest to a geneticist. When more than one allele exists at a locus, there is genetic variance, which produces phenotypic variance; and this can be exploited by breeding programmes.

The genetic make-up of each fish is called its "genotype." Because chromosomes occur as pairs, each gene occurs as a pair; this means the genotype is a paired entity (Figure 2). If the copy of the gene that a fish inherits from its father is the same as the copy that it inherits from its mother, that gene is an identical pair of alleles, and the fish is said to be "homozygous" at that locus (Figure 3). If the copy of the gene that a fish inherits from its father is different from the copy that it inherits from its mother, the gene is a non-identical pair of alleles, and that fish is said to be "heterozygous" at that locus. Consequently, every gene can exist in one of two genotypic states: homozygous (identical pair of alleles) and heterozygous (non-identical pair of alleles).

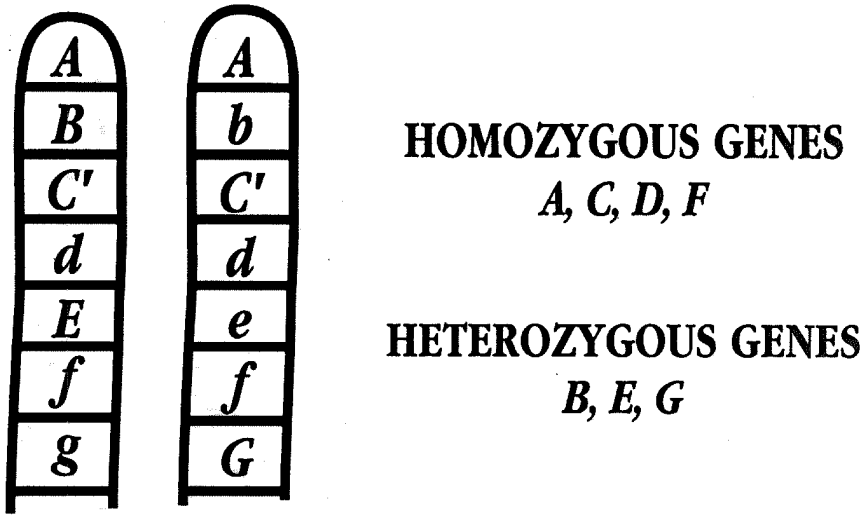
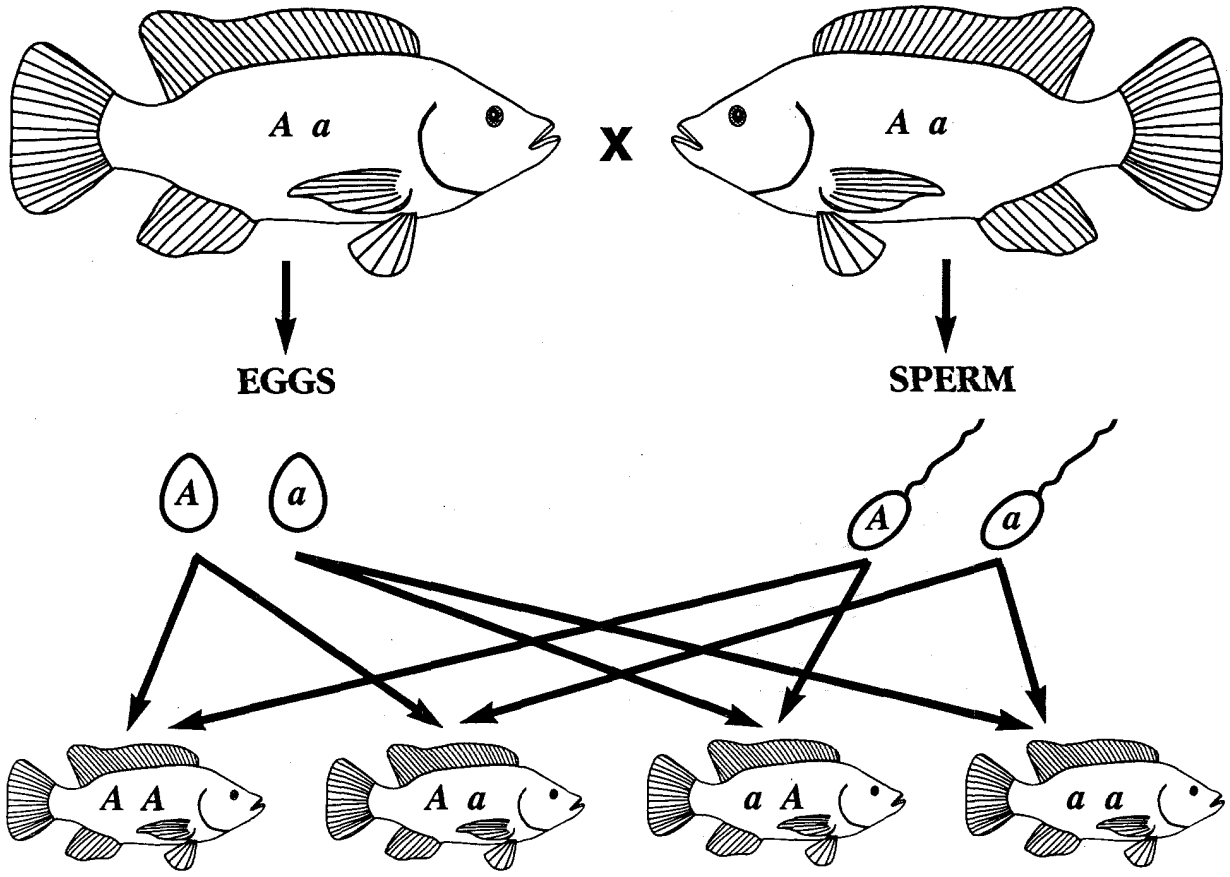


Figure 2. A small section of a pair of chromosomes, showing seven genes. Four of the genes exist in the homozygous state (A, C, D, and F), while three exist in the heterozygous state (B, E, and G).



PROGENY WITH *A A* AND *aa* GENOTYPES ARE HOMOZYGOUS; THEY INHERITED IDENTICAL ALLELES FROM BOTH PARENTS

PROGENY WITH *A a* AND *a A* GENOTYPES ARE HETEROZYGOUS; THEY INHERITED A DIFFERENT ALLELE FROM EACH PARENT

Figure 3. How homozygous and heterozygous fish are produced. In this example, both parents were heterozygotes (Aa), so each produced two types of gametes: half the eggs and half the sperm carried an A allele, while the other half carried an a allele. Some progeny were homozygous (AA or aa) because they inherited the same allele from both parents. Some progeny were heterozygotes (Aa) because they inherited a different allele from each parent.

The reason why it is important to make a distinction between the homozygous and heterozygous states, is they often produce different phenotypes. The number of phenotypes produced by a gene depends on the mode of gene action and the number of alleles.

A fish's genotype produces its phenotype. Consequently, if a breeder wants to conduct a breeding programme to control or alter phenotypic frequencies (using inbreeding or preventing it are both breeding programmes), he should understand how the genotype produces the phenotype.

GENETICS OF QUALITATIVE PHENOTYPES

Breeders divide phenotypes into qualitative (descriptive) phenotypes and quantitative (measured) phenotypes. Qualitative phenotypes are those that are described, such as body colour. Because these phenotypes are descriptive, individuals segregate themselves into descriptive categories: pink vs. normally pigmented; gold vs. bronze vs. black; normal vs. deformed.

In general, qualitative phenotypes are controlled by one or two genes. The alternate forms of a phenotype (pink vs. normally pigmented or normal vs. deformed) are produced by the alternate forms of a gene (the alleles).

Most qualitative phenotypes that have been described genetically in fish are controlled by a single autosomal gene with two alleles (autosomes are the pairs of chromosomes that males and females share in common; the pairs of chromosomes that are not the pair of sex chromosomes). Because of that, this discussion will concentrate on these phenotypes, especially since this type of inheritance helps explain how inbreeding works.

In general, genes express themselves in either an additive or a non-additive manner. When genes express themselves in a non-additive manner, one allele is dominant, while the other is recessive. The terms "dominant" and "recessive" carry no value judgement; the terms simply refer to the way the alleles produce their respective phenotypes.

There are two types of dominant alleles. When the dominant allele exhibits complete dominance, it always produces its phenotype, while the recessive allele can produce its phenotype only in the absence of the dominant allele. Thus, a gene that produces two phenotypes via complete dominance (it has a completely dominant allele and a recessive allele) has three genotypes, but the genotypes produce only two phenotypes:

Genotype	Phenotype
Homozygous dominant	Dominant
Heterozygous	Dominant
Homozygous recessive	Recessive

This type of gene action plays an important role in the genetics of inbreeding, and it helps explain inbreeding depression. This topic will be discussed later in this chapter.

A second type of dominance occurs when the dominant allele is incompletely dominant; i.e., the dominant allele always produces its phenotype, but it is unable to completely suppress the recessive allele in the heterozygous state. When this happens, a gene with incomplete dominant gene action (it has an incompletely dominant allele and a recessive allele) has three genotypes, and each genotype produces a unique phenotype:

Genotype	Phenotype
Homozygous dominant	Dominant
Heterozygous	Near-dominant
Homozygous recessive	Recessive

The heterozygous genotype produces a phenotype that resembles but is slightly different from the dominant phenotype.

When the mode of gene action is additive, neither allele is dominant, and each allele always produces its phenotype in a unidirectional step-wise manner. This means the heterozygous phenotype is intermediate between the two homozygous phenotypes. When this occurs, a gene with additive gene action produces a unique phenotype for each genotype:

Genotype	Phenotype
Homozygous allele 1	Homozygous phenotype 1
Heterozygous	Heterozygous
Homozygous allele 2	Homozygous phenotype 2

Only one additive gene has been discovered in fish; it controls golden, palomino, and normal body colours in rainbow trout, Oncorhynchus mykiss. All other qualitative phenotypes that are produced by a single autosomal gene are controlled either by complete dominance or incomplete dominance.

GENETICS OF QUANTITATIVE PHENOTYPES

Quantitative phenotypes are those that are measured, such as length, weight, and fecundity. The important production phenotypes are quantitative ones, although some qualitative phenotypes are quite important, and can greatly increase the value of a crop. Because quantitative phenotypes are measured, each phenotype is a single category, such as length. Individuals do not get segregated into alternate phenotypic categories, such as long vs. short; instead, they are distributed along a continuum, and differences among individuals are determined by the unit of measure that is used to assess the phenotype: millimeters, grams, etc.

Because each fish's phenotype is determined by a measurement, quantitative phenotypes form what are called "continuous distributions," which are described by the population's mean and the distribution about the mean (variance and standard deviation). In a population, these phenotypes form what are called "normal" or "bell-shaped" distributions.

The reason why quantitative phenotypes exhibit continuous distributions and why individuals do not get segregated into descriptive categories is that quantitative phenotypes are far more complicated genetically than qualitative phenotypes. Each quantitative phenotype is controlled by dozens to hundreds of genes, each of which makes a small contribution to the production of the phenotype. The exact number of genes is usually never known. Additionally, each phenotype is strongly influenced by the environment (e.g., date of birth, access to food, age of mother, etc.), and the influence varies both from family to family and from individual to individual. The simultaneous action of many genes and the environmental effects creates single phenotypic categories (e.g., length), in which the only way to describe an individual is to measure it.

Because each quantitative phenotype is controlled by numerous genes, as well as environmental variables, the only way to work with these phenotypes is to analyze the phenotypic variance that exists in the population and to divide the phenotypic variance into its component parts. Phenotypic variance (V_P) is the sum of the genetic variance (V_G), environmental variance (V_E), and genetic-environmental interaction variance (V_{G-E}) components:

$$V_P = V_G + V_E + V_{G-E}$$

When conducting a breeding programme, a geneticist tries to exploit V_G. Three distinct types of genetic variance combine to make V_G, and it is important to know what they are, because different breeding programmes are needed to exploit each type. Genetic variance is the sum of additive genetic variance (V_A), dominance genetic variance (V_D), and epistatic genetic variance (V_I):

$$V_G = V_A + V_D + V_I$$

These components of genetic variance do not refer to additive, dominance, and epistatic gene action; they refer to specific components of phenotypic variance that are produced by the entire genome, not that produced by one or two genes.

The major components are V_A and V_D. Epistatic genetic variance is usually considered to be unimportant, because it is difficult to exploit and improvements that occur by exploiting V_I plateau quickly.

Additive genetic variance is the component that is due to the additive effect of all the fish's alleles taken independently; i.e., the sum of the effects that each allele makes to the production of the phenotype. Additive genetic variance is the most important component of V_P, and the percentage of V_P that is controlled by V_A is called "heritability" (h^2):

$$h^2 = V_A/V_P$$

Additive genetic variance is the genetic component that can be exploited by selective breeding programmes. In general, phenotypes with h^2's $\gtrsim 0.25$ can be improved efficiently by individual (mass) selection; when h^2's are $\lesssim 0.15$, family selection is needed to improve a population. When h^2 is $\lesssim 0.10$, family selection is often ineffective, although some gains can be made when inbreeding is combined with family selection.

Dominance genetic variance is the other major component of V_G. Dominance genetic variance is the component that is due to the sum of each interaction that exists between the two alleles at each locus. Because V_D is produced by the interaction of the alleles at a locus, V_D cannot be inherited from either parent. Dominance genetic variance is a function of the diploid state (a function of the paired gene), and offspring inherit alleles that exist in the haploid state from each parent. Dominance genetic variance is created at fertilization when the haploid set of chromosomes from the mother pairs with the haploid set of chromosomes from the father. When this occurs, each gene exits in the paired state, and V_D is created. Consequently, V_D is destroyed by meiosis, and it is recreated anew and indifferent combinations at fertilization.

The breeding programme needed to exploit V_D is crossbreeding. When h^2 is $\lesssim 0.15$, crossbreeding is often prescribed to exploit V_G.

INBREEDING

Inbreeding is the mating of relatives. Genetically, all inbreeding does is increase homozygosity in the offspring (this means inbreeding also decreases heterozygosity in the offspring by an equal amount; in this manual, inbreeding will be defined and discussed as it relates to homozygosity). The increase in homozygosity occurs because related fish share alleles though one or more common ancestors; i.e., the parents may carry a copy of an allele that both inherited from a common ancestor. When relatives mate, the alleles that they share because of their common ancestor(s) can be paired in their offspring. This produces offspring that are more likely to be homozygous at one or more loci.

The mating of unrelated fish also produces offspring that have homozygous genes. Additionally, an inbred fish looks the same as one with no inbreeding; there is no distinguishing mark that separates fish into inbred vs. non-inbred categories. So why is a distinction made? How are they different genetically?

The answer to these questions are: The two types of homozygosity are identical; there is no genetic difference. The only difference between inbred and non-inbred fish is that the homozygosity of each is created in different ways, but this distinction is most important.

Inbred fish are homozygous because they have genes where the two alleles are identical by descent; non-inbred fish are homozygous because they have genes where the two alleles are identical because they are alike in kind (Figure 4). Inbred fish are homozygous at a locus because they inherited identical copies of an allele from both parents that the parents, in turn, inherited from a common ancestor. Non-inbred fish are homozygous at a locus because they just happened to inherit an identical pair of alleles from their parents. This difference in the type of homozygosity is not physical or chemical. The only difference is the paths that the alleles took before they were paired to create the homozygosity; i.e., how the alleles were inherited.

Since there is no difference, why do geneticists make a distinction between the two types of homozygosity? Why is inbreeding of any concern? The reason is that related fish are more alike genetically than unrelated fish. This means that when relatives mate, they produce offspring that have more homozygous loci than is the case when unrelated fish mate.

The mating of relatives produces offspring that tend to be more homozygous than the population average. This increase in homozygosity is called "inbreeding," and the coefficient of inbreeding (F) is a measure of how much more homozygous a fish is than the population average. The coefficient of inbreeding does not measure how many homozygous loci the fish has; it simply quantifies the percent increase in homozygosity. On a gene-by-gene basis, F is the probability that the two alleles will be identical by descent. Since F measures the percent increase in homozygosity, the same level of inbreeding can produce different amounts of homozygosity in different populations, depending on the level of homozygosity that existed before inbreeding began. The coefficient of inbreeding merely quantifies the increase in homozygosity that has occurred over a specific time interval (number of generations) due to identity by descent (due to the mating of relatives). Additionally, F is based on probability, so a given value of F is an average value; consequently, a given value of F means more homozygosity will have been produced in some fish, while less will have been produced in others.

The amount of inbreeding that is produced when relatives mate depends on the closeness of the relationship between the parents. Close relatives (e.g., brothers and sisters; parents and offspring) share many alleles inherited from common ancestors, so when they mate, they produce offspring with high levels of inbreeding. Distantly related individuals (e.g., fourth cousins; fifth cousins) share comparatively few alleles from a common ancestor, so when they mate, they produce offspring with comparatively little inbreeding. The way inbreeding is measured is explained in Chapters 3 and 4, and the inbreeding that is produced when various relatives mate is explained in Chapter 6.

Because inbreeding increases homozygosity, it changes genotypic frequencies, increasing the percentage of homozygous genotypes, while shrinking the percentage of heterozygous genotypes. If regular systems of inbreeding are conducted, the breeding programme creates a series of inbred families which do not interbreed. This subdivides a population into numerous lines, which further increases genotypic variance. Figure 5 shows how inbreeding changes genotypic frequency.

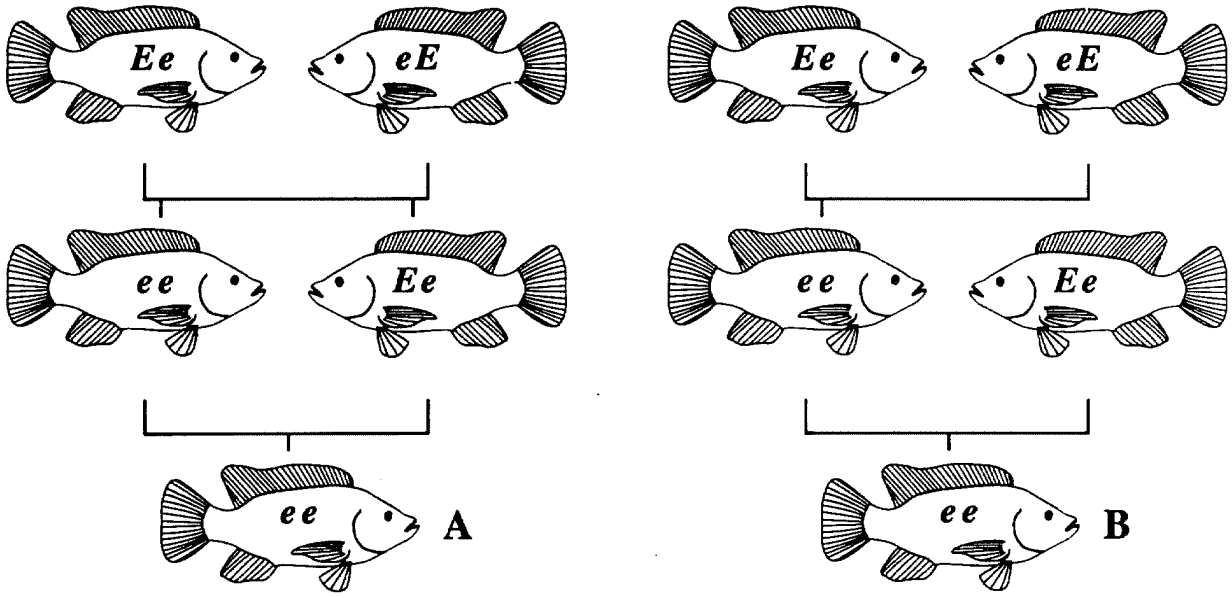
While inbreeding changes genotypic frequency, it does not change gene frequency (Figure 5). Selection, genetic drift (discussed in detail in Chapter 5), migration, and mutation are the evolutionary and breeding forces that change gene frequency. Inbreeding itself does not alter gene frequency, but by altering genotypic frequency inbreeding can, theoretically, accelerate selection.

During an inbreeding programme, genotypic frequencies can be drastically altered if relatively few inbred families are maintained (a type of selection). When this occurs, gene frequencies will also change.

Uses of inbreeding

Inbreeding is a breeding programme that can be used to produce superior animal and plant brood stock, and it can also be used to produce genetically improved animals and plants for grow-out. Inbreeding is the breeding programme that is often used to create new breeds or varieties that breed true for "type"; i.e., a particular body conformation or set of qualitative phenotypes. Linebreeding is a form of inbreeding that is used to increase an outstanding animal's contribution to a population. The use of herd bulls is a form of inbreeding that is used to quickly improve a population. Inbreeding can be used as a type of progeny testing to create defect-free

animals. Inbreeding can be used to improve response to selection when h^2 is small by combining inbreeding with between-family selection.



FISH A IS HOMOZYGOUS BY DESCENT; FISH A IS INBRED

FISH B IS HOMOZYGOUS IN KIND; FISH B IS NOT INBRED

Figure 4. Pedigrees of two fish with identical genotypes (ee), one of which is inbred and one of which is not. Fish A is inbred because its parents are related (brother and sister); it is homozygous by descent. Fish B is not inbred, because its parents are not related; it is homozygous in kind.

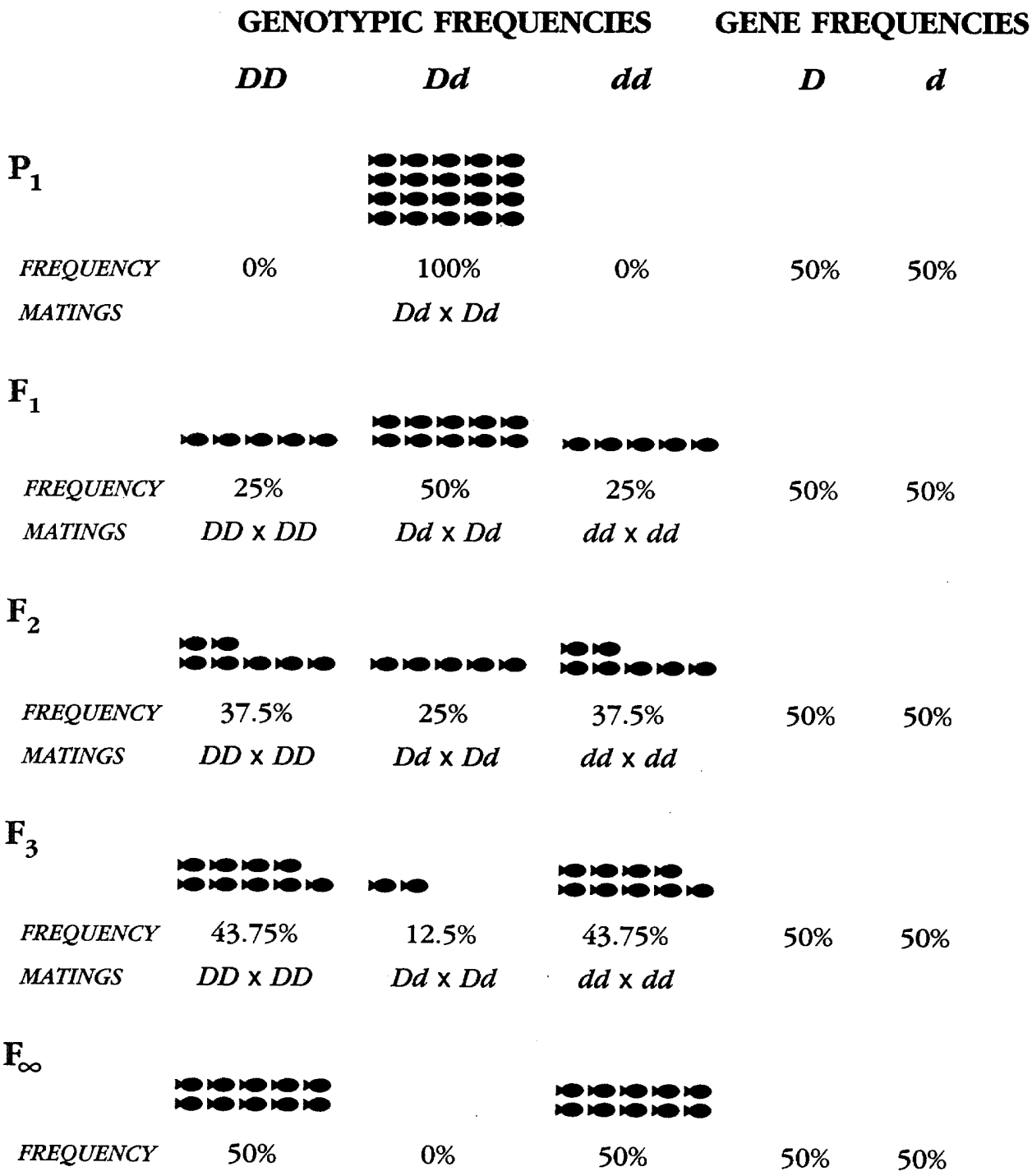


Figure 5. The effect of inbreeding on genotypic and gene frequencies. In the P_1 generation, all fish are heterozygotes (Dd). In every generation, the following matings are made: DD x DD; Dd x Dd; dd x dd. These matings reduce the percentage of heterozygotes and increase the percentage of homozygotes. In the F_3 generation, only 12.5% of the fish are heterozygotes, while 87.5% are homozygotes. Eventually, there will no heterozygotes. Although this mating pattern changes genotypic frequency, the frequencies of the two alleles do not change.

Finally, inbreeding is often combined with crossbreeding to increase hybrid vigour. Details about how inbreeding is used and the programmes needed to create inbred lines are described in Chapter 6.

Inbreeding depression

As inbreeding increases, it often causes a decrease in productivity which is termed "inbreeding depression." Inbreeding depression is a decrease in growth rate, fecundity, etc. that is observed in the inbred group when it is compared to a control population where there is no inbreeding. The severity of inbreeding depression depends on the level of inbreeding, the phenotype in question, and the population. Inbreeding depression is what gives inbreeding its bad reputation.

There are several explanations for why inbreeding depression occurs, and all contribute to inbreeding depression. The first two are qualitative genetic explanations.

The most common explanation is that inbreeding depression occurs because of the pairing and expression of detrimental recessive alleles. Although the terms "dominant" and "recessive" do not mean that one allele is good and the other undesirable, most of the alleles that produce abnormal phenotypes or that lower viability are recessive. Even though mutation rates are usually only one in 10,000 to one in 100,000 replications per gene, because each fish can produce many gametes (up to 500,000,000 sperm per spawn) and because each fish has tens of thousands of genes in its genome, each fish produces dozens to hundreds of gametes that contain a mutant copy of one or more genes. Most of these mutant alleles are recessive. Since recessive alleles can be expressed only when a fish is homozygous, these mutations tend to accumulate in a population. Consequently, many fish carry "hidden" copies of these mutant alleles in the heterozygous state.

Mutations do not have to be bad; some increase fitness or lead to the evolution of new species by creating new or improved phenotypes. But most mutations lower fitness, because they cause random changes in a phenotype that works and that works well as a result of natural selection. Many mutant alleles produce phenotypes that are so abnormal that they either cause death or reduce viability severely. Others cause only small reductions in viability.

Each fish carries a number of these detrimental recessive alleles, and inbreeding uncovers them. When relatives mate, they produce offspring with an increased level of homozygosity; consequently, some of the detrimental recessive alleles that the parents carry in the unexpressed heterozygous state are paired and expressed in the offspring. Detrimental recessive alleles can also be paired and expressed when unrelated fish mate. The difference is one of magnitude, and that is what causes inbreeding depression.

It has been estimated that each individual carries dozens of mutant alleles that lower viability. Most of these mutant alleles will produce only a small reduction in fitness, but several will produce phenotypes so abnormal that they are lethal or cause premature death. There are many of these unexploded genetic bombs in the gene pool. If two unrelated individuals mate, there is a good chance that they will not carry the same genetic dynamite. However, when relatives mate, there is a good chance that both parents will carry the same genetic time bombs, because they inherited them from a common ancestor. Even though the probability of producing a defective offspring is the same for each recessive allele (25% if both parents are heterozygotes), if both parents carry a large number of identical defective recessive alleles, the odds of producing defective offspring increase dramatically. The more closely related the parents, the more alike they are genetically, so it is more likely that defective offspring will be created.

Even if two parents do not carry any lethal recessive alleles, they can produce inbred offspring which exhibit inbreeding depression if the parents carry a number of recessive alleles that reduce viability, growth, or other production phenotypes. For example, if two relatives carry 10 recessive alleles, each of which depresses growth by 1%, the pairing and expression of these alleles will depress growth in the inbred offspring. Some offspring produced by these parents will not be homozygous for any of these genes, but most will be homozygous for one or more of these recessive alleles, and growth will be depressed from 1-10% in these offspring.

A second explanation for inbreeding depression is that by decreasing heterozygosity, inbreeding reduces what is called "overdominance." Overdominance occurs when the heterozygous genotype produces a phenotype that is superior to the two homozygous ones. Because inbreeding increases homozygosity, it decreases the number of heterozygous loci, which means that it reduces overdominance. The best known example of overdominance occurs in humans. In many populations, the gene which produces hemoglobin exists in two allelic forms: one produces normal hemoglobin, while the other produces sickle-cell hemoglobin. Individuals who are homozygous normal are susceptible to malaria and get quite ill and can die when infected. Individuals who are homozygous for sickle-cell get sickle-cell anemia and are sickly and often die. The heterozygotes are superior, even though they are slightly anemic, because they are resistant to malaria.

The final explanation for inbreeding depression is a quantitative genetic one. If a quantitative phenotype were controlled solely by V_A, inbreeding would not effect the phenotype, because the types of homozygosity that would be created at each locus would balance over the genome: some homozygous combinations would cause a slight decrease in the phenotypic mean, while other would cause a slight increase. Since quantitative phenotypes are controlled by hundreds of genes, and since the homozygosity that is created by inbreeding tends to be random, if a quantitative phenotype were solely controlled by V_A, inbreeding-produced homozygosity would neutralize itself, in terms of phenotypic expression. However, few if any quantitative phenotypes are controlled solely by V_A. When V_A controls just 30% of V_P for a particular phenotype, it produces a $h^2 = 0.3$, which is considered to be large.

Since inbreeding increases homozygosity, it also decreases heterozygosity. By doing this, inbreeding affects the interactions between alleles at each locus, which means that it affects V_D, and dominance effects can play a major role in the production of many quantitative phenotypes. When h^2 is small (<0.15), as it often is for viability and fecundity, dominance effects can be quite significant. The creation of excess homozygosity tends to disrupt dominance effects, which tend to be more unidirectional than the additive effects. Inbreeding can be considered to be the opposite of crossbreeding, which is used to exploit V_D. Consequently, if crossbreeding maximizes exploitation of V_D, inbreeding minimizes exploitation, which means that inbreeding makes phenotypes controlled by V_D worse (inbreeding decreases phenotypic mean).

This genetic explanation makes sense, because inbred lines are often used in crossbreeding programmes. The creation of inbred lines is done to produce opposite types of homozygosity that will be maximized when hybrids are created, which is how V_D is exploited. This explanation is further supported by the fact that crossbreeding, the breeding programme that is used to exploit V_D, can also be used to eliminate inbreeding and inbreeding depression.

INBREEDING STUDIES IN FISH

Unfortunately, there have been very few inbreeding studies with fish, so there is little specific information about the effects of inbreeding in aquacultured populations. In fact, inbreeding is the least studied aspect of fish genetics. Most of the studies that have been been conducted have been with salmonids, particularly rainbow trout. Most of the studies are singular experiments that were limited in scope, and many investigated only a single level of inbreeding-usually 25%. Furthermore, most studies looked at the effects of inbreeding in the range of 25-60%. No study has looked at the effects of inbreeding <12.5%. Such studies are needed, because inbreeding experiments with other animals have shown that mild levels of inbreeding can be beneficial.

Because of this, we know relatively little about the effects of inbreeding in fish culture. The studies have shown a few preliminary trends, but we do not know how different levels of inbreeding affect various phenotypes in all important cultured species of fish. We do not know what levels of inbreeding are beneficial and what levels are harmful. Finally, no inbreeding studies have been conducted on some important aquacultured species.

In general, inbreeding studies with fish have shown that inbreeding decreased production phenotypes such as growth rate, fecundity, and survival, while increasing the number of deformed offspring.

The most complete study was a long-term evaluation of the effects of six levels of inbreeding on rainbow trout. Some of the results of this study are summarized in Figures 6 and 7. The results show that the mildest level of inbreeding that was produced (12.5%; this is equal to one generation of half-sib matings) adversely affected percent hatch and survival, but it increased 77- to 150-day weight. Levels of inbreeding ≥25% severely depressed fecundity, growth, and survival (inbreeding of 25.0%, 37.5%, 50.0%, and 59.4% are produced by one, two, three, and four generations of brother-sister matings).

It is not known if these results apply to all species or to all populations of rainbow trout. Studies that have been conducted with other species of fish showed that inbreeding usually decreased means for production phenotypes, although the studies have produced mixed results: for example, inbreeding reduced the return rate of Atlantic salmon, Salmo salar; reduced growth and viability of Mozambique tilapia, Oreochromis mossambicus; increased growth of channel catfish, Ictalurus punctatus, in one study but reduced it in another. The only unambiguous result was that inbreeding produced some abnormal offspring.

It has been suggested that the breeding programmes used by most fish farmers will produce inbreeding of 3-5% per generation. If this occurs, inbreeding depression could begin to affect productivity and profits after only 3 to 5 generations.

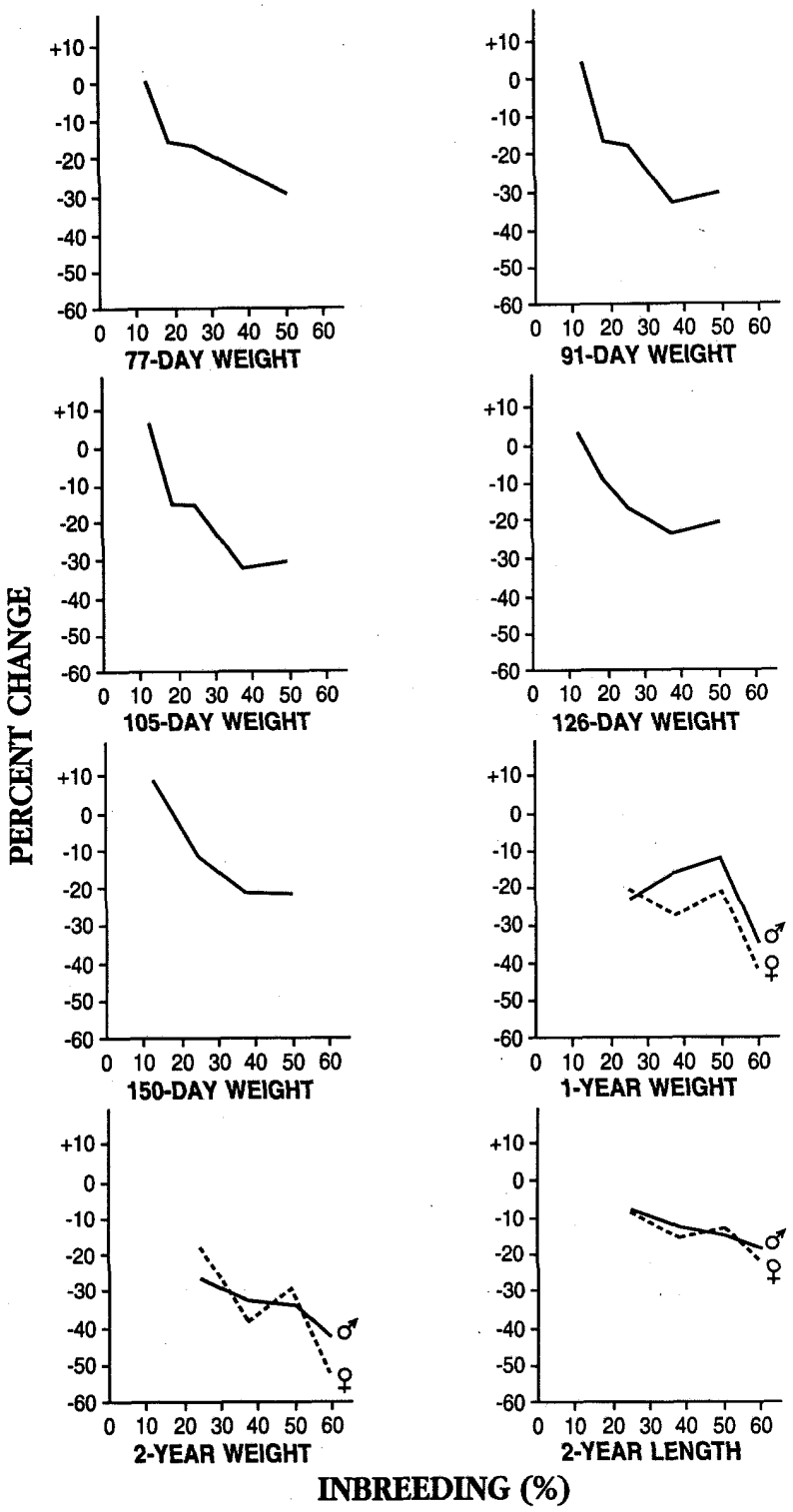


Figure 6. The effects of inbreeding on length and weight in rainbow trout. The results are presented as percent change when the inbred group was compared to a control group (inbreeding of 0%); a negative number means the inbred group was worse than the control and exhibited inbreeding depression. One generation of brother-sister mating produces inbreeding of 25%; two generations produce inbreeding of 37.5%; three generations produce inbreeding of 50%; and four generations produce inbreeding of 59.4%.

Data used to construct this figure are from: Kincaid, H.L. 1976. Effects of inbreeding on rainbow trout populations. Transactions of the American Fisheries Society 105:273-280; Kincaid, H.L. 1983. Inbreeding in fish populations used for aquaculture. Aquaculture 33:215-227.

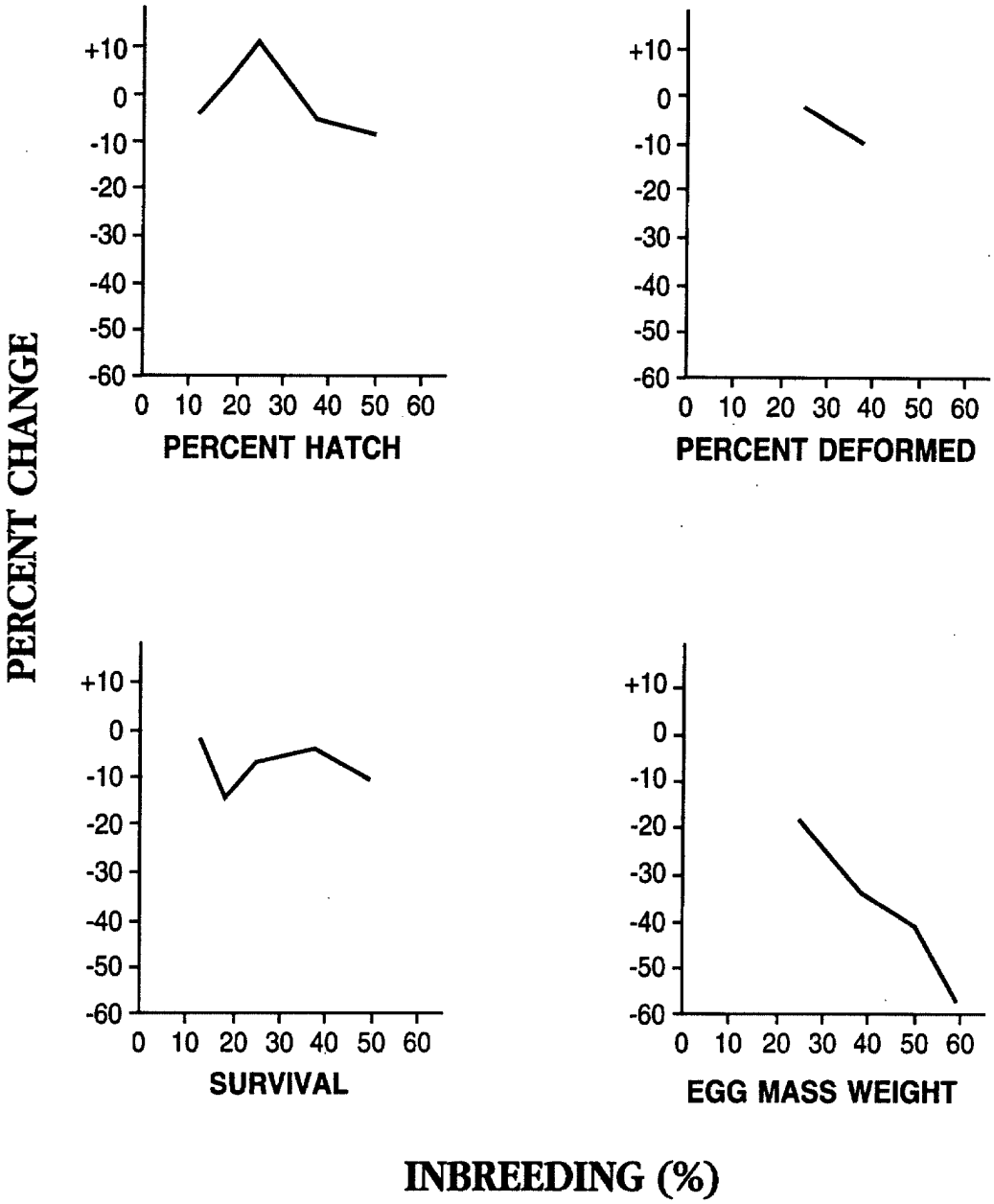


Figure 7. Effect of inbreeding on hatching rate, number of deformed offspring, survival, and fecundity (egg mass weight) in rainbow trout. The results are presented as percent change when the inbred group was compared to a control group (inbreeding of 0%); a negative number means the inbred group was worse than the control and exhibited inbreeding depression.

Data used to construct this figure are from: Kincaid, H.L. 1976. Effects of inbreeding on rainbow trout populations. Transactions of the American Fisheries Society 105:273-280; Kincaid, H.L. 1976. Inbreeding in rainbow trout (Salmo gairdneri). Journal of the Fisheries Research Board of Canada 33:2420-2426; Kincaid, H.L. 1983. Inbreeding in fish populations used for aquaculture. Aquaculture 33:215-227.

CHAPTER 3.

CALCULATING INDIVIDUAL INBREEDING VALUES

Calculating individual inbreeding values often requires information that a farmer or hatchery manager does not have-pedigrees. Those who culture fish usually do not know a fish's mother or father. Even if this information is known, it is unlikely that grandparents or cousins can be identified. This information is routinely gathered in livestock husbandry, and family pedigrees are recorded on forms or are entered into computer data bases.

It is far easier to record this information for cattle, pigs, sheep, and goats, because these animals are large, live on land, and can be branded or tagged with little effort at birth. This enables those who work with the marked livestock to identify each individual, to record matings and births, and thus to create pedigrees. Few aquaculturists record such information with fish, because fish are difficult to mark, and marking techniques often kill a large percentage of the fish. Additionally, while this information has been considered important for centuries with livestock, it is still considered to be of little value by most aquaculturists.

Even though it is impossible for most farmers or research scientists to determine individual inbreeding values, it is important to know how these values are calculated and to learn the techniques that are used. An understanding of the methods and protocols needed to determine individual inbreeding values explains how inbreeding is created. It helps explain how an individual can become homozygous by descent, which is what occurs when relatives mate and produce offspring. Learning how to trace family trees and how to calculate individual inbreeding values demonstrates how an ancestral allele can be inherited, both maternally and paternally, from a common ancestor. An understanding of these techniques also shows how inbreeding can be prevented, demonstrates how inbreeding can be reduced to zero, and helps lay the groundwork for the next chapter, the determination of average inbreeding values in hatchery populations.

Two methods can be used to determine individual inbreeding values: path analysis and covariance analysis. Both methods produce the same answer, but some feel more comfortable with one than the other. Both methods use simple arithmetic. Path analysis is quicker and requires fewer steps, but it is easier to make a mistake using this technique. Covariance analysis can take far more time if the pedigree is complicated, but once complete, the information generated by covariance analysis can be used to quickly predict inbreeding values that will be produced from any possible mating between individuals in the pedigree.

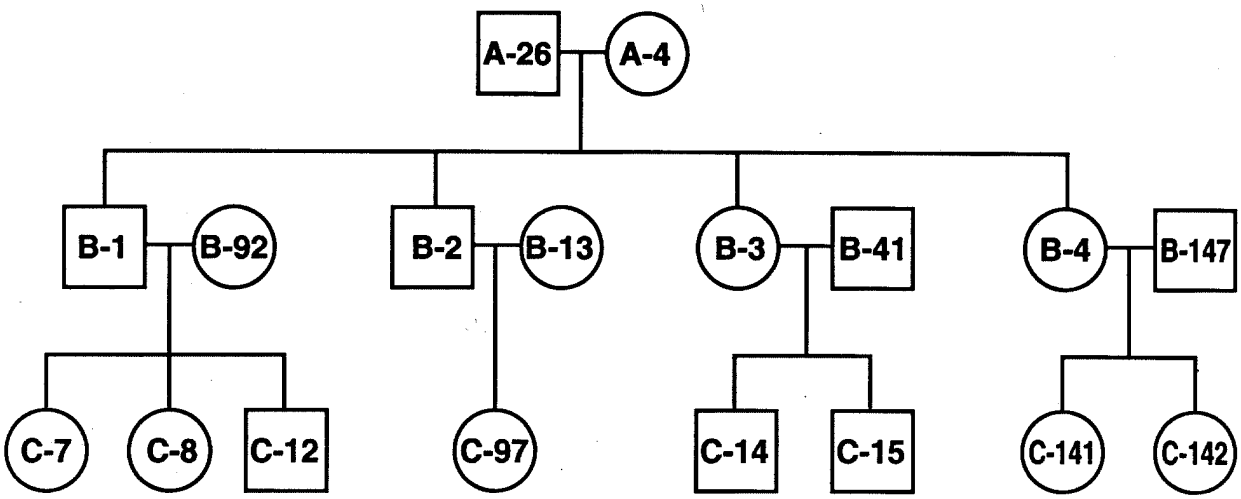
Those who are not interested in learning how to calculate individual inbreeding values can skip this chapter and go to Chapter 4, which describes the techniques that are used to determine average inbreeding values in hatchery populations.

CREATING A PEDIGREE

The first step in determining an individual fish's inbreeding is to create its pedigree. A pedigree is a family tree. It lists an individual's brothers and sisters, parents, aunts and uncles, grandparents, great-grandparents, nephews and nieces, and cousins. The information is usually streamlined; often, only direct ancestors are included; and the family tree is often traced back four or fewer generations, only going back to an individual's great-grandparents or occasionally including the great-great-grandparents.

More generations can be listed, but with each additional ancestral generation, the genetic contribution of each fish in the oldest generation to the present generation decreases to the point where it becomes genetically meaningless. This is because an animal's genetic contribution to its descendants is halved each generation: Each parent contributes 50% of a individual's genes; each grandparent contributes 25%; each great-grandparent contributes 12.5%; and each great-great-grandparent contributes 6.25%. The contribution of each great-great-great-great-great-grandparent (seven ancestral generations) is only 0.78%. These values are determined by the number of direct ancestors an individual has in each ancestral generation: two parents, four grandparents, eight great-grandparents, and 16 great-great-grandparents. All fish have 128 great-great-great-great-great-grandparents, which means the contribution from each is so small (0.78%) that it is of no value in most breeding programmes.

Figure 8 contains two pedigrees. Traditionally, males are represented by squares and females are represented by circles. Various symbols can be added to the squares and circles to convey phenotypic information, such as body colour, genetic diseases, etc., but this information is not needed to determine inbreeding. Other information that can be added to a pedigree are: names, birth and death dates, and weights at specific periods. When letters are used to designate individuals, the letter F is usually omitted, because F is the symbol for coefficient of inbreeding; its omission prevents confusion.



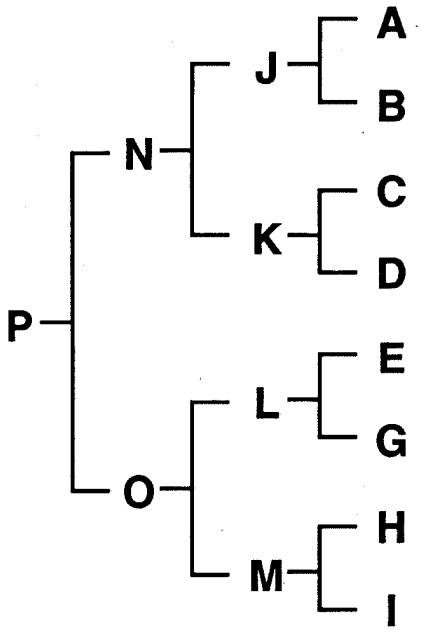


Figure 8. Examples of two pedigrees. The top pedigree uses squares to represent males and circles to represent females. Fish name is inside each square or circle. The other pedigree simply uses letter symbols to represent fish. Information about the fish, such as birth date, weight, etc., would be kept in ledgers or on data sheets. When letters are used to designate individuals, the letter F is usually omitted, because F is the symbol for coefficient of inbreeding; this prevents confusion.

PATH ANALYSIS

In path analysis, a pedigree is converted to a path diagram (Figure 9). A path diagram differs from a pedigree in that arrows between parents and offspring replace the tier-step decent brackets that are used in a pedigree. Each arrow represents a gamete, which means that each arrowhead represents the path or way an individual received 50% of its genes, and the arrow shaft touches the parent that contributed those genes. Thus, an arrow shows how a fish received one homologue of each chromosome pair, which means it also shows how a fish received one allele of each allelic pair (each gene).

Each fish in a path diagram can be touched by zero, one, or two arrowheads. If a fish's parents are unknown, no arrowhead will touch it; if one parent is known, a single arrowhead will touch the fish; if both parents are known, the fish will be touched by two arrowheads. A fish cannot be touched by more than two arrowheads, because no fish can have more than two biological parents. If this occurs, you have made an error. (Eventually, various biotechnological manipulations will enable research scientists to create fish that have three or four parents; however, for everyday practical breeding work, this situation can be ignored.) Many arrows can leave an individual, if that fish produced a number of offspring that are listed in the family tree that is being analyzed.

The arrows, or paths, flow from the oldest to the youngest generation, and they show how the genes were transmitted from generation to generation. They do not always flow in a straight line and sometimes cross or go sideways, depending on the complexity of the family tree and the matings that have occurred. Because of this, it is crucial to make the path diagram large enough to house the arrows and to accurately place the arrows. If arrows are incorrectly drawn, inbreeding values generated by the path diagram will be incorrect.

An individual's inbreeding value is calculated by determining all possible paths that the individual has with one or more common ancestors. A common ancestor is an individual that occurs on both sides of the family tree or pedigree; i.e., an individual that contributes genes through both the mother and the father. If just one common ancestor exists, the individual's parents are related and the fish is inbred. If there are no common ancestors, the inbreeding of that fish is zero.

Obviously, if family trees are traced back far enough, all species of fish, like all humans, are related though a common ancestor. However, for practical breeding purposes, common ancestors that are five or six ancestral generations removed are of little importance in any breeding programme, so the genetic contribution of such distant relatives is usually ignored. In most western societies, the legal distance for consanguineous marriages is second cousins; i.e., it is illegal to marry a first cousin or someone more related than that, but is lawful to marry a second cousin or someone less related than that. Western societal norms figure that the inbreeding produced by second cousin marriages is insignificant and therefore acceptable, while that produced by first cousin marriages is not. The inbreeding that is produced by matings between various relatives is listed in Figure 23 (page 57).

The following formula is used to determine individual inbreeding values from a path diagram:

$$F_X = \sum [(0.5)^N(1 + F_A)]$$

where:
- F_X = the inbreeding of an individual;
- $\sum$ = the symbol for "sum of" or "add";
- N = the number of individuals in a path that is determined by tracing a path from one parent back to the common ancestor and forward from the common ancestor to the other parent; if more than one common ancestor exists, the term $(0.5)^N$ is repeated for each common ancestor; if more than one path exists between the individual and a common ancestor, the term $(0.5)^N$ is repeated for each unique path;
- F_A = the inbreeding of the common ancestor.

If the inbreeding value of the common ancestor is zero or unknown, in which case you must assume that it is zero, the formula is simplified to:

$$F_X = \sum (0.5)^N$$

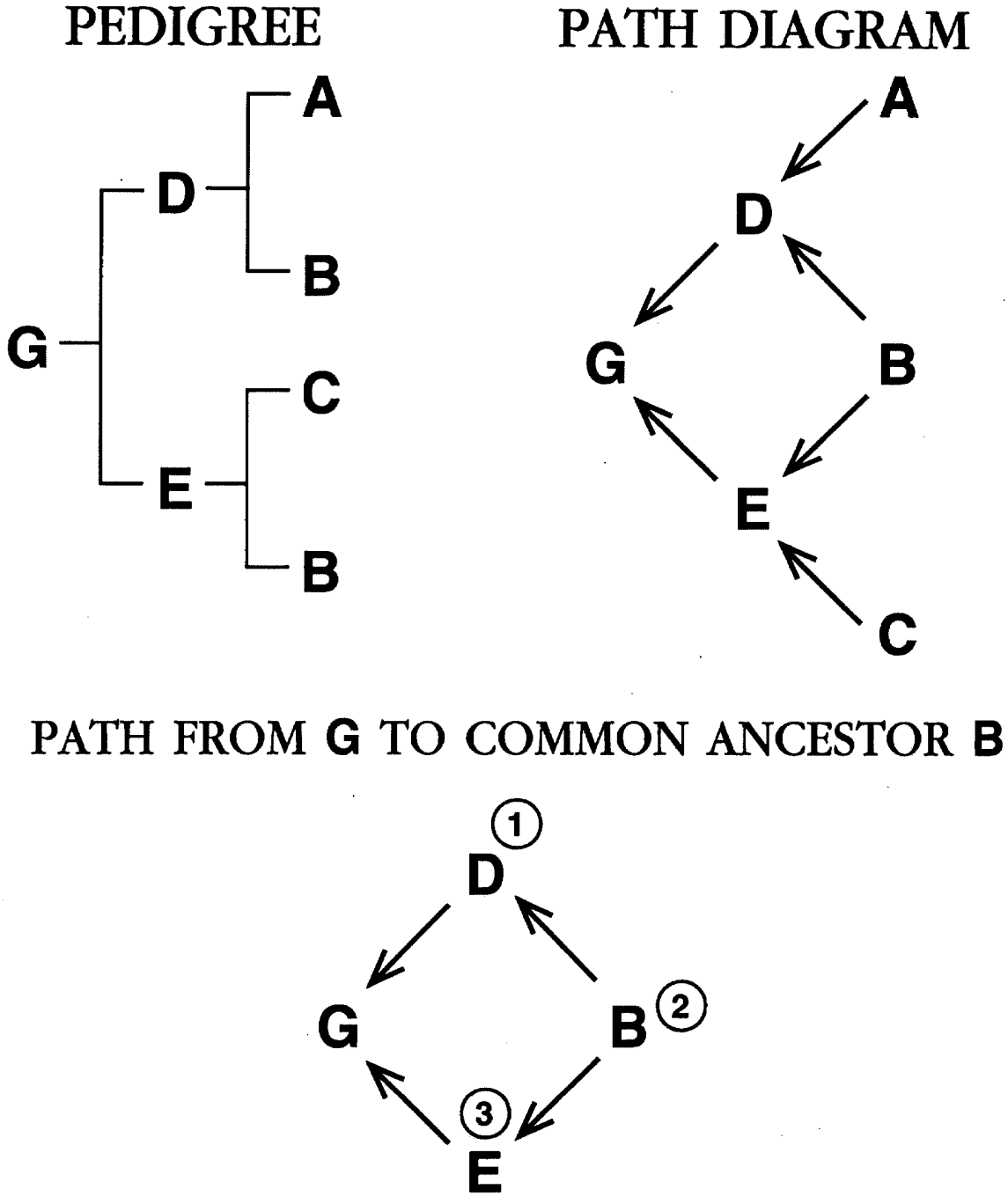


Figure 9. A simple pedigree and the path diagram that can be used to calculate inbreeding. The path that is used to calculate inbreeding of fish G is shown.

The formula may look like complicated math, but it is simple arithmetic that can be easily done with basic, inexpensive hand-held calculators; the term $(0.5)^N$ can be determined by using the "y^x" button.

In Figure 9, fish G is the only fish that has a common ancestor. An inspection of the pedigree shows that fish B is on both sides of fish G's family tree; this means that fish G is inbred. No other fish depicted in the pedigree has a common ancestor.

To determine an individual's inbreeding, a path is traced from one parent back to the common ancestor and from the common ancestor up to the other parent. Consequently, fish G's (Figure 9) inbreeding is determined by tracing the path that can be drawn from fish G's parents to fish B; i.e., from fish D (one parent) to fish B (the common ancestor) and from fish B to fish E (the other parent). Therefore, the path that is traced goes:

D-B-E

There are three individuals in this path, so N = 3. Since fish B (the common ancestor) is not inbred, the simplified formula can be used to calculate the inbreeding value of fish G:

$$F_G = (0.5)^3$$

$$F_G = 0.125$$

Thus, $F_G = 0.125$ or 12.5%. This means that fish G is expected to have 12.5% more homozygous loci than the average fish in the population. This also means that 12.5% of the loci that were heterozygous became homozygous as as result of inbreeding.

Figure 10 shows a more complicated pedigree and its path diagram. Fish J and fish N are inbred, because both have common ancestors. Fish A is the common ancestor of fish J. The inbreeding of fish J is determined by tracing a path to fish A through fish J's parents (fish H and fish I). The path is:

H-A-I

There are three individuals in the path, so N = 3. Since fish A (the common ancestor) is not inbred, we can use the simplified formula, and the inbreeding value for fish J is:

$$F_J = (0.5)^3$$
$$F_J = 0.125$$

Thus, $F_J = 0.125$ or 12.5%.

Fish N has more than one common ancestor. This means that more than one path will determine the inbreeding of fish N. Fish A and fish C are both common ancestors of fish N. A glance at the pedigree reveals that fish A and fish C are the only fish to appear on both sides of fish N's pedigree. Fish J is inbred, but since it does not appear on both sides of fish N's pedigree, its inbreeding value does not contribute to fish N's inbreeding value.

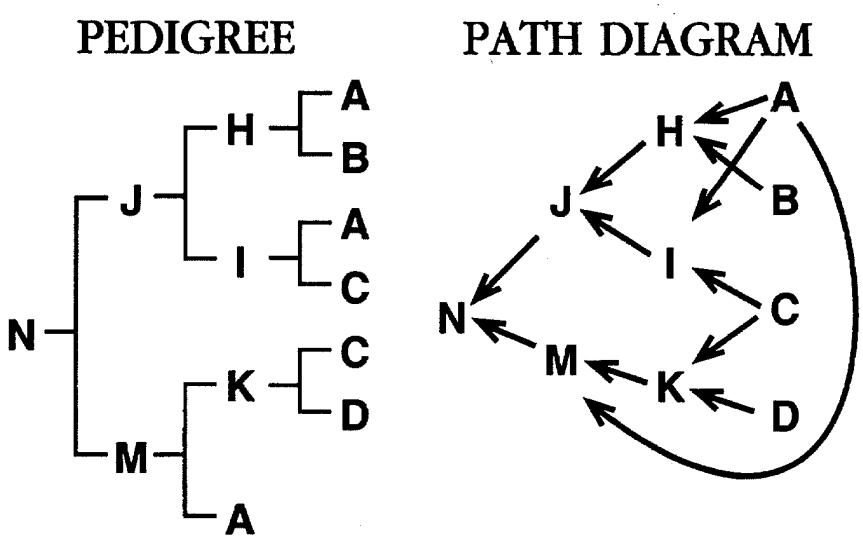
The path that can be traced from fish J to fish M (the parents of fish N) though common ancestor fish C is:

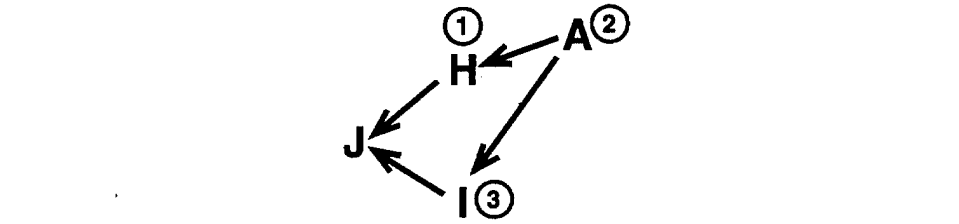
J-I-C-K-M

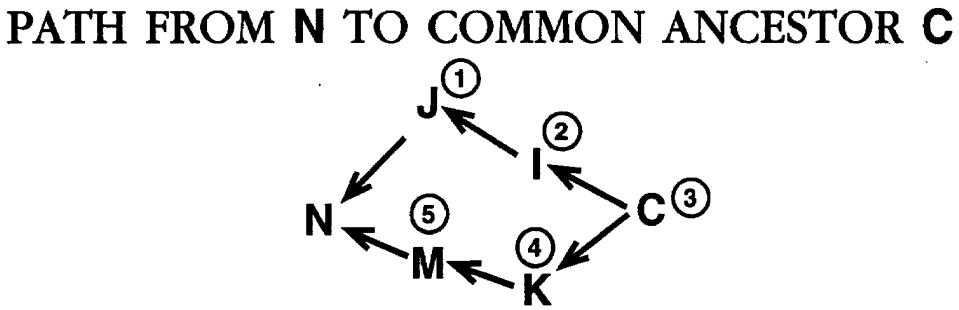
There are five individuals in this path, so N = 5.

Two different paths can be drawn from fish J to fish M (the parents of fish N) though common ancestor fish A:

1) J-H-A-M
2) J-I-A-M







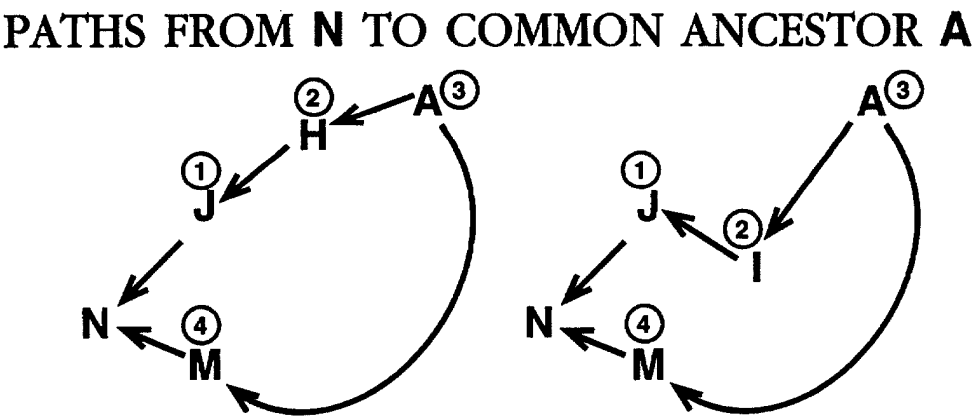


Figure 10. A pedigree and the path diagram that is used to calculate inbreeding. The paths that are used to calculate inbreeding in fish J and fish N are shown.

There are four individuals in each path, so N = 4 for both. Since neither common ancestor is inbred, the simplified formula can be used. The only difference that occurs when there is more than one common ancestor or when more than one path exits for a particular common ancestor is that the inbreeding value is determined by adding the value derived from each path:

$$F_N = (0.5)^5 + (0.5)^4 + (0.5)^4$$

$$F_N = 0.03125 + 0.0625 + 0.0625$$

$$F_N = 0.15625$$

Thus, $F_N = 0.15625$ or 15.62%.

There are two important rules about tracing paths:

Rule 1:

You cannot retrace a path; i.e., you cannot go though any individual twice in a given path.

That is why you cannot use the path J-I-C-I-A-M to determine inbreeding of fish N in Figure 10. Fish I occurs twice in that path.

Rule 2:

A path is traced backward from one parent to the common ancestor, and then forward from the common ancestor to the other parent.

The part of the path that goes from one parent to the common ancestor will travel only in a backward direction; i.e., the path it traces will always start at the arrowhead and go towards the shaft. The part of the path that returns from the common ancestor to the other parent will travel only in a forward direction; i.e., the path it traces will always start at an arrow's shaft and go to the arrowhead. A path that travels in both directions, either on the way to the common ancestor or on the way back, is an erroneous path; i.e, two arrowheads or the ends of two shafts cannot touch in a given path. This is why you cannot use the path J-H-A-I-C-K-M when determining the inbreeding of fish N in Figure 10. This path goes forward and backward while going from common ancestor A to parent M; two arrowheads touch when going A-I-C.

COVARIANCE ANALYSIS

In covariance analysis, a pedigree is converted to a covariance table, which is illustrated in Figure 11. Each individual in the pedigree is listed at the top of each column and to the left of each row. The parents of each individual are listed at the far left of the table. If one or both parents are unknown, that information is represented by a dash in the parents' column.

The cells that make up a covariance table will contain either the covariance values between two individuals or the covariance value of an individual. The cells that form the diagonal that starts with the upper left cell and ends with the lower right cell and that are the intersections produced by each individual's row and column (e.g., cells AA, BB, CC, etc.) are the cells that contain individual covariance values. The cells that lie below this diagonal are the cells that contain covariance values between two individuals.

Because all individuals are listed both in the rows and in the columns, the cells above the diagonal and those below the diagonal will contain the same information. It is not necessary to record the information twice, so the cells above the diagonal are left blank.

COVARIANCE TABLE

PARENTS	A	B	C	D	E	G	H	I	J	K
/ A										
/ B										
/ C										
/ D										
/ E										
/ G										
/ H										
/ I										
/ J										
/ K										

FORMULAE USED TO COMPLETE COVARIANCE TABLE

COVARIANCE VALUES BETWEEN INDIVIDUALS (BELOW-DIAGONAL CELLS ⁝⁝⁝)

$$COV_{BI} = \frac{\text{COV IND 1 WITH SIRE IND 2 + COV IND 1 WITH DAM IND 2}}{2}$$

INDIVIDUAL COVARIANCE VALUES (DIAGONAL CELLS ▨)

$$COV_{IND} = 1 + \frac{\text{COV OF THE PARENTS}}{2}$$

Figure 11. A covariance table that can be used to calculate individual inbreeding values. Each fish is listed in a row and in a column. The parents that produced each fish are listed to the left of each row. If a parent is not known, it is represented by a dash. The formula that determines covariance values between individuals will be used to fill in the below-diagonal cells (dotted cells). The formula that determines individual covariance values will be used to fill in the diagonal cells (lined cells). The cells above the diagonal contain the same information as those below the diagonal, so they are not used.

Two simple math formulae are needed to calculate the covariance values. The formula that determines covariance values between two individuals (the values below the diagonal) is:

$$Cov_{BI} = \frac{\text{Cov Ind 1 with Sire Ind 2} + \text{Cov Ind 1 with Dam Ind 2}}{2}$$

where: Cov_{BI} = the covariance between two individuals;
Cov Ind 1 with Sire Ind 2 = the covariance of individual 1 with the sire (father) of individual 2;
Cov Ind 1 with Dam Ind 2 = the covariance of individual 1 with the dam (mother) of individual 2.

The formula that determines individual covariance values (the values along the diagonal) is:

$$Cov_{Ind} = 1 + \frac{\text{Cov of the Parents}}{2}$$

where: Cov_{Ind} = the covariance of an individual;
Cov of the Parents = Cov_{BI} of the parents.

A few rules are needed to complete the table:

Rule 1 for determining Cov_{BI} values:
If individual 2's sire or dam is not known, the covariance of individual 1 with the unknown parent = 0.0.

Rule 2 for determining Cov_{BI} values:
Cov_{BI} values range from 0.0 to 2.0.

Rule 1 for determining Cov_{Ind} values:
If one or both parents are unknown, $Cov_{parents} = 0.0$. Consequently, $Cov_{Ind} = 1.0$.

Rule 2 for determining Cov_{Ind} values: Cov_{Ind} values range from 1.0 to 2.0.

Cov_{Ind} values (the diagonal values) can be used to determine individual inbreeding values. Inbreeding is determined by subtracting 1.0 from Cov_{Ind}:

$$F_{Ind} = Cov_{Ind} - 1.0$$

Thus, an individual that is 100% (1.0) inbred will have a $Cov_{Ind} = 2.0$:

$$F = 2.0 - 1.0 = 1.0$$

while one with no inbreeding will have a $Cov_{Ind} = 1.0$:

$$F = 1.0 - 1.0 = 0.0$$

Rule 1 for determining Cov_{Ind} values states that if you do not know both parents $Cov_{parents}$ = 0.0. The practical aspect of this rule means that if you do not know both parents, an individual automatically has inbreeding of zero. This assumption may be false, but it must be made. This rule simplifies calculations for many covariance tables because most hatcheries do not have good breeding records.

The covariance table for the pedigree and path diagram illustrated in Figure 9 is shown in Figure 12. The calculations that were used to complete that covariance table are given in Figure 13.

PARENTS		A	B	C	D	E	G
- / -	A	1.00					
- / -	B	0.0	1.00				
- / -	C	0.0	0.0	1.00			
A / B	D	0.5	0.5	0.0	1.00		
C / B	E	0.0	0.5	0.5	0.25	1.00	
D / E	G	0.25	0.5	0.25	0.625	0.625	1.125

Figure 12. Covariance table for the pedigree given in Figure 9.

$$Cov_{AA} = 1 + \frac{Cov\ -/-}{2} = 1 + \frac{0.0}{2} = 1.0$$

$$Cov_{AB} = \frac{Cov\ A- + Cov\ A-}{2} = \frac{0.0 + 0.0}{2} = 0.0$$

$$Cov_{BB} = 1 + \frac{Cov\ -/-}{2} = 1 + \frac{0.0}{2} = 1.0$$

$$Cov_{AC} = \frac{Cov\ A- + Cov\ A-}{2} = \frac{0.0 + 0.0}{2} = 0.0$$

$$Cov_{BC} = \frac{Cov\ B- + Cov\ B-}{2} = \frac{0.0 + 0.0}{2} = 0.0$$

$$Cov_{CC} = 1 + \frac{Cov\ -/-}{2} = 1 + \frac{0.0}{2} = 1.0$$

$$Cov_{AD} = \frac{Cov\ AA + Cov\ AB}{2} = \frac{1.0 + 0.0}{2} = 0.5$$

$$Cov_{BD} = \frac{Cov\ BA + Cov\ BB}{2} = \frac{0.0 + 1.0}{2} = 0.5$$

$$Cov_{CD} = \frac{Cov\ CA + Cov\ CB}{2} = \frac{0.0 + 0.0}{2} = 0.0$$

$$Cov_{DD} = 1 + \frac{Cov\ AB}{2} = 1 + \frac{0.0}{2} = 1.0$$

$$Cov_{AE} = \frac{Cov\ AC + Cov\ AB}{2} = \frac{0.0 + 0.0}{2} = 0.0$$

$$Cov_{BE} = \frac{Cov\ BC + Cov\ BB}{2} = \frac{0.0 + 1.0}{2} = 0.5$$

$$Cov_{CE} = \frac{Cov\ CC + Cov\ CB}{2} = \frac{1.0 + 0.0}{2} = 0.5$$

$$Cov_{DE} = \frac{Cov\ DC + Cov\ DB}{2} = \frac{0.0 + 0.5}{2} = 0.25$$

$$Cov_{EE} = 1 + \frac{Cov\ CB}{2} = 1 + \frac{0.0}{2} = 1.0$$

$$Cov_{AG} = \frac{Cov\ AD + Cov\ AE}{2} = \frac{0.5 + 0.0}{2} = 0.25$$

$$Cov_{BG} = \frac{Cov\ BD + Cov\ BE}{2} = \frac{0.5 + 0.5}{2} = 0.5$$

$$Cov_{CG} = \frac{Cov\ CD + Cov\ CE}{2} = \frac{0.0 + 0.5}{2} = 0.25$$

$$Cov_{DG} = \frac{Cov\ DD + Cov\ DE}{2} = \frac{1.0 + 0.25}{2} = 0.625$$

$$Cov_{EG} = \frac{Cov\ ED + Cov\ EE}{2} = \frac{0.25 + 1.0}{2} = 0.625$$

$$Cov_{GG} = 1 + \frac{Cov\ DE}{2} = 1 + \frac{0.25}{2} = 1.125$$

Figure 13. Calculations used to complete the covariance table in Figure 12.

This method is somewhat tedious, but the arithmetic is easy, and covariance values from the top half of the table are used to determine those in the bottom half. This method is more time consuming than a path diagram, because most of the table must be completed in order to determine the inbreeding of a fish at the end of a pedigree. But this method uses simple arithmetic, and when path diagrams become complicated, it is easy to overlook a path or to make a mistake when tracing a path.

In the covariance table in Figure 12, the covariance values for fish A, fish B, and fish C (Cov_{AA}, Cov_{BB}, and Cov_{CC}) are all 1.0 because we do not know their parents. The covariance values for fish D (Cov_{DD}) and fish E (Cov_{EE}) are both 1.0 because their parents are not related.

The Cov_{BI} values in the covariance table shown in Figure 12 describe the relationships between two individuals (also shown in the pedigree in Figure 9): Cov_{AD} and Cov_{BD} = 0.5 because fish A and fish B are the parents of fish D, and each parent contributes half (0.5) of fish D's genes. Cov_{DE} = 0.25, because fish D and fish E are half-sibs in that they share a single parent-fish B. (If fish D and fish E had shared both parents [full-sibs], Cov_{DE} would have been 0.5, or twice that for half-sibs.) Cov_{AG} = 0.25, because fish A is fish G's grandparent, and a grandparent contributes 25% of an individual's genes. Fish G receives genes from grandparent B via both parents (fish G is a double grandchild of fish B), which is why Cov_{BG} = 0.5, while Cov_{AG} and Cov_{CG} (fish A and fish C are fish G's other grandparents) = 0.25. Cov_{DG} = 0.625 because fish G is more related to that parent (fish D) than is normally the case (0.5): fish B appears on both sides of fish G's pedigree (fish B is fish G's maternal and paternal grandmothers). Fish G is related to its mother (fish D) in the normal manner, but it is also related to her through its father (fish E). Cov_{BC} = 0.0 because fish B and fish C are not related.

To determine inbreeding values, 1.0 is subtracted from Cov_{Ind} values. Fish A, B, C, D, and E are not inbred (F = 0.0), because all have Cov_{Ind} = 1.0. Fish G is inbred. Its inbreeding is:

$$F_G = Cov_{GG} - 1.0$$

$$F_G = 1.125 - 1.0$$

$$F_G = 0.125$$

Thus, F_G = 0.125 or 12.5%.

The covariance table and calculations needed to complete the table for the pedigree listed in Figure 10 are shown in Figures 14 and 15.

One tremendous advantage that a covariance table has over a path diagram is that a covariance table can be used to predict inbreeding from any mating among the individuals listed in the pedigree. The inbreeding that would be produced by any mating is simply half the Cov_{BI} value for the two fish. This information can then be used to decide if certain matings should be avoided. For example, using the covariance table in Figure 14, the inbreeding that would be produced by mating fish H and fish N would be 21.875% (Cov_{HN} = 0.4375). The inbreeding that would be produced by mating fish D with fish J would be 0% (Cov_{DJ} = 0.0).

If a farmer creates a table of covariance values for all female and male brood fish, he can tell at a glance what the inbreeding of any possible mating would be. An example of such a table is shown in Table 1. (Note: a table of covariance values is not a covariance table. Individuals are not listed in both rows and columns. In a table of covariance values, males are listed in columns and females are listed in rows [or vice versa].) All values in a table of covariance values are Cov_{BI} values, which means the inbreeding that will be produced by any mating combination is half the Cov_{BI} value for those individuals. For example, the mating of female 1 with male 2 (Table 1) will produce offspring with F = 6.25% ($Cov_{\text{female 1 with male 2}}$ = 0.125). If the farmer wants to produce a generation of fish with F = 0%, all he has to do is mate fish where the covariance value = 0.0. Thus, he could mate female 1 with male 1, female 2 with male 3, etc.

PARENTS		A	B	C	D	H	I	J	K	M	N
- / -	A	1.00									
- / -	B	0.0	1.00								
- / -	C	0.0	0.0	1.00							
- / -	D	0.0	0.0	0.0	1.00						
A / B	H	0.5	0.5	0.0	0.0	1.00					
A / C	I	0.5	0.0	0.5	0.0	0.25	1.00				
H / I	J	0.5	0.25	0.25	0.0	0.625	0.625	1.125			
D / C	K	0.0	0.0	0.5	0.5	0.0	0.25	0.125	1.00		
K / A	M	0.5	0.0	0.25	0.25	0.25	0.375	0.3125	0.5	1.00	
J / M	N	0.5	0.125	0.25	0.125	0.4375	0.5	0.71875	0.3125	0.65625	1.15625

Figure 14. Covariance table for the pedigree given in Figure 10.

$$Cov_{AA} = 1 + \frac{Cov\ -/-}{2} = 1 + \frac{0.0}{2} = 1.0$$

$$Cov_{AB} = \frac{Cov\ A- + Cov\ A-}{2} = \frac{0.0 + 0.0}{2} = 0.0$$

$$Cov_{BB} = 1 + \frac{Cov\ -/-}{2} = 1 + \frac{0.0}{2} = 1.0$$

$$Cov_{AC} = \frac{Cov\ A- + Cov\ A-}{2} = \frac{0.0 + 0.0}{2} = 0.0$$

$$Cov_{BC} = \frac{Cov\ B- + Cov\ B-}{2} = \frac{0.0 + 0.0}{2} = 0.0$$

$$Cov_{CC} = 1 + \frac{Cov\ -/-}{2} = 1 + \frac{0.0}{2} = 1.0$$

$$Cov_{AD} = \frac{Cov\ A- + Cov\ A-}{2} = \frac{0.0 + 0.0}{2} = 0.0$$

$$Cov_{BD} = \frac{Cov\ B- + Cov\ B-}{2} = \frac{0.0 + 0.0}{2} = 0.0$$

$$Cov_{CD} = \frac{Cov\ C- + Cov\ C-}{2} = \frac{0.0 + 0.0}{2} = 0.0$$

$$Cov_{DD} = 1 + \frac{Cov\ -/-}{2} = 1 + \frac{0.0}{2} = 1.0$$

$$Cov_{AH} = \frac{Cov\ AA + Cov\ AB}{2} = \frac{1.0 + 0.0}{2} = 0.5$$

$$Cov_{BH} = \frac{Cov\ BA + Cov\ BB}{2} = \frac{0.0 + 1.0}{2} = 0.5$$

$$Cov_{CH} = \frac{Cov\ CA + Cov\ CB}{2} = \frac{0.0 + 0.0}{2} = 0.0$$

$$Cov_{DH} = \frac{Cov\ DA + Cov\ DB}{2} = \frac{0.0 + 0.0}{2} = 0.0$$

$$Cov_{HH} = 1 + \frac{Cov\ AB}{2} = 1 + \frac{0.0}{2} = 1.0$$

$$Cov_{AI} = \frac{Cov\ AA + Cov\ AC}{2} = \frac{1.0 + 0.0}{2} = 0.5$$

$$Cov_{BI} = \frac{Cov\ BA + Cov\ BC}{2} = \frac{0.0 + 0.0}{2} = 0.0$$

$$Cov_{CI} = \frac{Cov\ CA + Cov\ CC}{2} = \frac{0.0 + 1.0}{2} = 0.5$$

$$Cov_{DI} = \frac{Cov\ DA + Cov\ DC}{2} = \frac{0.0 + 0.0}{2} = 0.0$$

$$Cov_{HI} = \frac{Cov\ HA + Cov\ HC}{2} = \frac{0.5 + 0.0}{2} = 0.25$$

$$Cov_{II} = 1 + \frac{Cov\ AC}{2} = 1 + \frac{0.0}{2} = 1.0$$

$$Cov_{AJ} = \frac{Cov\ AH + Cov\ AI}{2} = \frac{0.5 + 0.5}{2} = 0.5$$

$$Cov_{BJ} = \frac{Cov\ BH + Cov\ BI}{2} = \frac{0.5 + 0.0}{2} = 0.25$$

$$Cov_{CJ} = \frac{Cov\ CH + Cov\ CI}{2} = \frac{0.0 + 0.5}{2} = 0.25$$

$$Cov_{DJ} = \frac{Cov\ DH + Cov\ DI}{2} = \frac{0.0 + 0.0}{2} = 0.0$$

$$Cov_{HJ} = \frac{Cov\ HH + Cov\ HI}{2} = \frac{1.0 + 0.25}{2} = 0.625$$

$$Cov_{IJ} = \frac{Cov\ IH + Cov\ II}{2} = \frac{0.25 + 1.0}{2} = 0.625$$

$$Cov_{JJ} = 1 + \frac{Cov\ HI}{2} = 1 + \frac{0.25}{2} = 1.125$$

$$Cov_{AK} = \frac{Cov\ AD + Cov\ AC}{2} = \frac{0.0 + 0.0}{2} = 0.0$$

$$Cov_{BK} = \frac{Cov\ BD + Cov\ BC}{2} = \frac{0.0 + 0.0}{2} = 0.0$$

$$Cov_{CK} = \frac{Cov\ CD + Cov\ CC}{2} = \frac{0.0 + 1.0}{2} = 0.5$$

$$Cov_{DK} = \frac{Cov\ DD + Cov\ DC}{2} = \frac{1.0 + 0.0}{2} = 0.5$$

Figure 15 (part 1). Calculations used to complete the covariance table in Figure 14.

$$Cov_{HK} = \frac{Cov\,HD + Cov\,HC}{2} = \frac{0.0 + 0.0}{2} = 0.0$$

$$Cov_{IK} = \frac{Cov\,ID + Cov\,IC}{2} = \frac{0.0 + 0.5}{2} = 0.25$$

$$Cov_{JK} = \frac{Cov\,JD + Cov\,JC}{2} = \frac{0.0 + 0.25}{2} = 0.125$$

$$Cov_{KK} = 1 + \frac{Cov\,DC}{2} = 1 + \frac{0.0}{2} = 1.0$$

$$Cov_{AM} = \frac{Cov\,AK + Cov\,AA}{2} = \frac{0.0 + 1.0}{2} = 0.5$$

$$Cov_{BM} = \frac{Cov\,BK + Cov\,BA}{2} = \frac{0.0 + 0.0}{2} = 0.0$$

$$Cov_{CM} = \frac{Cov\,CK + Cov\,CA}{2} = \frac{0.5 + 0.0}{2} = 0.25$$

$$Cov_{DM} = \frac{Cov\,DK + Cov\,DA}{2} = \frac{0.5 + 0.0}{2} = 0.25$$

$$Cov_{HM} = \frac{Cov\,HK + Cov\,HA}{2} = \frac{0.0 + 0.5}{2} = 0.25$$

$$Cov_{IM} = \frac{Cov\,IK + Cov\,IA}{2} = \frac{0.25 + 0.5}{2} = 0.375$$

$$Cov_{JM} = \frac{Cov\,JK + Cov\,JA}{2} = \frac{0.125 + 0.5}{2} = 0.3125$$

$$Cov_{KM} = \frac{Cov\,KK + Cov\,KA}{2} = \frac{1.0 + 0.0}{2} = 0.5$$

$$Cov_{MM} = 1 + \frac{Cov\,KA}{2} = 1 + \frac{0.0}{2} = 1.0$$

$$Cov_{AN} = \frac{Cov\,AJ + Cov\,AM}{2} = \frac{0.5 + 0.5}{2} = 0.5$$

$$Cov_{BN} = \frac{Cov\,BJ + Cov\,BM}{2} = \frac{0.25 + 0.0}{2} = 0.125$$

$$Cov_{CN} = \frac{Cov\,CJ + Cov\,CM}{2} = \frac{0.25 + 0.25}{2} = 0.25$$

$$Cov_{DN} = \frac{Cov\,DJ + Cov\,DM}{2} = \frac{0.0 + 0.25}{2} = 0.125$$

$$Cov_{HN} = \frac{Cov\,HL + Cov\,HM}{2} = \frac{0.625 + 0.25}{2} = 0.4375$$

$$Cov_{IN} = \frac{Cov\,IJ + Cov\,IM}{2} = \frac{0.625 + 0.375}{2} = 0.5$$

$$Cov_{JN} = \frac{Cov\,JJ + Cov\,JM}{2} = \frac{1.125 + 0.3125}{2} = 0.71875$$

$$Cov_{KN} = \frac{Cov\,KJ + Cov\,KM}{2} = \frac{0.125 + 0.5}{2} = 0.3125$$

$$Cov_{MN} = \frac{Cov\,MJ + Cov\,MM}{2} = \frac{0.3125 + 1.0}{2} = 0.65625$$

$$Cov_{NN} = 1 + \frac{Cov\,JM}{2} = 1 + \frac{0.3125}{2} = 1.15625$$

Figure 15 (part 2). Calculations used to complete the covariance table in Figure 14.

Table 1. Example of a table of covariance values for brood fish at a fish station. Males are listed across the top, and females are listed along the left. This table is a compilation of Cov_{BI} values among all brood fish, so all cells are filled; the information above the diagonal is not the same as that below the diagonal, and the diagonal values are not Cov_{Ind} values. This table can be used to predict the inbreeding that will be produced by any mating combination. The inbreeding that will be produced by any mating is simply half the Cov_{BI} value. The table lists only 10 males and 10 females.

	Males									
Females	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
1	**0.0**	**0.125**	**0.125**	**0.125**	**0.0**	**0.5**	**0.5**	**0.5**	**0.25**	**0.25**
2	**0.75**	**0.75**	**0.0**	**0.0**	**0.25**	**0.0**	**0.625**	**0.625**	**0.0**	**0.25**
3	**0.0**	**0.0**	**0.0**	**0.25**	**0.0**	**0.0625**	**0.0625**	**0.625**	**0.0**	**0.25**
4	**0.125**	**0.125**	**0.5**	**0.5**	**0.5**	**0.0625**	**0.0625**	**0.25**	**0.25**	**0.25**
5	**0.0**	**0.25**	**0.0**	**0.0**	**0.75**	**0.75**	**0.75**	**0.0**	**0.625**	**0.0**
6	**0.0**	**0.125**	**0.125**	**0.25**	**0.0**	**0.625**	**0.625**	**0.625**	**0.625**	**0.0**
7	**0.25**	**0.125**	**0.125**	**0.0**	**0.5**	**0.0**	**0.0**	**0.625**	**0.625**	**0.625**
8	**0.0**	**0.25**	**0.25**	**0.0**	**0.5**	**0.0**	**0.0**	**0.625**	**0.0625**	**0.0625**
9	**0.0**	**0.25**	**0.25**	**0.0**	**0.5**	**0.0**	**0.0**	**0.625**	**0.125**	**0.125**
10	**0.0**	**0.025**	**0.25**	**0.0**	**0.0**	**0.625**	**0.625**	**0.0**	**0.5**	**0.5**

CONCLUSION

Individual inbreeding values can be determined only if a farmer has detailed breeding records which can be turned into pedigrees. There are many reasons why farmers and hatchery managers should maintain breeding records. The ability to determine individual inbreeding values is but one reason. If such information exists, it is easy to calculate individual inbreeding values and to use them to predict future inbreeding values from various mating combinations.

Individual inbreeding values can be determined by two techniques: path analysis and covariance analysis. Both techniques provide accurate results, so the method that is used is determined by personal choice. Path analysis requires less work, but following paths can be tricky, so it is easier to make a mistake using this method. Covariance analysis is more tedious, but the math is very simple, and the completed table can be used to quickly predict any inbreeding that would be produced by mating any fish that are included in the table.

Both techniques clearly demonstrate an important fact: An individual is inbred if and only if its parents are related through one or more common ancestors. Both parents can be highly inbred, but if they are not related, their offspring will have F = 0.0. Figure 16 clearly illustrates this concept. Fish J and fish U are inbred; F_J = 0.25 and F_U = 0.375. However, the mating of fish J and fish U will produce offspring with no inbreeding, because fish J and fish U are not related through a common ancestor. F_Z = 0.0 because no fish appears on both the maternal and paternal sides of fish Z's pedigree.

This fact has important consequences for brood stock management and for breeding programmes. Inbreeding can be reduced to zero in a single generation by mating unrelated fish. Even if inbreeding has reached levels that cause problems, inbreeding can be eliminated and prevented in subsequent generations simply by examining pedigrees and by mating unrelated fish.

This can also be be accomplished by acquiring fish from another hatchery and by making hybrids. If the fish from the two hatcheries are unrelated, the F_1 hybrids will have inbreeding of zero. F_1 hybrids produced by this technique always have inbreeding of zero, provided the parents are unrelated. In fact, many hybrid seeds that are produced commercially are produced by crossbreeding highly inbred, but unrelated, lines. This reduces inbreeding to zero in the plants that will be grown and also produces some hybrid vigour, as well as uniform crops.

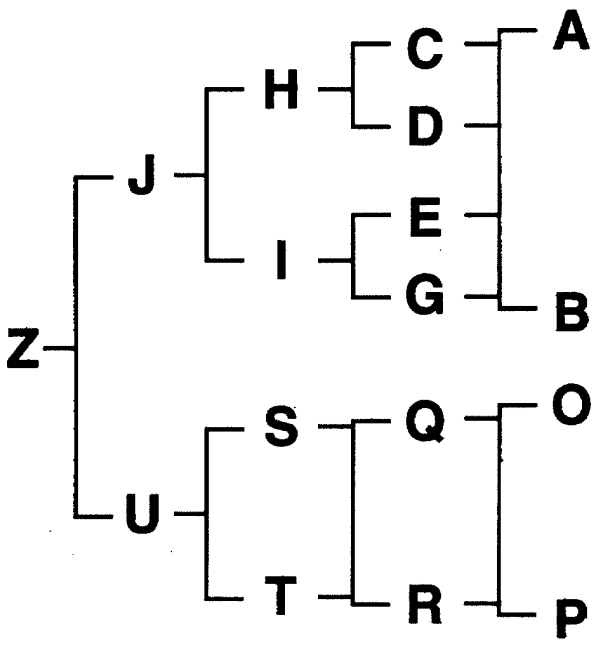


Figure 16. Pedigree illustrating the fact that the mating of two inbred, but unrelated, fish will produce offspring with no inbreeding: F_J = 25% and F_U = 37.5%, but because they are not related, F_Z = 0%.

CHAPTER 4.

CALCULATING AVERAGE INBREEDING VALUES IN HATCHERY POPULATIONS

Most farmers and hatchery managers cannot calculate individual inbreeding values for fish that they culture, because individual fish cannot be identified and because pedigrees are not recorded. However, this does not mean that inbreeding values cannot be determined for hatchery fish. Even though individual inbreeding values cannot be calculated, population averages can be and should be determined. Average inbreeding values should be determined for all hatchery populations every generation, and the acquisition and use of these data should be an integral part of hatchery management. The average inbreeding value is as important as average harvest weight or any other data that can be gathered.

The average inbreeding value of a population is determined from the population's effective breeding number (N_e). Effective breeding number is one of the most important bits of information that can be gathered about a hatchery population, and N_e should be determined every generation for all hatchery populations.

The first part of this chapter will define N_e, will describe what factors influence N_e, and will then show how N_e is calculated. Effective breeding number is traditionally calculated by counting the number of males and number of females that produce viable offspring. This direct approach will be the technique described in this chapter.

Recently, protocols have been developed to assess N_e indirectly by examining changes in gene frequency and by assessing something called "linkage disequilibrium," which is the difference between the actual and expected co-occurrence of two alleles at two loci. In some cases, these indirect procedures may provide a more accurate estimate of N_e, because they account for differential reproduction and offspring survival, which can have a marked impact on N_e. However, these techniques are more expensive because they require detailed biochemical work and they also require highly trained scientists who are skilled at biochemical population genetics, requirements that are not available at most farms or hatcheries.

Consequently, the traditional approach of enumerating the males and females that spawn and that produce viable offspring will be the only technique that will be described in this chapter. This approach is easy and the math is relatively simple. Those who wish to learn about the indirect procedures will find papers that describe these techniques in Recommended Reading.

The second part of the chapter will show how N_e is used to determine the average inbreeding value of a hatchery population.

The techniques outlined in this chapter are fairly simple. Subsistence-level farmers do not need to know how to determine N_e and average inbreeding, but any farmer or hatchery manager who wants to increase yields and productivity or to manage populations that are being cultured in order to restock lakes and rivers should learn how to determine these values and should incorporate these protocols into yearly work plans.

Effective breeding number and the average inbreeding value are important population descriptors, because they help explain trends in yield, fecundity, and other important production phenotypes. These values also enable a farmer to predict if problems will occur as a result of inbreeding depression or the loss of genetic variance. Proper brood stock management cannot be accomplished without this information.

The information generated in this chapter will be used in Chapter 5 to show how N_e affects genetic drift-random changes in gene frequency-and in Chapters 7 and 8 which will describe techniques that can be used to manage N_e and inbreeding.

EFFECTIVE BREEDING NUMBER (N_e)

The N_e of a hatchery population is one of the most important bits of information about the population. Unfortunately, most hatchery managers do not know what N_e is, do not know how to determine N_e, and do not know how N_e influences inbreeding and brood stock management. If asked to describe the size of their hatchery population, most farmers or hatchery managers would produce a census or an approximate number. Those with good records would p robably be able to give the number of male and female brood fish. This information is used to determine the number of fingerlings that can be produced, to calculate the amount of feed that must be ordered, or to estimate yield. As important as this information is, it does not describe the population genetically. In order to describe a population genetically, one must determine N_e.

Effective breeding number is one of the most important concepts in brood stock management, because it gives an indication about the genetic stability or genetic health of the population. This is because N_e is inversely related to inbreeding and to genetic drift. The relationship between N_e and inbreeding will be described later in this chapter. That with genetic drift will be described in Chapter 5.

If hatchery populations were infinitely large, an understanding of N_e would be unnecessary. However, hatchery populations are usually small and are often closed. A closed population is one where immigration (the introduction of fish from another population) is not allowed; consequently, fish from other populations are not allowed to mate with or hybridize with fish from a closed population. Hatchery managers often maintain closed populations for various reasons; chief among them is the desire to minimize health problems by preventing the introduction of diseases that often accompany acquired fish.

When working with a closed, finite population, the best way to describe it is not by total number of fish, but by N_e. Effective breeding number is determined by the number of male and female brood fish that produce viable offspring, the sex ratio of the brood fish that spawned, the variance of family size, and the mating system that is used.

In most situations where fish cannot be identified and where mating is random (fish are paired without regard to phenotypic value, or fish swim free in a pond and choose their own mates), N_e can be determined by using the following formula:

$$N_e = \frac{4(\text{number of males})(\text{number of females})}{\text{number of males} + \text{number of females}}$$

where: number of males and number of females are the number of male and female brood fish that produce viable offspring. If all offspring for a brood fish die, that male or female is not included when determining N_e.

If matings and offspring production cannot be monitored, N_e cannot be determined. If fish spawn in ponds and eggs are allowed to hatch in the ponds and if offspring are not harvested until they are mixed schools of fry or fingerlings, N_e will be difficult, if not impossible, to determine.

The formula shows that N_e is determined both by the number of male and female brood fish and by the sex ratio. For example, if 53 female brood fish produced eggs and if 25 males were used to fertilize those eggs and if all brood fish produced viable offspring, N_e is:

$$N_e = \frac{4(53)(25)}{53 + 25}$$

$$N_e = \frac{5300}{78}$$

$$N_e = 67.95$$

This example illustrates a fundamental concept of brood stock management: The genetic size of the population (N_e) and the number of fish that produce offspring are not always the same; the genetic size is usually smaller. Seventy-eight brood fish produced offspring, but N_e was only 67.95. The reason N_e was smaller is because the sex ratio was skewed; in this example, the sex ratio was 2.12 females:1 male (53 females:25 males).

Effective breeding number and the number of brood fish that produce offspring will be the same only when the sex ratio is 1:1. In the above example, there were 78 total brood fish, so a 1:1 sex ratio would have been 39 females:39 males. If these numbers were used to produce offspring, N_e would have been:

$$N_e = \frac{4(39)(39)}{39 + 39}$$

$$N_e = \frac{6084}{78}$$

$$N_e = 78$$

Figure 17 shows the effect of number of males and females and of the sex ratio in determining N_e. The information presented in Figure 17 clearly shows that the best way to increase N_e is to increase the number of males and the number of females that spawn and produce viable offspring and to bring the sex ratio closer to a 1:1 ratio. Increasing one sex while keeping the other sex at a fixed number has little effect on increasing N_e after a certain point; this can be seen by examining the curves for 1, 2, 10, and 25 males in Figure 17.

Unequal reproductive success can affect N_e. If some fish produce thousands of viable offspring while others produce only dozens, N_e will be less than expected. Variance in family size is a major reason why N_e is often smaller than expected. Variance in family size effects N_e as follows: When there are single pair matings, variance in family size is equal for both sexes, and N_e becomes:

$$N_{eUR} = \frac{4(N_e)}{\text{variance of offspring production}+2}$$

Where: N_{eUR} is effective breeding number when there is unequal reproductive success and variance in offspring production is variance in family size.

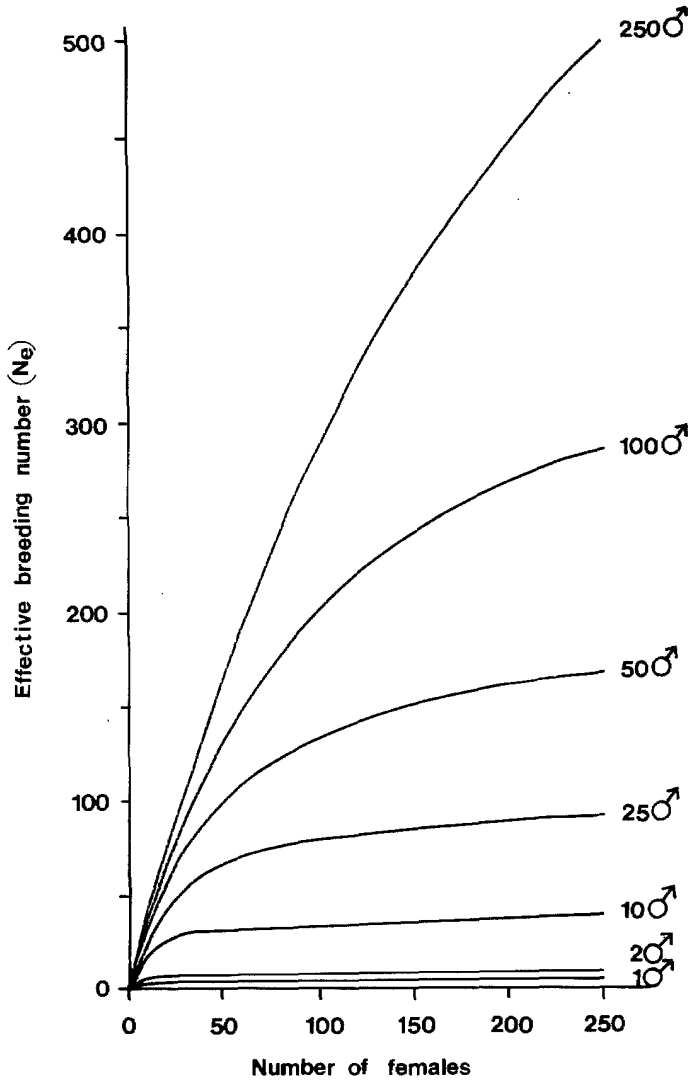


Figure 17. Effective breeding numbers produced by various combinations of males and females.

From: Tave, D. 1993. Genetics for Fish Hatchery Managers, 2nd ed. Van Nostrand Reinhold, New York, New York, USA.

For example, if N_e in a population is 100, there are single pair matings (50 males and 50 females), and the variance in family size (variance in offspring production) is 5, N_e becomes:

$$N_{eUR} = \frac{4(100)}{5+2}$$

$$N_{eUR} = \frac{400}{7}$$

$$N_{eUR} = 57.14$$

The variance in family size reduced predicted N_e by 43%, and yielded an effective breeding population of just 28 males and 28 females.

When males mate with more than one female and/or females mate with more than one male, there is unequal reproductive success for the two sexes, and N_e becomes:

$$N_{eUR} = \frac{8(N_e)}{\begin{array}{l}\text{variance of female} \\ \text{offspring production} +\end{array} \quad \begin{array}{l}\text{variance of male} \\ \text{offspring production} + 4\end{array}}$$

Family size often assumes what statisticians call a "Poisson distribution," and when this occurs the mean and the variance are the same. If this occurs, mean family size can be used.

The N_e that has been calculated in the previous examples is that for a single generation. It is important to determine N_e for every generation, because the values are not independent. The average N_e for a series of generations is determined by calculating the harmonic mean:

$$\frac{1}{N_{e\cdot mean}} = \frac{1}{t}\left(\frac{1}{N_{e1}} + \frac{1}{N_{e2}} + ... + \frac{1}{N_{et}}\right)$$

where: $N_{e\ mean}$ is the mean effective breeding number over t generations; and N_{e1}, N_{e2}, and Net are the effective breeding numbers in generations 1, 2, and t, respectively. The math needed to determine mean N_e is fairly simple and can be done "by hand"; however, inexpensive hand-held calculators are preprogrammed to determine inverses (the "1/x" key), so their use reduces this formula to simple arithmetic.

Because the mean is determined by the harmonic average, the generation with the smallest N_e will have a controlling influence on average N_e. This means that if a disease or other calamity reduces N_e, subsequent increases in N_e will have little effect on increasing the average.

For example, if the N_e's for five generations are 50, 10, 65, 85, and 100, the mean N_e is:

$$\frac{1}{N_{e\ mean}} = \frac{1}{5}\left(\frac{1}{50} + \frac{1}{10} + \frac{1}{65} + \frac{1}{85} + \frac{1}{100}\right)$$

$$\frac{1}{N_{e\ mean}} = 0.2(0.02 + 0.1 + 0.01538 + 0.01176 + 0.01)$$

$$\frac{1}{N_{e\ mean}} = 0.031428$$

$$N_{e\ mean} = 31.82$$

In this example, N_e went below 50 only once, but mean N_e was 31.82 because N_e dropped to 10 for a single generation. Even though N_e was 85 and 100 in the final two generations, if just 16 males and 16 females had been spawned each of the five generations, mean N_e would have been slightly larger ($N_{e\ mean} = 32$).

This example illustrates one of the most important concepts of brood stock management: N_e must be managed each and every generation. The population's N_e must be considered to be one of its most important descriptors. If N_e declines to a low level for just a single generation, this will have a devastating impact on mean N_e, even if N_e is maintained at high levels before and after the single-generation reduction. When a harmonic mean is used to determine the mean, the generation with the lowest value has a disproportionate effect on the mean. The implications of this on the genetic health of a hatchery populations will be discussed in Chapter 7.

INBREEDING

Once N_e has been determined, a simple formula can be used to calculate the average inbreeding value in the population:

$$F = \frac{1}{2N_e}$$

where F is the average inbreeding value in the population. For example, if N_e = 100, the average inbreeding value for the fish in the population is:

$$F = \frac{1}{2(100)}$$

$$F = 0.005$$

This value is assigned to every offspring produced by those brood fish.

The formula shows that F is inversely related to N_e: a large N_e produces a small F; a small N_e produces a large F. Figure 18 shows how much inbreeding will be produced by various N_e's for a single generation. The relationship between F and N_e in this formula clearly demonstrates why it is important to calculate N_e and why management of N_e is of paramount importance in the management of hatchery populations.

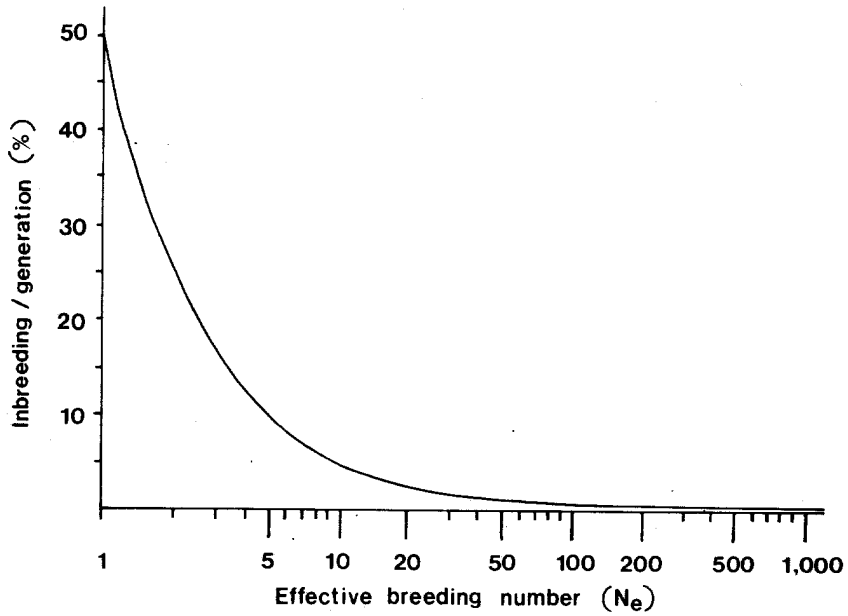


Figure 18. The relationship between effective breeding number (N_e) and average inbreeding (F) in a population. This is the average inbreeding produced by a single generation of mating.

From: Tave, D. 1990. Effective breeding number and broodstock management: I. How to minimize inbreeding. Pages 27-38 in R.O. Smitherman and D. Tave, eds. Proceedings Auburn Symposium on Fisheries and Aquaculture. Alabama Agricultural Experiment Station, Auburn University, Alabama, USA.

The inbreeding that is calculated in this manner is genetically identical to that which was determined in Chapter 3; it is a measure of the increase in homozygosity that occurred as a result of the mating of relatives. The only difference is: when individual inbreeding values are determined, the values are customized for each individual, based on its family pedigree; when N_e is used to determine inbreeding, the value that is calculated is the population average. Consequently, some fish will be more inbred than the average while others will be less inbred, and there is no easy and inexpensive way to determine which fish are above the mean and which are below. But if pedigrees do not exist, this is the only way to produce an estimate of the inbreeding that has accumulated in the population.

The reason why F and N_e are inversely related is that chance encounters between relatives increase in small populations, and inbreeding is the mating of relatives. When mating is random, closed populations produce consanguineous matings by chance, and the probability of consanguineous matings is inversely related to N_e. For example, if a fish has 50 relatives in a population of 1,000,000, the odds of that fish mating with a relative (assuming there is a 50:50 sex ratio) is 25/500,000 or 0.00005 if mates are chosen at random without regard to pedigree. If a fish has 50 relatives in a population of 1,000 fish, the odds of mating with a relative jumps to 25/500 or 0.05, which is 1,000 times greater. Consequently, the probability of mating with a relative and of producing inbred offspring increases as N_e decreases.

Once inbreeding is produced in a small closed population as a result of a small N_e, the inbreeding itself lowers future N_e in a positive feedback cycle:

$$N_{eF} = \frac{N_e}{1+F}$$

where: N_{eF} is the effective breeding number in an inbred population, and F is the average inbreeding in the population.

The positive feedback illustrated in this formula means that reductions in N_e increase inbreeding, which in turn lowers N_e, which in turn increases inbreeding, etc. Because it is a positive feedback, the system takes a bad situation and continually makes it worse.

CONCLUSION

Farmers and hatchery managers should determine the N_e of a hatchery population every generation. A population's N_e is one of the most important pieces of information about that population because it describes its genetic size. An understanding of N_e and what it means is of major importance because N_e helps determine the genetic stability of the population in that it determines the average inbreeding in the population and influences genetic drift. Once N_e has been quantified, it can be used to determine the average inbreeding value in the population. The link between N_e and genetic drift will be discussed in Chapter 5.

The average inbreeding value in the population is inversely related to N_e, which means that inbreeding can become a problem in small, closed populations, which are typical of those found at most farms and fish culture stations. Once inbreeding occurs, the problem can quickly become worse as inbreeding and N_e are linked in a *positive feedback loop. Consequently, small N_e's will produce levels of inbreeding which will cause growth* rate, fecundity, and other traits to decline over succeeding generations.

It is important to understand what N_e is, how it is determined, and how it affects inbreeding so that it can be managed to prevent unwanted inbreeding from decreasing productivity and profits. Techniques that can be used to manage N_e and thus prevent inbreeding from reaching levels that cause problems will be discussed in Chapter 7, and recommended N_e's that should be maintained to prevent inbreeding from reaching levels that cause problems will be presented in Chapter 8.

CHAPTER 5.

GENETIC DRIFT

Genetic drift is the second important genetic concept that is a function of N_e. Genetic drift is random changes in gene frequency; it is a major factor in evolution and population biology, but most aquaculturists have not heard of it. However, like inbreeding, it too must be considered if hatchery populations are to be properly managed.

Genetic drift can be as important as selection in altering a population's gene pool. The effects of genetic drift can be devastating. Genetic drift can irreversibly alter gene frequencies and eliminate alleles, which can decrease a population's ability to survive or to adapt to an altered environment, and it can preclude future selection. The effect that genetic drift can have on a population's gene pool can make many management goals impossible to achieve.

Those who do not wish to learn about genetic drift, how it is measured, and the effects it has on hatchery populations can skip this chapter and go to: Chapter 6, which describes how inbreeding programmes can be used to improve populations; Chapter 7, which describes techniques that can be used to manage N_e to prevent inbreeding from reaching detrimental levels and which can prevent genetic drift from eliminating rare alleles; Chapter 8, which prescribes N_e's that are needed to prevent inbreeding- and genetic drift-related problems.

EFFECTIVE BREEDING NUMBER AND GENETIC DRIFT

Genetic drift is random changes in gene frequency that occur because of sampling error. Sampling error can be natural, or it can be manmade. Natural sampling errors are those which occur when earthquakes, floods, landslides, or other natural disasters subdivide a population and isolate small groups of organisms. This process is a major force in the evolution of new species. Manmade sampling errors are inaccurate collections: sampling only a portion of the population, sampling only a single age class, sampling only fish that possess a certain phenotype or that spawn on a particular day, etc.

When a population is sampled, there is a chance that the sample does not accurately reflect the make-up of the population. This inaccuracy can include length, sex ratio, body colour, and gene frequencies. The smaller the sample, the greater the likelihood that inaccuracies in the sample will occur. Changes in gene frequency that occur as a result of sampling error are called "genetic drift."

When culturing fish, the important changes that can occur in gene frequency as a result of genetic drift occur during the creation of the next generation (during spawning season) or during the acquisition of the population. This is when the genes are transferred from parents to offspring (the transfer of genes across time; from one generation to the next) or when they are transferred from one hatchery to another (the transfer of genes across space). As was the case with inbreeding, N_e is the factor that determines the magnitude of genetic drift. The relationship between N_e and genetic drift is:

$$\sigma^2_{\Delta q} = \frac{pq}{2N_e}$$

where: $\sigma^2_{\Delta q}$ is the variance in the change of gene frequency, and p and q are the frequencies of alleles p and q for a given gene. The variance of the change in gene frequency is the way genetic drift is measured.

Like average inbreeding value, genetic drift is also inversely related to N_e. A comparison of the two formulae shows that they are similar. This means that large N_e's produce small changes in gene frequency, while small N_e's produce large changes in gene frequency.

Many factors can cause changes in gene frequency; among them are: selection by the farmer or hatchery manager; domestication (selection caused by the hatchery and the techniques used to culture the fish); genetic drift. The changes in gene frequency caused by selection and domestication usually produce genetic improvements, in that the fish grow faster, are more disease resistant, are calmer, accept pelleted feed more readily, have more efficient feed conversions, and are easier to spawn. In contrast, the changes in gene frequency that are caused by genetic drift are random, which means they can be counterproductive.

If the frequency of an allele changes from, say, 0.5 to 0.45 or from 0.4 to 0.3 as a result of genetic drift, the genetic effects on the population might not be that great. The major damage that is caused by genetic drift occurs when the frequency of an allele goes to 0.0; i.e., the allele is lost and no longer exists in the population. The odds of losing alleles via genetic drift are related to their frequencies; i.e., rare alleles (the frequency is low; usually ≤ 0.01) are lost more easily than common ones.

The probability of losing an allele by genetic drift is determined by using the following formula:

$$P = (1.0 - q)^{2N_e}$$

where: P is the probability of losing an allele, and q is the frequency of the allele.

For example, if N_e is 50, the probability of losing an allele whose frequency is 0.1 ($q = 0.1$) is:

$$P = (1.0 - 0.1)^{2(50)}$$

$$P = (0.9)^{100}$$

$$P = 0.0000265$$

If N_e is 10, the probability of losing the same allele is:

$$P = (1.0 - 0.1)^{2(10)}$$

$$P = (0.9)^{20}$$

$$P = 0.12158$$

These examples clearly show that the probability of losing an allele is inversely related to N_e; the probability of losing the allele was 4,588 times greater when N_e decreased from 50 to 10.

Table 2 lists the probabilities of losing alleles of various frequencies via genetic drift for N_e's that range from 2 to 6,880. For example, an N_e of 10 produces a probability of losing an allele of $P = 0.000001$ (1×10^{-6}) for an allele whose frequency is 0.5. This means that there is a virtual guarantee (99.9999% chance) of keeping an allele whose frequency is 0.5 (guarantee of keeping the allele = 1.0 - the probability of losing the allele). On the other hand, the same N_e produces a probability of losing an allele of $P = 0.81791$ for an allele whose frequency is 0.01; the guarantee of keeping such an allele with an N_e of 10 is only 18.2%. These values demonstrate that rare alleles are more likely to be lost than common ones when N_e is small.

Table 2. Probabilities of losing an allele via genetic drift for eight allelic frequencies at various effective breeding numbers (N_e). These probabilities are for a single event (spawning season or acquisition of brood stock). The guarantee of keeping the allele is: 1.0 - the probability of losing the allele. Once the probability of losing an allele reaches 1×10^{-6} (a 99.9999% guarantee of keeping it), no further probabilities are listed.

	Allelic frequency							
N_e	0.5	0.4	0.3	0.2	0.1	0.05	0.01	0.001
2	0.06250	0.12960	0.24010	0.40960	0.65610	0.81451	0.96060	0.99601
3	0.01562	0.04666	0.11765	0.26214	0.53144	0.73509	0.94148	0.99402
4	0.00391	0.01680	0.05765	0.16778	0.43047	0.66342	0.92274	0.99203
5	0.00098	0.00605	0.02825	0.10737	0.34868	0.59874	0.90438	0.99004
6	0.00024	0.00218	0.01384	0.06872	0.28243	0.54036	0.88638	0.98807
7	0.00006	0.00078	0.00678	0.04398	0.22877	0.48768	0.86875	0.98609
8	0.00002	0.00028	0.00332	0.02815	0.18530	0.44013	0.85146	0.98412
9	4×10^{-6}	0.00010	0.00163	0.01801	0.15009	0.39721	0.83451	0.98215
10	1×10^{-6}	0.00004	0.00080	0.01153	0.12158	0.35849	0.81791	0.98019
14		6×10^{-7}	0.00005	0.00193	0.05233	0.23783	0.75472	0.97237
15			0.00002	0.00124	0.04239	0.21464	0.73970	0.97043
20			6×10^{-7}	0.00013	0.01478	0.12851	0.66897	0.96077
25				0.00001	0.00515	0.07694	0.60501	0.95121
30				2×10^{-6}	0.00180	0.04607	0.54716	0.94174
31				1×10^{-6}	0.00146	0.04158	0.53627	0.93985
35					0.00063	0.02758	0.49484	0.93236
40					0.00022	0.01652	0.44752	0.92308
45					0.00008	0.00989	0.40473	0.91389
50					0.00003	0.00592	0.36603	0.90479
55					9×10^{-6}	0.00354	0.33103	0.89578
60					3×10^{-6}	0.00212	0.29938	0.88687
66					9×10^{-7}	0.00115	0.26537	0.87628
70						0.00076	0.24487	0.86930
75						0.00046	0.22145	0.86064
80						0.00027	0.20028	0.85208
85						0.00016	0.18113	0.84359
90						0.00010	0.16381	0.83520
95						0.00006	0.14814	0.82688
100						0.00004	0.13398	0.81865
125						3×10^{-6}	0.08106	0.77870
135						1×10^{-6}	0.06630	0.76328
150							0.04904	0.74071
175							0.02967	0.70456
200							0.01795	0.67019
225							0.01086	0.63748
230							0.00982	0.63114
250							0.00657	0.60638
275							0.00398	0.57679
300							0.00240	0.54865
325							0.00146	0.52188
350							0.00088	0.49641
375							0.00053	0.47219
400							0.00032	0.44915
425							0.00019	0.42723
450							0.00012	0.40639
475							0.00007	0.38656
500							0.00004	0.36770
685							1×10^{-6}	0.25393
1498								0.04991
2302								0.00999
6880								1×10^{-6}

From: Tave, D. 1993. Genetics for Fish Hatchery Managers, 2nd ed. Van Nostrand Reinhold, New York, New York, USA.

The probabilities listed in Table 2 are for a single generation (single spawning season with complete brood stock replacement or the acquisition of brood stock). Additionally, they are the probability for losing a single allele of the frequencies that are listed. Consequently, if there is a 20% chance of losing an allele of a given frequency, 20% of all alleles with that frequency will probably be lost while 80% will remain, although the frequencies will probably be different as a result of genetic drift.

The loss of alleles via genetic drift has two effects: First, it increases homozygosity; consequently, it has an effect similar to that seen for inbreeding. The simultaneous effect of an increase in inbreeding and the loss of alleles via genetic drift as a result of a decrease in N_e can cause severe genetic problems. Secondly, the loss of alleles reduces genetic variance. Genetic variance is the raw material with which selection works. A reduction in genetic variance can make selection difficult, if not impractical. If there is no genetic variance, there will be no heritable differences, which means that selection cannot improve a phenotype. Equally important, if the population is being cultured for stocking lakes and rivers, the loss of genetic variance may doom the project to failure. Natural populations need broad gene pools (i.e., they need as much genetic variance as possible), because it is impossible to predict what genotypes and what alleles will be needed to ensure survival. Populations with narrow genetic bases are less likely to survive in the long term.

The problems caused by genetic drift are measurable, and genetic drift has been shown to have damaged the gene pool of several hatchery populations. Research has shown that brood stock acquisition drastically altered gene frequencies, even when measures were taken to prevent genetic drift. Genetic drift has been shown to have robbed an important aquacultured population of Nile tilapia of so many alleles that there was no detectable heterozygosity and there was virtually no heritable variance for growth. Finally, genetic drift-induced changes in gene frequency may be a major reason why many stocking programmes have been unsuccessful.

Techniques that can be used to manage N_e to prevent the loss of alleles are described in Chapter 7.

CONCLUSION

The genetic stability of a closed hatchery population depends on the population's N_e. As was the case with average inbreeding, genetic drift is inversely related to N_e. Consequently, small hatchery populations can cause random changes in gene frequency. The ultimate effect of a small N_e is the loss of alleles via genetic drift. Rare alleles will be lost more easily, but common alleles can also be lost. The loss of genetic variance can produce irreversible damage to a population's gene pool. This loss can prevent future improvements via selection, and it can reduce viability in populations that are stocked in lakes and rivers.

CHAPTER 6.

USING INBREEDING TO IMPROVE GROWTH AND OTHER PHENOTYPES

A farmer or hatchery manager who wants to manage the genetic aspects of his population must know how inbreeding can be used to improve productivity and profits. Inbreeding is one of the three major traditional breeding programmes that breeders have used for centuries to improve animals and plants. While it is not as important as selection or crossbreeding, inbreeding is used to produce genetically improved livestock, plants, and laboratory animals. Inbreeding might be the most important breeding technique used in the production of laboratory animals, because genetically uniform lines of rats, mice, etc. are desired for biological and medical research.

Inbreeding will never be as important in animal husbandry as it is in agronomy, because many plants can be self-fertilized; it is easy to create matings by artificial pollination; and many individuals can be raised cheaply in a small plot. However, inbreeding has been used to create better, faster growing livestock, and new technologies have improved our ability to create and use highly inbred fish to improve a population or for research purposes. Inbreeding programmes should be far easier with fish than with livestock, because many species can be stripped, which enables fish culturists to create mating combinations livestock farmers can only imagine. Additionally, fish are highly fecund when compared to livestock, so different types of inbreeding programmes can be used.

In order to use an inbreeding programme to improve productivity and profits, a farmer or hatchery manager must understand how inbreeding works and what it does. The genetics of inbreeding was discussed in Chapter 2, and the techniques that are used to calculate individual inbreeding values were outlined in Chapter 3. This chapter will explain how inbreeding can be used to improve populations by itself, or how inbreeding can be combined with selection and crossbreeding to improve growth rate and other phenotypes. Finally, regular systems of inbreeding that can be used to produce inbred fish for breeding programmes are described.

Those who are not interested in learning how inbreeding programmes can be used to improve a population but only want to learn how to prevent unwanted inbreeding can skip this chapter and go to Chapter 7.

INBREEDING PROGRAMMES

Although selection and crossbreeding are the breeding programmes that are usually considered when plans are made to improve a population genetically, inbreeding is a third option that can be used to produce good results. Inbreeding is generally shunned because it is a two-edged sword that can mortally wound a population. However, when used properly, inbreeding can be an effective and efficient breeding programme. In general, inbreeding programmes are used when you have superior animals. If you inbreed average animals, you produce average animals. But if you inbreed superior animals, you can create outstanding animals.

Some of the ways inbreeding programmes can be used are described in this section.

Creation of new breeds

Inbreeding is often used when a new breed, strain, or variety is founded. In many cases it is inevitable and unavoidable. New breeds can be formed as the result of a fortuitous hybrid, or they can originate from a single individual with an unusual or desirable phenotype, as was the case with the Morgan horse, an American breed founded by a stallion named Justin Morgan. When breeds are new and small, inbreeding is inevitable. When breeds or strains are first created, there may be only a few males in the breed, and one is considered to be far superior to all others. When this happens, that male is bred to many females and to a good percentage of his daughters and granddaughters in order to produce a population of animals that resembles him. This is how a breed "type" (a particular body conformation) is created. A second round of inbreeding can occur if only one

or two of the male's sons are used. When the size of the breed increases, inbreeding will be less important, but it is the breeding technique that is often used to develop a new breed or strain that breeds true for type.

Linebreeding

Linebreeding is an inbreeding programme whereby an individual is mated to its descendants. Traditionally, a single male is bred to many females. Most linebreeding programmes that are used in livestock husbandry only linebreed males, because fecundity is low and gestation periods are long. However, in aquaculture the fecundity of fish should enable farmers and hatchery managers to use females as well as males in linebreeding programmes.

Linebreeding can be used when the breed is in its infancy to develop the breed, or it can also be used in an established breed or strain when an outstanding animal is discovered. Linebreeding is used to increase an outstanding individual's contribution to a population, especially when a farmer thinks that the animal is so superior that it is unlikely that he will ever find a better one.

Linebreeding is used by many livestock farmers, even when they say that they do not want inbred animals. Linebreeding is often not considered to be inbreeding, because the major goal is not to produce inbred animals but to increase an outstanding individual's contribution to the population and to succeeding generations. However, this will occur only because linebreeding is inbreeding.

Even if inbreeding reaches fairly high levels in a population, if the inbreeding is planned it can be used to produce outstanding individuals, and linebreeding is an inbreeding programme that is designed to accomplish that goal. Inbreeding depression is a measure of the population, not of an individual. Inbreeding depression is the difference between the mean of the inbred group and the mean of a control population with no inbreeding. Since inbreeding depression is a populational value, it represents the average value, which means an individual with a high level of inbreeding can be outstanding. If a breeder linebreeds a superior male only with superior females, this programme can quickly produce a superior population.

Linebreeding is the breeding programme that is needed if a farmer wants to keep the relationship of the next generation with a particular ancestor at a high level. The genetic contribution that a fish makes to a population is halved each generation: 50% in its offspring, 25% in its grandchildren, 12.5% in its great-grandchildren, etc. After seven generations, a fish contributes less than 1% of its descendants' genes. Linebreeding is the breeding programme that can be used to reverse this trend. For example, if a fish is mated with its grandchildren (the offspring would be both its great-grandchildren and its children), its contribution to those offspring would rise would rise from the normal value of 12.5% to 62.5%.

If the male that is being linebred mates only once or twice with his descendants, the linebreeding is considered to be mild linebreeding (Figure 19). If the male that is being linebred is repeatedly mated to his female descendants or is mated to them using a regular pattern, the linebreeding is considered to be intense linebreeding (Figure 20).

Intense linebreeding is a pre-biotechnology breeding programme that can be used to create a "near clone." By repeatedly mating a male to his female descendants a farmer can create an "almost identical" genetic twin of the male. For example, in Figure 20, 93.75% of individual G's genes come from individual A.

Use of a herd bull

This type of inbreeding programme is a variation on linebreeding. In this type of inbreeding programme, a farmer usually purchases an outstanding bull and uses it as a "herd bull"; i.e., it is the only male in the herd. This bull is mated to all females and many female descendents. This inbreeding programme is used to bring a herd of animals up to the herd bull's phenotypic standards.

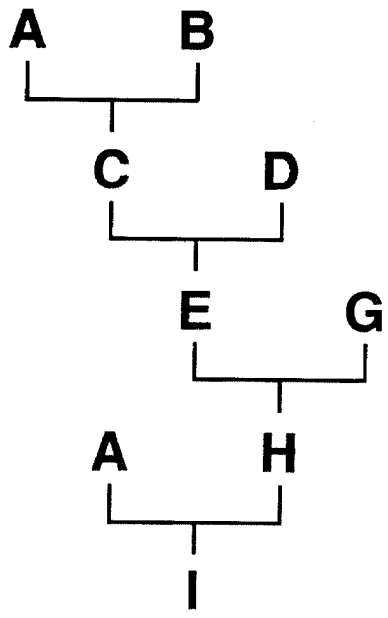


Figure 19. An example of a linebreeding programme. This example can be considered to be mild linebreeding. Individual A is brought back and mated with his great-grandchild. Individual A can be a male or female, but linebreeding usually uses males. Consequently, individual A is the father and great-great-grandfather of individual I. This is used to increase the percentage of A's genes in his descendants; 56.25% of I's genes come from A; $F_I = 6.25\%$.

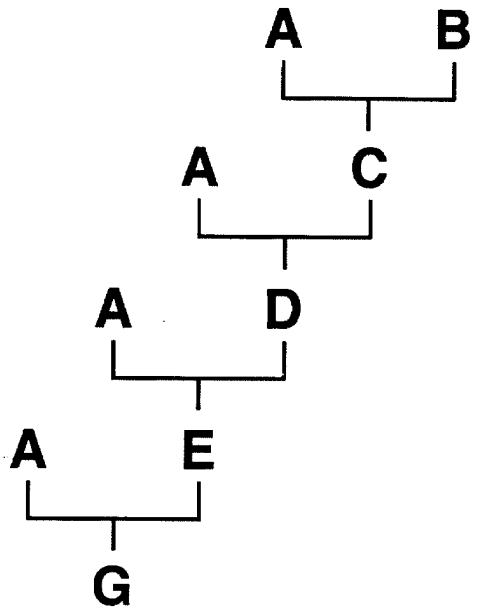


Figure 20. An example of a linebreeding programme. This can be considered to be intense linebreeding. Individual A is repeatedly mated to his daughters. In this example, individual A is the father, grandfather, great-grandfather, and great-great-grandfather of individual G. This breeding programme is used to increase the percentage of A's genes in his descendants; 93.75% of G's genes come from A; $F_G = 43.75\%$.

This breeding programme will create herds that are highly inbred, so some farmers use several herd bulls to reduce the level of inbreeding and thus minimize inbreeding depression. The inbreeding that will be produced by this inbreeding programme can be determined by the following equation:

$$F = \frac{1}{8(\text{number of males})} + \frac{1}{8(\text{number of females})}$$

where: number of males and number of females are the number that produce viable offspring. The formula shows that inbreeding is controlled by the sex that is least numerous, so the rate of inbreeding is governed by the number of herd bulls. For example, if 50 females are used, the inbreeding that will be produced per generation when 1, 2, 3, 4, and 5 herd bulls are used is:

Number of herd bulls mated to 50 females	Inbreeding per generation
1	12.75%
2	6.50%
3	4.42%
4	3.38%
5	2.75%

Inbreeding to expose and cull detrimental recessive alleles

Inbreeding can be used as an extreme form of progeny testing to expose detrimental recessive alleles and eliminate families that carry these undesired genetic bombs. Progeny testing is a selective breeding programme that is often used to accomplish this, but progeny testing is usually used to eliminate only one recessive allele. Progeny testing is a breeding programme where fish with the dominant phenotype are mated to a test fish (one that is homozygous recessive) to identify and cull the heterozygotes and to identify and save the homozygotes.

By mating relatives and developing highly inbred families, a farmer can unmask many detrimental recessive alleles simultaneously and cull families that carry many hidden genetic defects. Since most animals carry several to several dozen detrimental recessive alleles that are hidden in the heterozygous state, this type of breeding programme is a severe form of progeny testing, but families that exhibit no problems are genetically superior and defect-free. Because this type of breeding programme is so severe, many inbred families must be created in order to identify and save the few that will be defect-free. Although this is costly, it is a breeding programme that will produce outstanding animals, in terms of qualitative phenotypes, within a few generations.

Inbreeding to improve the results of between-family selection

Inbreeding can be used to improve the results of selection when the h^2 for a trait is small. When h^2 is ≤ 0.15, it is difficult to improve the population by individual selection, because most of the phenotypic differences that can be measured are not heritable. When this occurs, between-family selection is more efficient because this selective breeding programme magnifies the heritable differences (those due to V_A) by minimizing environmental effects (V_E).

When h^2 is small, inbreeding can be used to create inbred families which will further magnify the heritable differences among the families. Inbreeding makes it easier to assess the heritable differences among families, by minimizing some of the non-heritable sources (V_D, V_I, and V_E). Inbreeding does not change the absolute amount of V_A; it changes relative amount, which improves the breeder's ability to identify and save families which are superior because of V_A. This makes between-family selection more efficient.

Creation of inbred lines for crossbreeding programmes

Once a breed is established, perhaps the most important use of inbreeding is to develop inbred lines that will be used in crossbreeding programmes to produce outstanding hybrids for grow-out. Crossbreeding and inbreeding are mating extremes along a continuum: inbreeding is the mating of animals that are more closely related than the average in a population, and crossbreeding is the mating of animals that are less related than the average in a population.

The genetics that controls phenotypic expression for both inbreeding and crossbreeding programmes is a function of heterozygosity, which means it is a function of V_D. Inbreeding can be used to develop inbred lines that produce better hybrids-fish that exhibit more hybrid vigour. The creation of inbred lines improves the results of hybridization by "stretching" the genetic distance between lines; i.e., inbreeding can be used to create opposite types of homozygosity, and this will enable hybrids to be more heterozygous and hopefully to exhibit more hybrid vigour. Additionally, the development of inbred lines can be used to fix one or two qualitative traits in each line, so all F_1 hybrids will have two or more desired qualitative traits.

There are three ways this type of breeding programme can be conducted: The first two types hybridize inbred lines. In one, inbred lines are created in two strains that were previously shown to produce outstanding F_1 hybrids. The other option is to create inbred lines in a number of strains and then hybridize them in an attempt to discover a combination that works. The more efficient way is to create inbred lines in two strains that were previously shown to produce outstanding F_1 hybrids.

This approach makes sense, because producing superior F_1 hybrids is a hit-or-miss proposition. You cannot predict which mating combinations will nick and produce great hybrids and which mating combinations will produce duds. If you create inbred lines in two strains that have already been shown to produce outstanding F_1 hybrids, it is likely that some of the inbred line combinations will also produce better F_1 hybrids. This type of breeding programme is illustrated in Figure 21.

Inbreeding can also be used to create an inbred line that will be used in a topcrossing programme. Topcrossing is a crossbreeding programme in which an inbred line is hybridized with a randomly bred line to produce F_1 hybrids. This type of breeding programme has been traditionally used by animal breeders to produce outstanding animals for judging contests.

Even though inbreeding can be used to improve a population, it does cause inbreeding depression. Fecundity is often adversely affected by inbreeding. When fecundity declines, it may not be possible to produce enough F_1 hybrids for grow-out. This problem can be circumvented by developing four inbred lines and mating them to create di-hybrids.

The first step in this breeding programme is the creation of four inbred lines. The second step is the creation of two F_1 hybrids. The third step is the mating of the F_1 hybrids to produce a di-hybrid. The creation of the F_1 hybrids reduces F to 0%, so fecundity will return to normal. The F_1 hybrids are used to produce the seeds or animals that farmers will use for grow-out. This is how modern agribusinesses produce seed corn. If each inbred line is used to fix one or two qualitative phenotypes, the di-hybrids will exhibit heterosis, and all will also have several desired qualitative phenotypes. This type of breeding programme is illustrated in Figure 22.

INBREEDING PRODUCED WHEN RELATIVES MATE

The ability to use inbreeding in some of these breeding programmes depends on a farmer's or hatchery manager's ability to produce inbred lines quickly and efficiently. This can be accomplished only if breeders know how much inbreeding will be produced by a particular mating programme and the rate at which the inbreeding is created. The amount of inbreeding that will be produced by a given inbreeding programme depends on the relationship that exists between the fish that are mated. Consequently, it is important to know how much inbreeding is produced when relatives mate and produce offspring. This information can then be

used to develop regular systems of inbreeding that will enable a farmer to produce predictable levels of inbreeding in a predictable time frame.

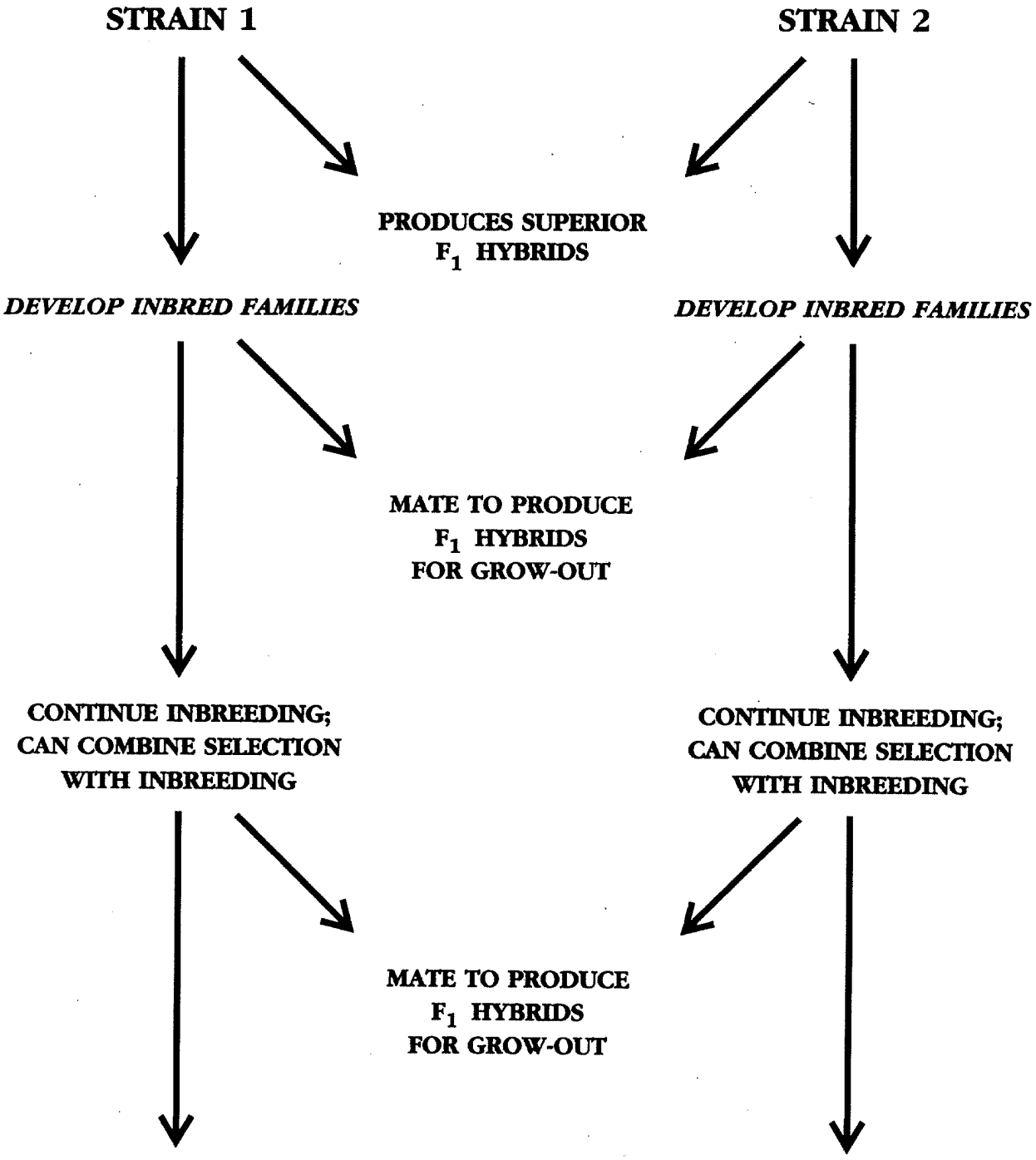


Figure 21. Breeding programme illustrating how an inbreeding programme can be combined with hybridization. Inbred lines of fish are created in two strains that have been previously shown to produce outstanding F_1 hybrids. Once created, the inbred lines are hybridized to produce F_1 hybrids. The use of inbred lines will improve hybrid vigour. This type of breeding programme is the way to maximize exploitation of V_D. Once identified, selection can be conducted in the inbred lines to improve growth; this will exploit V_A.

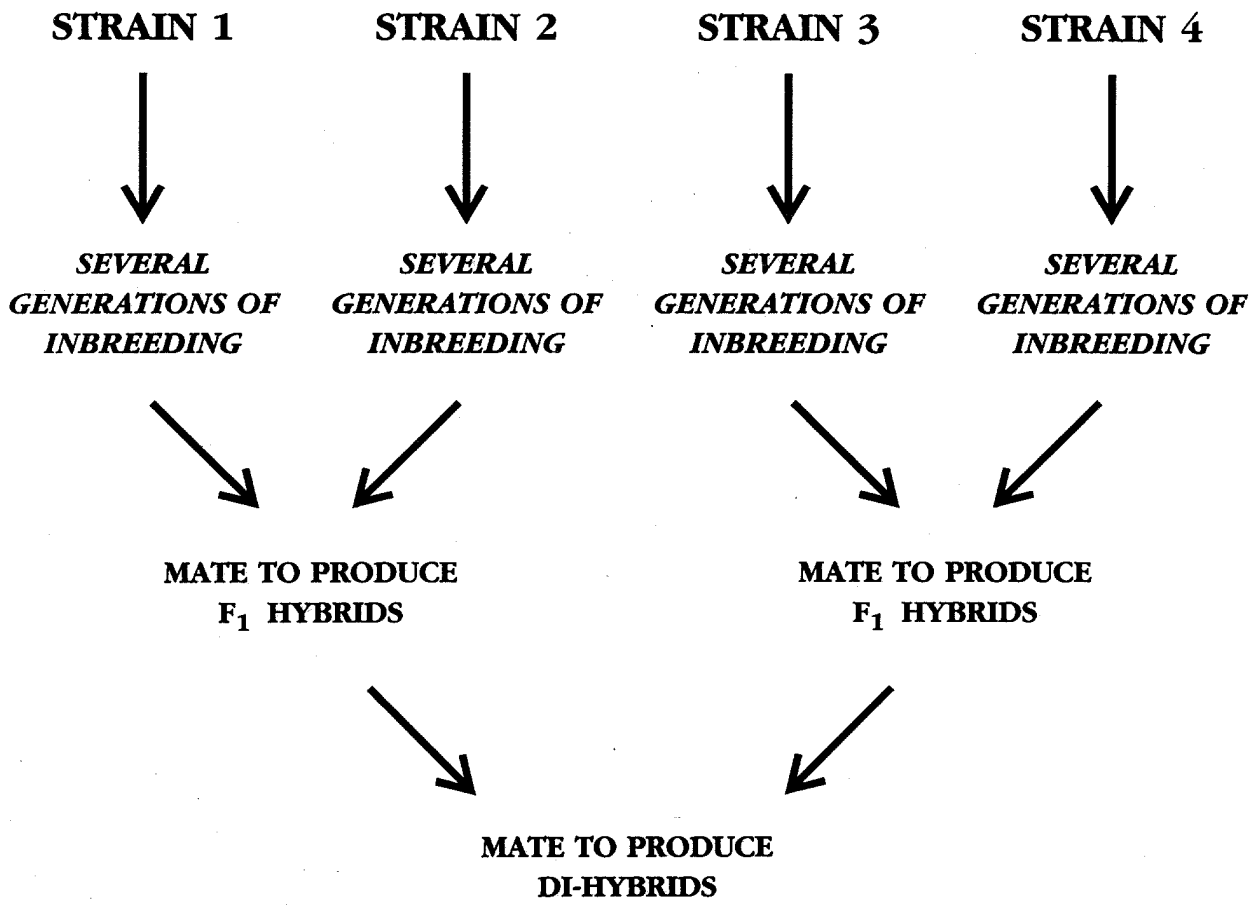


Figure 22. Breeding programme illustrating how inbreeding can be used to produce di-hybrids for grow-out. In this breeding programme, inbreeding is used to create inbred lines in four strains that have been previously shown to produce outstanding F_1 hybrids. The F_1 hybrids are then crossed to produce di-hybrids. This breeding programme is used when inbreeding depression decreases fecundity in the inbred lines to the point where it is impossible to produce enough F_1 hybrids for grow-out. The F_1 hybrids have F = 0%, which restores fecundity; the F_1 hybrids are hybridized to produce the seed or animals that will be used for grow-out.

This section will show how much inbreeding can be produced when relatives are allowed to mate. In order to do this, it will be assumed that no previous inbreeding exists, so the amounts that are mentioned are those that are produced when inbreeding first occurs. Figure 23 shows a family tree with 41 individuals that could be mated to produce offspring with F ranging from 0% to 25%. The values presented in Figure 23 show that the matings of close relatives, such as parents with offspring and brothers with sisters, produce fish with high levels of inbreeding (25%) and that the inbreeding produced steadily decreases as the distance between relatives increases. For example, matings between third cousins, matings between fourth cousins, and matings between fifth cousins produce very little inbreeding (0.39%, 0.097% and 0.024%, respectively). Fifth cousins share so few genes that the inbreeding produced when they mate is insignificant; thus, on a practical level, many geneticists consider fifth cousins to be unrelated.

The inbreeding values listed in Figure 23 explain why first cousin marriages are forbidden in many cultures while second cousin marriages are sanctioned. First cousin marriages produce children with F = 6.25%, while second cousin marriages produce children with F = 1.56%.

One type of cousin marriage will produce a large amount of inbreeding. A double first cousin marriage (the offspring from the marriages of a pair of sibs that married a pair of sibs; first cousins are individuals that are not sibs but that share two grandparents in common; double first cousins are individuals that are not sibs but that share all four grandparents in common-individuals 22 and 23 in Figure 23 are double first cousins). Double first cousin matings produce as much inbreeding as half-sib, grandparent-grandchild, and aunt-nephew (or uncle-niece) matings-12.5%.

Figure 23 shows that some matings will produce F = 0%. It should be obvious that the mating of unrelated individuals such as 13 and 14 would produce offspring with no inbreeding, but other matings which would usually be considered to be between relatives would also produce offspring with no inbreeding. For example, if individuals 9 and 27 were mated, the offspring would have F = 0%, even though this would be considered to be an uncle-niece marriage. The reason why the 10 x 26 mating produces offspring with F = 12.5% while the 9 x 27 mating produces offspring with F = 0% is individual 9 is individual 27's uncle by marriage. Individuals 9 and 27 share no genes in common; they have no common ancestor and are not related, which is the requirement for the production of inbred offspring. The 9 x 27 mating might be incestuous in most societies, but it produces no inbreeding.

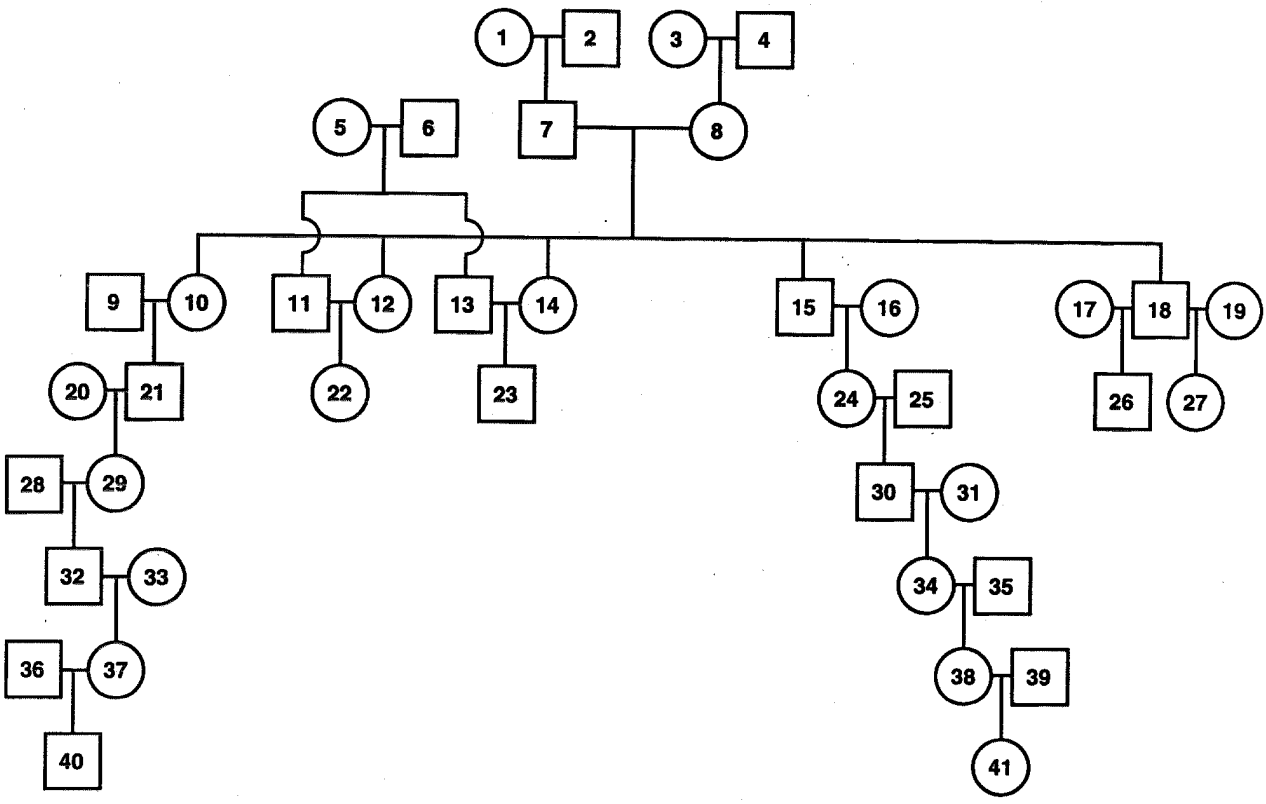
REGULAR SYSTEMS OF INBREEDING

The relationships illustrated in Figure 23 and the amount of inbreeding produced by matings among those individuals can be used to develop regular systems of inbreeding in order to produce inbred lines of fish for breeding purposes. The reason why regular systems of inbreeding need to be used is simple: breeding programmes are expensive and take years, so random, haphazard breeding systems that produce unknown and unpredictable amounts of inbreeding are expensive lessons in poor planning and futility. All breeding programmes require meticulous planning, and inbreeding is no exception. If farmers or hatchery managers are going to use inbreeding to improve a population, they must know how to conduct regular systems of inbreeding. Regular systems of inbreeding enable a farmer or hatchery manager to know what matings must be made generation after generation, and this allows them to predict the amount of inbreeding that will be produced each generation.

There are a number of regular systems of inbreeding. Some are relatively simple, but others are so complex or produce so little inbreeding that their use is impractical. A major goal of an inbreeding programme is to produce F = 40-50% in a number of families and to do it fairly rapidly. The only liability of inbreeding programmes that quickly produce large levels of inbreeding is that inbreeding depression will reduce viability and fecundity, and this will cause some of the inbred lines to go extinct. Consequently, many inbred lines must created so that some will survive the ravages of inbreeding depression.

Figure 23 shows that there are six types of matings that produce large amounts of inbreeding. Some of these matings are difficult to make generation after generation (e.g., grandparent-grandchild), which limits their use in practical inbreeding programmes. Relatively simple regular systems of inbreeding that will produce F = 40-50% in four to five generations are: parent-offspring, full-sib, half-sib, and double first cousin.

Regular systems of inbreeding divide a population into a series of inbred families. This occurs because the only way a regular system of inbreeding can be maintained is by continually mating a specific combination of relatives, which means mating fish within a family. The size of the family is determined by the inbreeding programme: the closer the relationship between mates, the smaller family size can be.



MATING	INDIVIDUALS IN FAMILY TREE	F PRODUCED BY MATING
Parent-Offspring	7 x 10	25.0%
Brother-Sister (full sibs)	10 x 15	25.0%
Half-brother-Half-sister (half sibs)	26 x 27	12.5%
Grandparent-Grandchild	2 x 10	12.5%
Aunt-Nephew (or Uncle-Niece)	10 x 26	12.5%
Double first cousins	22 x 23	12.5%
Great-grandparent-Grandchild	21 x 37	6.25%
First cousins	21 x 24	6.25%
Second cousins	29 x 30	1.56%
Third cousins	32 x 34	0.39%
Fourth cousins	37 x 39	0.097%
Fifth cousins	40 x 41	0.024%
Unrelated	13 x 14	0.0%

Figure 23. A family tree with 41 individuals. Males are represented by squares and females by circles. This family tree is used to illustrate the amount of inbreeding that would be produced by matings between relatives. Possible mating combinations and the inbreeding that would be produced by these matings range from F = 0% to F = 25%. Individuals 1, 2, 3, and 4 have F = 0% and are not related, and they are not related to individuals 5 and 6. Individuals 5 and 6 have F = 0% and are not related.

Parent-offspring inbreeding programme

A breeding programme of parent-offspring matings **is** an inbreeding programme that is traditionally used to quickly produce highly inbred animals. A single generation of parent-offspring mating will produce fish with F = 25%, and three generations will produce fish with F = 50%; eventually, F will approach 100%. A parent-offspring inbreeding programme is illustrated in Figure 24. Five generations of parent-offspring matings, along with the inbreeding that is produced, is illustrated. The inbreeding that is created by a parent-offspring inbreeding programme is contrasted to that which can be created by other regular systems of inbreeding in Table 3.

This type of inbreeding programme is simple and requires the fewest numbers of individuals in each inbred family. Only two individuals are needed in each generation-a parent (usually the younger parent) and an offspring. Consequently, this is the least expensive regular inbreeding programme. The major liability of this inbreeding programme is that it is often difficult to mate parents with their offspring generation after generation. A number of fish species spawn only once and then die. When this happens, the only way parent-offspring matings can occur is by using cryopreserved gametes.

Table 3. Percent inbreeding produced by regular systems of inbreeding: parent-offspring; full-sib; half-sib; double first cousin. There are two half-sib inbreeding programmes: a single male is mated to two half-sisters each generation (A) (Figure 26); a single male is mated to many half-sisters each generation (B) (Figure 27). Generation 1 is the first generation in which inbreeding is produced.

Generation	Parent-offspring	Full- sib	Half-sib (A)	Half-sib (B)	Double first cousin
0	0.00	0.00	0.00	0.00	0.00
1	25.00	25.00	12.50	12.50	12.50
2	37.50	37.50	25.00	21.88	18.75
3	50.00	50.00	34.38	30.47	25.00
4	59.38	59.38	42.97	38.09	31.25
5	67.19	67.19	50.39	44.87	36.72
6	73.44	73.44	56.84	50.92	41.80
7	78.52	78.52	62.45	56.30	46.48
8	82.62	82.62	67.23	61.09	50.78
9	85.94	85.94	71.58	65.35	54.74
10	88.62	88.62	75.28	69.15	58.37
∞	100.00	100.00	100.00	100.00	100.00

After: Wright, S. 1921. Systems of mating. II. The effects of inbreeding on the genetic composition of a population. Genetics 6:124-143.

Full-sib inbreeding programme

Full-sibs are brothers and sisters that share two parents. This regular system of inbreeding is as effective in producing inbreeding as parent-offspring matings, and it is equally simple and inexpensive. A breeding programme of full-sib matings is illustrated in Figure 25; five generations of full-sib matings along with the inbreeding that is produced is illustrated. As was the case with parent-offspring matings, full-sib matings produce F = 25% in the first generation and F = 50% after three generations; eventually, F will approach 100% (Table 3).

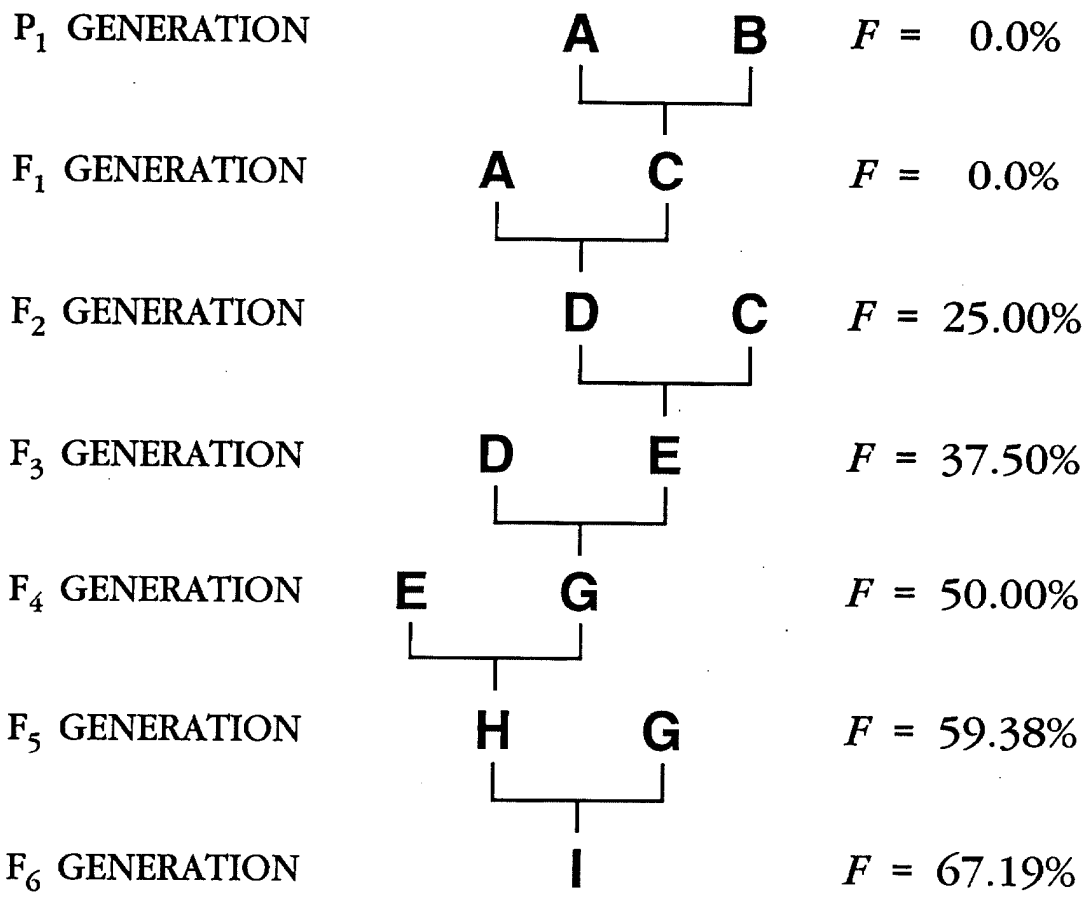


Figure 24. A regular inbreeding programme of parent-offspring matings. Five generations of parent-offspring matings and the inbreeding produced by this breeding programme are illustrated. The first generation of parent-offspring mating occurs in the F_1 generation, and their offspring (F_2 generation) is the first generation of inbred fish. Fish in the P_1 generation have F = 0% and are not related. In subsequent generations, inbreeding values apply only to individuals which are produced in that generation; e.g., F_C = 0%, not 25%.

Only two individuals are needed per inbred family per generation, but more can be used. Even though family size of parent-offspring and full-sib inbreeding programmes can be identical, a full-sib inbreeding programme is usually less expensive, because when parents are mated to offspring fish have to be cultured for two spawning seasons. When sibs are mated, broodstock can be turned over more rapidly. A major difference between regular parent-offspring and full-sib inbreeding programmes is the fact that it is far easier to make full-sib matings generation after generation. Because full-sib matings are rather easy to make and because this type of inbreeding programme quickly produces large levels of inbreeding, full-sib matings is probably the most commonly used regular system of inbreeding.

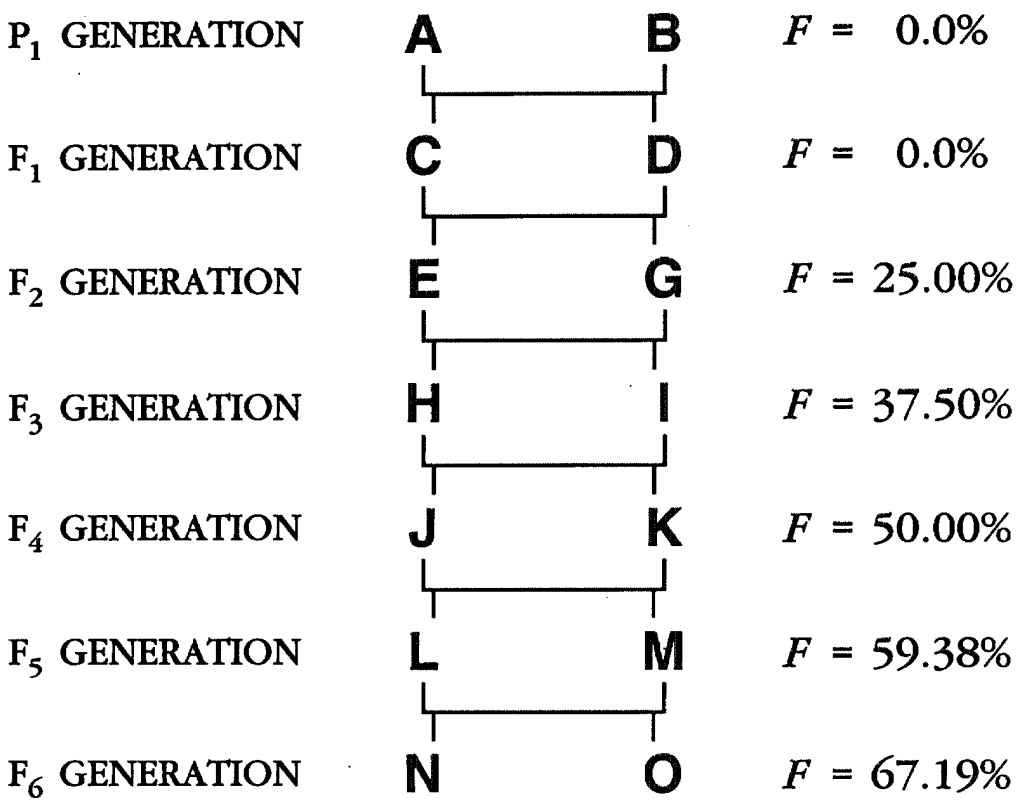


Figure 25. A regular inbreeding programme of full-sib (brother-sister) matings. Five generations of full-sib matings and the inbreeding produced by this breeding programme are illustrated. The first generation of full-sib mating occurs in the F_1 generation, and their offspring (F_2 generation) is the first generation of inbred fish. Fish in the P_1 generation have F = 0% and are not related.

Half-sib inbreeding programme

Half-sibs are brothers and sisters that share only a single parent in common. Regular programmes of half-sib matings are used when the same ultimate level of inbreeding is wanted, but a slower rate of increase is desired. A single generation of half-sib mating produces offspring with F = 12.5%, but the amount produced thereafter depends on the half-sib inbreeding programme that is used; F will reach 40-50% after four to five generations (Table 3).

There are several types of regular half-sib inbreeding programmes. The two simplest are illustrated in Figures 26 and 27. The breeding programme outlined in Figure 26 is one whereby a breeding set of three individuals is established to create an inbred family, and a single male is mated to two half-sisters each generation. There are two matings each generation: one produces the male and the other produces the two half-sisters.

The half-sib inbreeding programme illustrated in Figure 27 is similar to that illustrated in Figure 26, except a single male is mated to many half-sisters each generation. The inbreeding produced after the first generation in this programme is less than that produced by the smaller half-sib breeding family (Figure 26; Table 3); the additional females in this breeding programme moderates the accumulation of inbreeding. The inbreeding programme illustrated in Figure 27 shows that the size of the family decreases each generation, but this is only because the illustration shows that each mating produces a single offspring that is used in the next generation. To keep family size from decreasing, one mating each generation that does not produce the male brood fish must produce two half-sisters.

The cost and complexity of half-sib inbreeding programmes depend on the programme that is used. The one illustrated in Figure 26 is not much more expensive than a full-sib inbreeding programme. The half-sib inbreeding programme illustrated in Figure 27 is more expensive; the expense for this inbreeding programme is determined by the number of half-sisters that are maintained.

Half-sib mating programmes are used because large levels of inbreeding can be produced-but at a slower rate than is the case for parent-offspring or full-sib inbreeding programmes. This means regular half-sib inbreeding programmes take longer and are more expensive than full-sib and parent-offspring inbreeding programmes.

Double first cousin inbreeding programme

Double first cousins are first cousins that are twice as related as regular first cousins because the parents that produced them are a pair of full-sibs that mated with a pair of full-sibs (often a pair of brothers that mate with a pair of sisters). A regular inbreeding programme of double first cousins is illustrated in Figure 28. The inbreeding produced by mating double first cousins is similar to that produced by regular systems of half-sib matings (Table 3). The breeding programme illustrated in Figure 28 may look complicated, but in reality it is a fairly simple regular mating scheme. Each inbred family in a breeding programme of double first cousin matings requires a minimum of four individuals per generation. Consequently, the cost of raising fish each generation is about twice that of a programme of full-sib matings.

Although this inbreeding programme produces levels of inbreeding that are similar to those produced by half-sib matings, it is less efficient and more expensive. When double first cousins are mated, no inbreeding will be produced until F_3-generation fish are produced, because the F_2 generation is the first generation in which double first cousins can be mated (Figure 28).

CHROMOSOMAL MANIPULATION

Chromosomal set manipulation can be used to produce highly inbred fish in a relatively short period. Individual fish with F = 100% can be produced in a single generation, while inbred lines where all fish have F = 100% can be produced in two generations.

Chromosomal set manipulation to produce inbred fish can be done in one of two basic ways, but regardless of the technique used, the fish that are produced have only a single parent. The first technique is to prevent the first mitotic division that occurs when the zygote nucleus and zygote itself divides to become a two-celled embryo. To create inbred fish, this technique is done with haploid zygotes. This technique is called either "mitotic gynogenesis" or "mitotic androgenesis," depending on the whether the haploid set of chromosomes of the zygote comes from the mother or from the father.

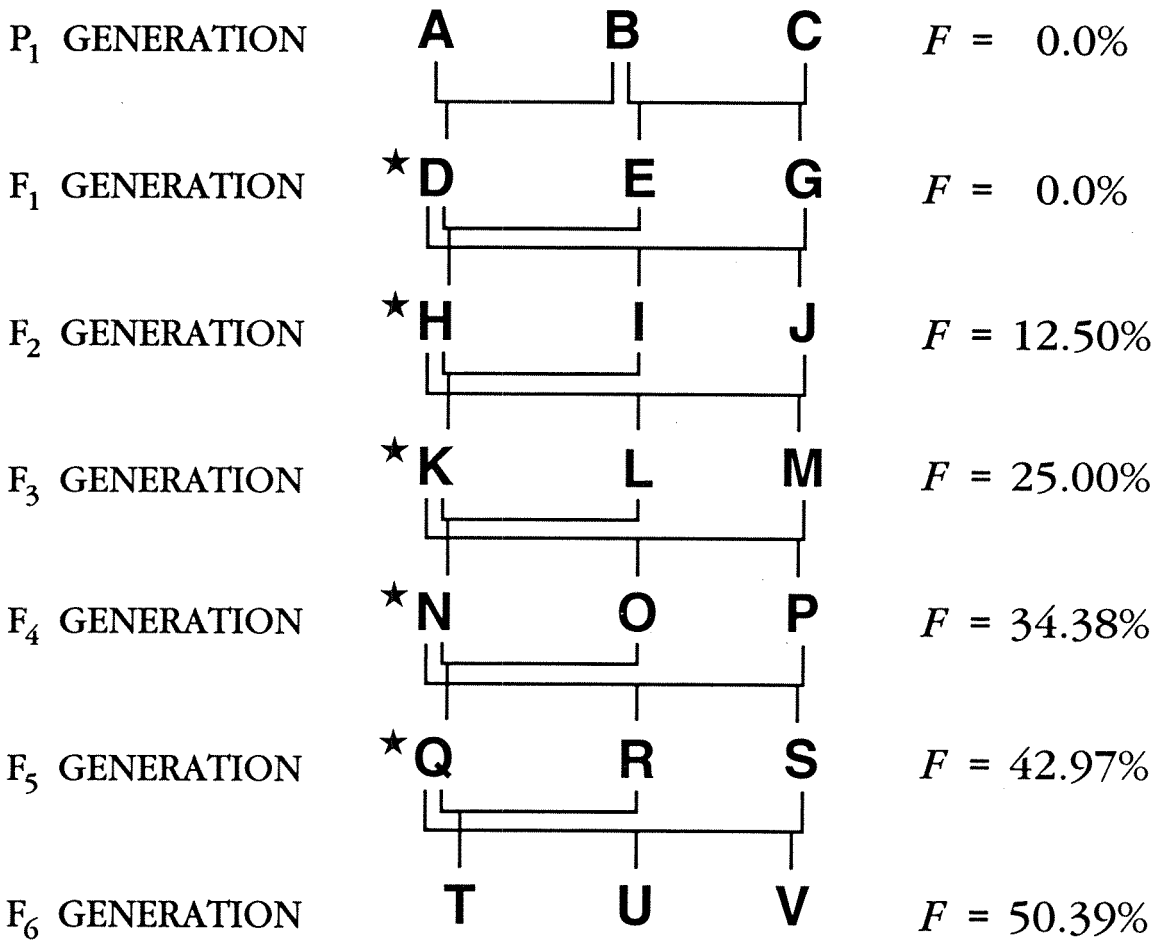


Figure 26. A regular inbreeding programme of half-sib matings. In this type of regular half-sib breeding programme, a single male each generation (marked with a star) is mated to two half-sisters. This is the simplest and least expensive type of half-sib inbreeding programme. Each inbred family is composed of three individuals per generation. There are two matings per generation: one produces the male and the other produces the two half-sisters. Five generations of half-sib matings and the inbreeding produced by this breeding programme are illustrated. The first generation of half-sib mating occurs in the F_1 generation, and their offspring (F_2 generation) is the first generation of inbred fish. Fish in the P_1 generation have F = 0% and are not related.

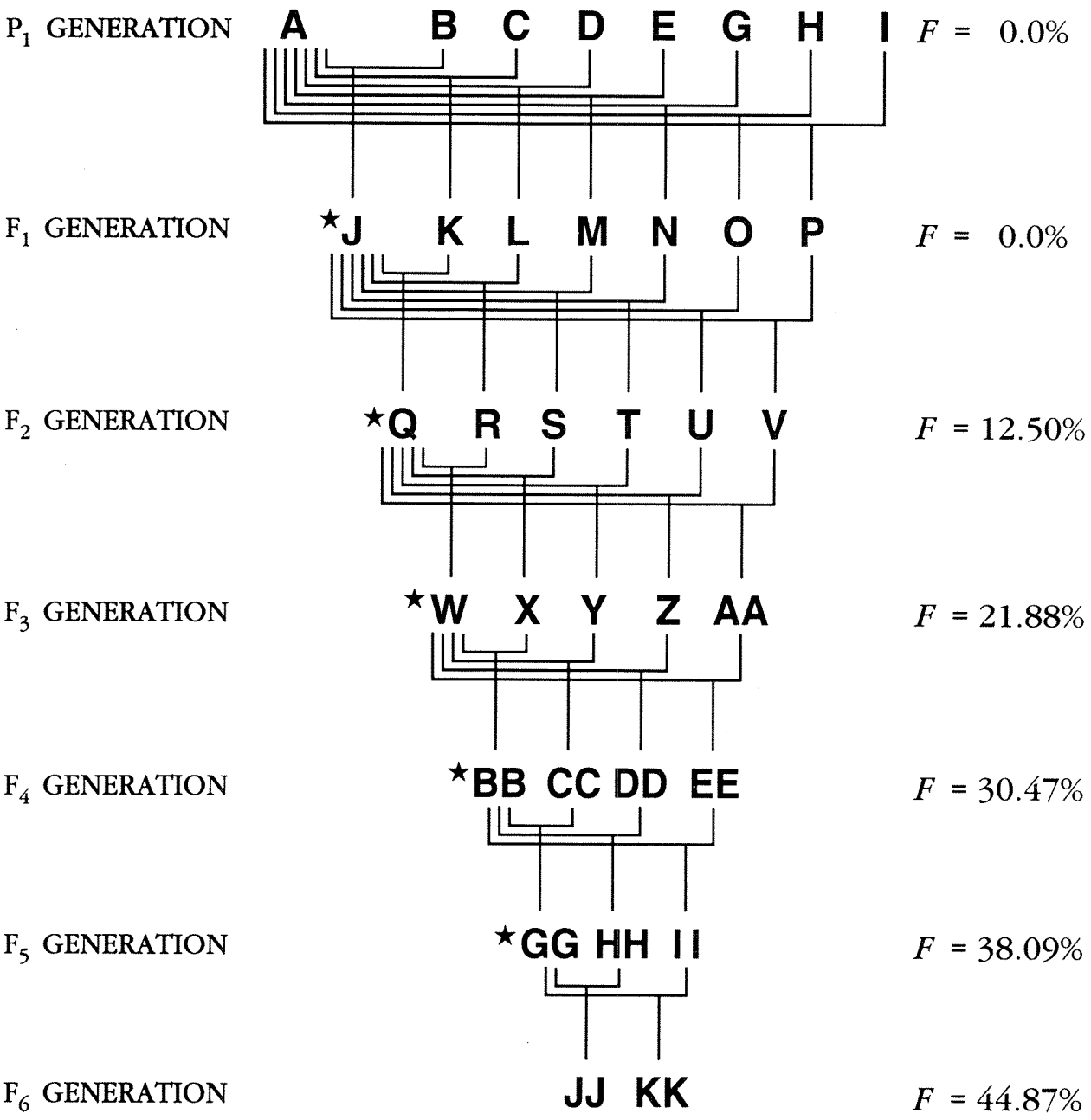


Figure 27. A regular inbreeding programme of half-sib matings. In this type of regular half-sib breeding programme, a single male each generation (marked with a star) is mated to many half-sisters. Five generations of half-sib matings and the inbreeding produced by this breeding programme are illustrated. The first generation of half-sib mating occurs in the F_1 generation, and their offspring (F_2 generation) is the first generation of inbred fish. Fish in the P_1 generation have F = 0% and are not related. This illustration shows that the size of the family decreases each generation. This is because each mating in the figure produces a single offspring that is used in the breeding programme. To keep family size from decreasing, one mating that does not produce the male must produce two half-sisters.

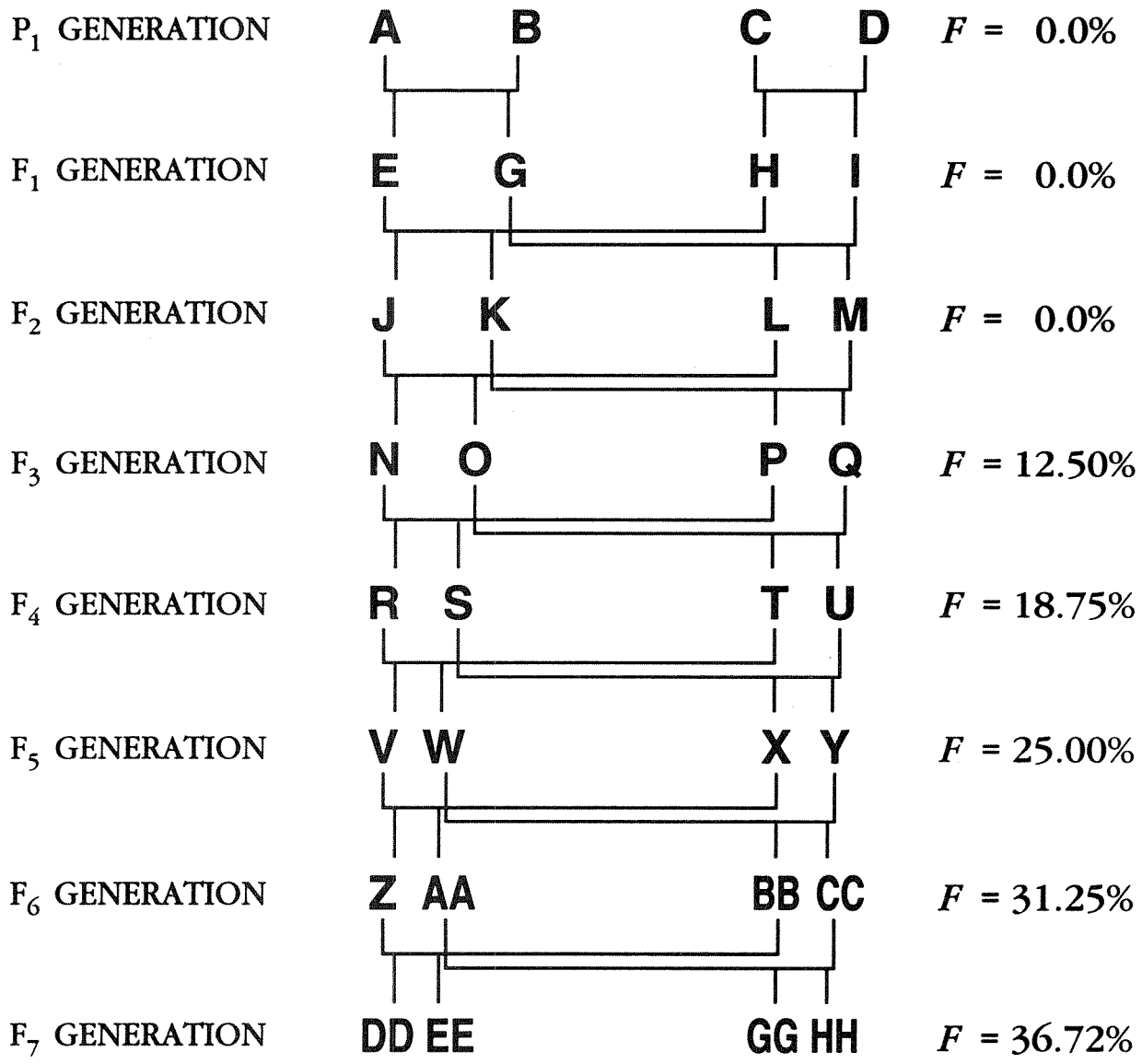


Figure 28. A regular inbreeding programme of double first cousin matings. Five generations of double first cousin matings and the inbreeding produced by the breeding programme are illustrated. The first generation of double first cousin matings occurs in the F_2 generation, and their offspring (F_3 generation) is the first generation of inbred fish. Fish in the P_1 generation have F = 0% and are not related.

The second technique is to prevent equational division (second meiotic division) of the secondary oocyte (egg) after sperm penetration; this prevents the second polar body from leaving the egg. This technique is called "meiotic gynogenesis," because chromosomal set manipulation is accomplished by disrupting a meiotic division; it produces fish called "meiotic gynogens," because all chromosomes in the offspring come from the mother.

This technology requires highly skilled labour, and the methodologies have not been perfected. At present, these breeding programmes are important for some kinds of genetic research, but their practical use has not been quantified. Consequently, these breeding programmes should be done only by scientists who work at agribusinesses or research institutions that are capable of conducting sophisticated genetics experiments. The techniques and procedures needed to create the fish described in this section are species specific, so a detailed description for each important farmed species is beyond the scope of this manual.

Mitotic gynogenesis

Mitotic gynogenesis can be used to create mitotic gynogens (all genes come from the mother), fish that are 100% inbred. The technique that is used to accomplish this with species that have the XY sex-determining system (females are XX and males are XY; virtually all aquacultured species have this system of sex determination) is outlined in Figure 29.

The first step in this breeding programme is the production of first-generation mitotic gynogens. Ultraviolet radiation is used to destroy the DNA (the genes) in sperm. The irradiated sperm are then used to activate eggs. An irradiated sperm cannot fertilize an egg because its genes have been destroyed. The activation causes the egg to undergo the equational division (second meiotic division) and to extrude the second polar body. The egg now contains only a haploid egg nucleus; this produces a haploid zygote (the zygote contains only a single chromosome (homologue) from each chromosome pair, and each chromosome comes from the mother, which is why they are called "gynogens").

When the haploid zygote undergoes first cleavage, a pressure or temperature shock is used to prevent the haploid zygote nucleus from dividing into two daughter nuclei. If the shock is timed perfectly, the haploid zygote nucleus has replicated its chromosomes so that each daughter nucleus will have a full and identical set of chromosomes, but the haploid zygote nucleus has not divided. By preventing first cleavage, the zygote remains a zygote, but the chromosome number of the zygote has doubled from the haploid state to the normal diploid state, which means that each chromosome occurs as a pair. Since mitosis (first cleavage is a mitotic cell division) produces two identical sets of chromosomes, each chromosome pair is composed of two identical chromosomes. Consequently, every gene comes from the mother, and every gene is homozygous; the mitotic diploid gynogen is 100% homozygous and 100% inbred.

This technique does not produce many viable fish. Since each fish is 100% homozygous, every detrimental recessive allele that reduces viability, produces an abnormality, or causes death will be expressed. However, mitotic gynogens that do survive are fish that are free from detrimental recessive alleles, which means they carry no recessive alleles that produce genetic defects.

If this is done with species that have the XY sex-determining system, each mitotic gynogen is a female. The only sex chromosome that exists in mitotic gynogens are those that were contributed by the mother, and females have only X sex chromosomes (all genetic material, including all sex chromosomes, in sperm were destroyed by UV irradiation). When the diploid chromosome number is restored by the shock, all fish go from X zygotes to XX zygotes, and all are females.

Each 100% homozygous mitotic gynogen is unique because there are thousands of genes. Consequently, each first-generation mitotic gynogen is a singular inbred line.

MITOTIC GYNOGENESIS

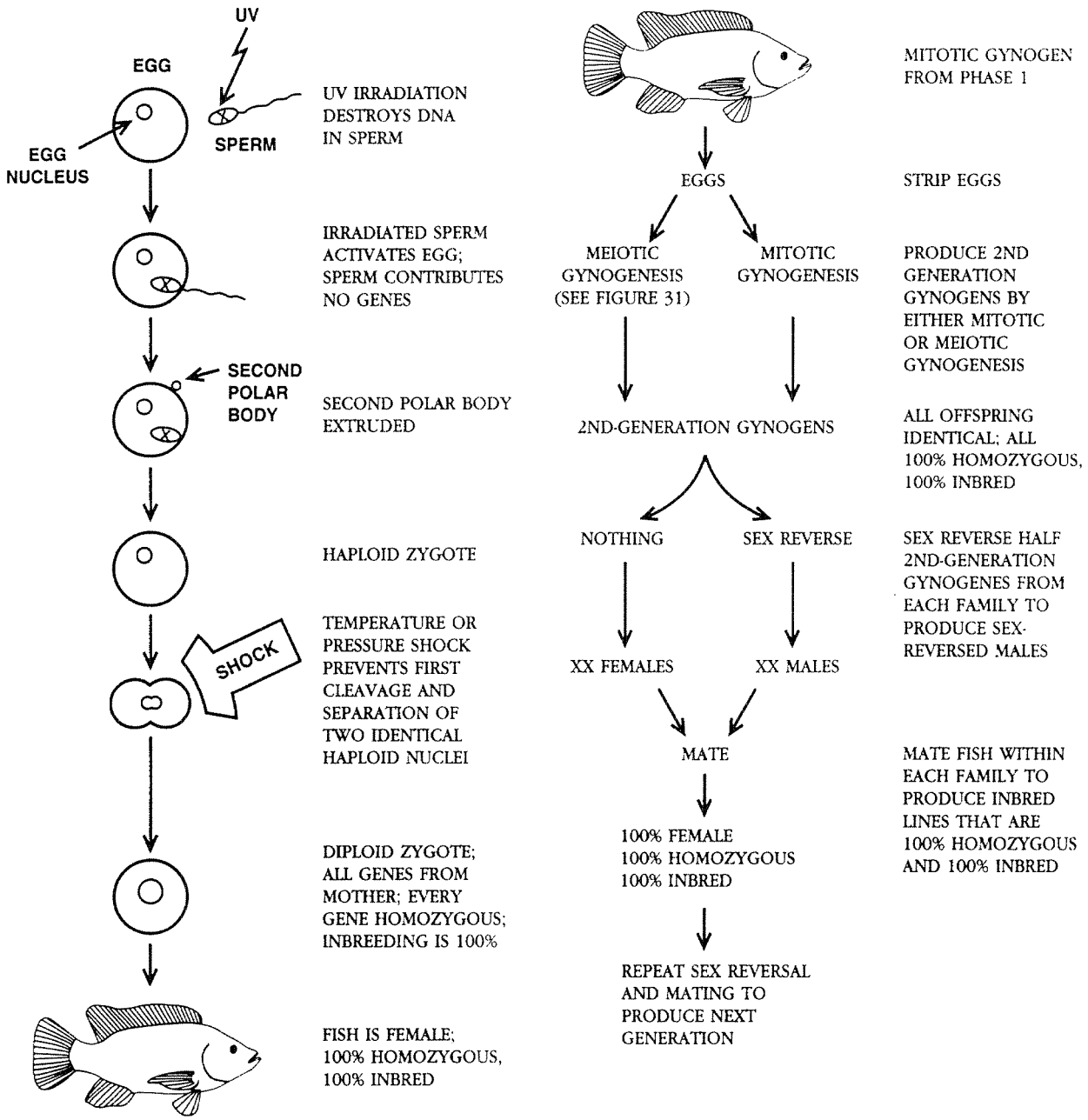


Figure 29. A schematic flow chart of the production of an inbred line of genetically identical 100% homozygous and 100% inbred fish by mitotic gynogenesis (all genes come from the mother) in species with the XY sex-determining system. In phase 1, first-generation mitotic gynogens are produced by preventing first cleavage (a mitotic nuclear division) of haploid zygotes. The end result is females that are 100% homozygous and 100% inbred. Each gynogen is genetically unique and is a singular inbred line. Phase 2 of the breeding programme is needed to produce a family of genetically identical, 100% homozygous, and 100% inbred fish that are capable of mating to perpetuate the inbred line. Second-generation gynogens can be produced either by a second round of mitotic gynogenesis or by meiotic gynogenesis (see Figure 31). Second-generation gynogens produced from each first-generation gynogen are divided into two lots, and one is sex-reversed to produce sex-reversed males. Within each family, sex-reversed males and their genetically identical sisters will be mated to produce an inbred line of genetically identical females that is 100% homozygous and 100% inbred.

If first-generation mitotic gynogens are to be used in a breeding programme to create inbred lines, a second phase of gynogenesis followed by sex reversal is needed in order to produce the lines of 100% inbred fish, in which all fish within each inbred line are genetically identical. Each first-generation mitotic gynogen will be used to create a unique line of 100% homozygous and 100% inbred fish by utilizing either of two possible types of gynogenesis. When the first-generation mitotic gynogens mature, their eggs are stripped, and either mitotic gynogenesis is repeated to produce second-generation gynogens or meiotic gynogenesis is used to create second generation gynogens. If meiotic gynogenesis is used, a shock is used to prevent the second polar body from leaving the activated but unfertilized eggs (meiotic gynogenesis will be described in more detail in a later section). Regardless of the technique used during phase 2 of this breeding programme, the second-generation gynogens are considered to be mitotic gynogens because that was the technique used during phase 1. Both techniques produce families (inbred lines) of identical fish that are 100% homozygous and 100% inbred but, in general, survival will be better if meiotic gynogenesis is used during phase 2.

The eggs and offspring from each first-generation mitotic gynogen must be isolated from all other second-generation eggs and offspring, because each first-generation mitotic gynogen is going to form a "cloned" line. The second round of gynogenesis produces hundreds of genetically identical copies of each first-generation mitotic gynogen (a form of cloning).

Half of the second-generation gynogens from each family are sex-reversed with anabolic androgens (steroid hormones) to produce XX sex-reversed males. The sex-reversed males are genetic females but phenotypic males. The fish that are not treated with hormones are raised normally. Within each family, the sex-reversed males and their sisters are genetically identical (genetically, they are all identical sisters); when they mate, they produce an inbred line of genetically identical fish that is 100% female, 100% homozygous, and 100% inbred. Sex-reversed males must be created every generation, because it is the only way males can be produced, and it is the only way each inbred line can be perpetuated without additional chromosomal manipulation.

If each inbred line is isolated from all other inbred lines, it will breed true and produce genetically identical 100% inbred fish. This breeding programme produces 100% inbred fish in one generation and 100% inbred fish that are capable of producing inbred lines of 100% inbred fish in two generations.

If this is done with species with the WZ sex-determining system (females are WZ and males are ZZ; the only major aquacultured species with this system of sex determination are: blue tilapia, Oreochromis aureus; Wami tilapia, O. urolepis; Japanese eel, Anguilla japonica), half the first-generation mitotic gynogens will be mitotic gynogenetic males (ZZ) and half will be mitotic gynogenetic superfemales (WW). Phase 2 of the breeding programme needed for mitotic gynogenetic WW superfemales is the same as that described previously in this section (Figure 29). ZZ mitotic gynogenetic males, on the other hand, must undergo mitotic androgenesis during phase 2 (Figure 30).

Mitotic androgenesis

Mitotic androgenesis can be used to produce mitotic androgens (all genes come from the father), fish that are 100% inbred. The technique that is used to produce mitotic androgens for species with the WZ sex-determining system is outlined in Figure 30.

In phase 1, eggs whose DNA has been destroyed by ultraviolet radiation are fertilized by normal sperm. This produces a haploid zygote, and all chromosomes come from the sperm (from the father), so these zygotes are called "mitotic androgens." When the zygote undergoes first cleavage, a pressure or temperature shock is used to prevent nuclear division, which produces a diploid androgenetic zygote. In species with the WZ sex-determining system, males are homogametic (ZZ), so all sperm contain a Z chromosome. Consequently, all haploid zygotes have a Z sex chromosome. The disruption of first cleavage changes Z zygotes into ZZ zygotes, and all are males. As was the case with mitotic gynogens, all mitotic androgens are 100% homozygous and 100% inbred, but in this case all fish are males. Each androgen is a singular inbred line.

MITOTIC ANDROGENESIS

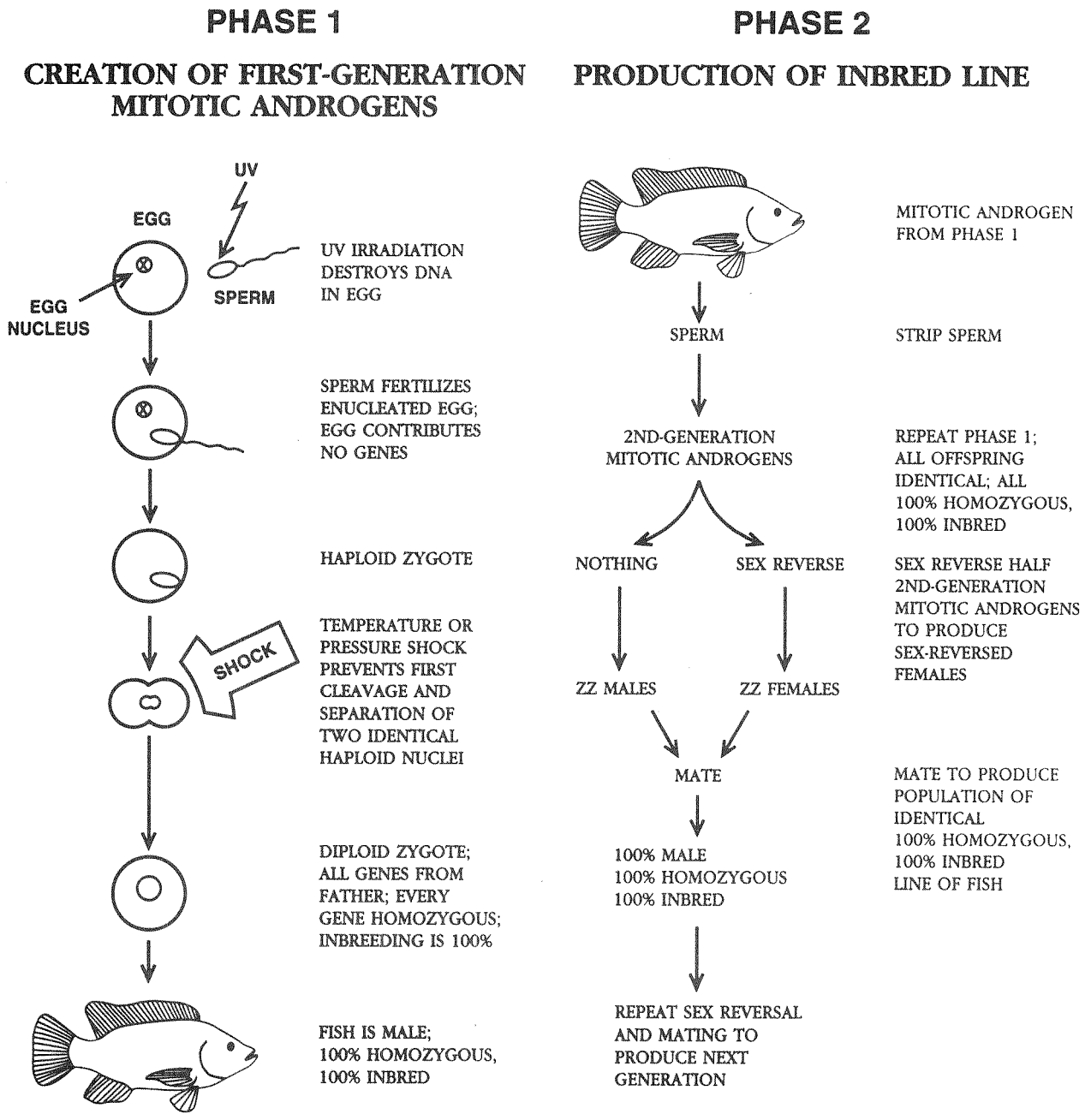


Figure 30. A schematic flow chart of the production of an inbred line that is 100% homozygous and 100% inbred by mitotic androgenesis (all genes come from the father) for species with the WZ sex-determining system. In phase 1, first-generation mitotic androgens are produced by preventing first cleavage of haploid zygotes. All such fish are 100% homozygous and 100% inbred, and all are males. Each androgen is a singular inbred line. In phase 2, a second round of mitotic androgenesis is needed to produce a family of genetically identical 100% homozygous and 100% inbred fish that are capable of mating to perpetuate the inbred line. Second-generation androgens produced from each first-generation androgen are divided into two lots, and one is sex-reversed to produce sex-reversed females. Within each family, the sex-reversed females and their genetically identical untreated brothers will be mated to produce an inbred line of genetically identical males that is 100% homozygous and 100% inbred.

As was the case with mitotic gynogenesis, mitotic androgenesis does not produce many viable fish. Because each androgen is 100% homozygous, every detrimental recessive allele will be able to produce its phenotype. However, mitotic androgens that survive are free from detrimental recessive alleles.

Phase 2 of this breeding programme uses a second round of mitotic androgenesis, and half the offspring from each family are sex-reversed with anabolic estrogens to produce sex-reversed ZZ females. These females are genetic males but phenotypic females. Within each family, the sex-reversed females are mated to their genetically identical brothers to produce each inbred line; fish in each inbred line are genetically identical, 100% homozygous, and 100% inbred and all are males. As was the case with mitotic gynogens, each line is genetically unique.

Mitotic androgenesis can be done with species that have the XY sex-determining system. In this case, half the first-generation mitotic androgens will be XX androgenetic females and half will be YY androgenetic supermales. Second-generation androgens and inbred lines can be produced as follows: the phase 2 protocol needed for the YY androgenetic supermales is the same as that outlined in Figure 30; the phase 2 protocol needed for XX androgenetic females is that which is outlined in Figure 29.

Meiotic gynogenesis

Gynogenesis can be used to create another type of inbred fish-meiotic gynogens. This type of chromosomal manipulation is easier than mitotic gynogenesis, and meiotic gynogens have a higher survival rate than mitotic gynogens because they have less inbreeding. Meiotic gynogenesis is less useful in producing inbred lines because it is difficult to accurately predict the exact amount of inbreeding produced, and the inbreeding produced each generation is quite variable. Since regular systems of inbreeding are most useful when they produce reliable and predictable amounts of inbreeding, meiotic gynogenesis is less useful than mitotic gynogenesis for producing inbred lines. However, one to three generations of meiotic gynogenesis can be used to produce highly inbred fish.

The procedure needed to create meiotic gynogens for species that have the XY sex-determining system is outlined in Figure 31. Ultraviolet irradiated sperm are used to activate eggs. A pressure or temperature shock is applied shortly after activation to prevent the second polar body from leaving the egg during the equational meiotic division. The shock produces an activated egg that has a haploid egg nucleus and a haploid second polar body nucleus. These haploid nuclei fuse, and the egg "fertilizes" itself to produce a meiotic gynogenetic diploid zygote.

At first glance, it appears as if meiotic gynogenesis is a form of self-fertilization, which should theoretically produce fish with F = 50% in the first generation, F = 75% in the second generation, and F >99% in the seventh generation. However, this isn't a form of self-fertilization because the the genes in a haploid egg nucleus and the haploid second polar body nucleus are not truly random with respect to each other, as is the case with two randomly chosen haploid egg nuclei. A haploid egg nucleus and the haploid second polar body nucleus are created after the reduction division. Two randomly chosen haploid egg nuclei differ from each other in that independent assortment randomly distributed the maternal and paternal homologues of each chromosome pair. An egg haploid nucleus and the second polar body haploid nucleus have already undergone independent assortment, so both receive a replicated homologue from each pair of sister chromatids; consequently, they differ less from each other in that respect than two randomly chosen egg nuclei. When the egg haploid nucleus and the haploid second polar body nuclei fuse, the sister chromatids that were separated during the equational division are reunited. These sister chromatids tend to be homozygous with respect for genes near the centromere and heterozygous for genes further away due to crossing over. Because of this, the exact amount of inbreeding produced by one generation of meiotic gynogenesis is difficult to predict.

MEIOTIC GYNOGENESIS

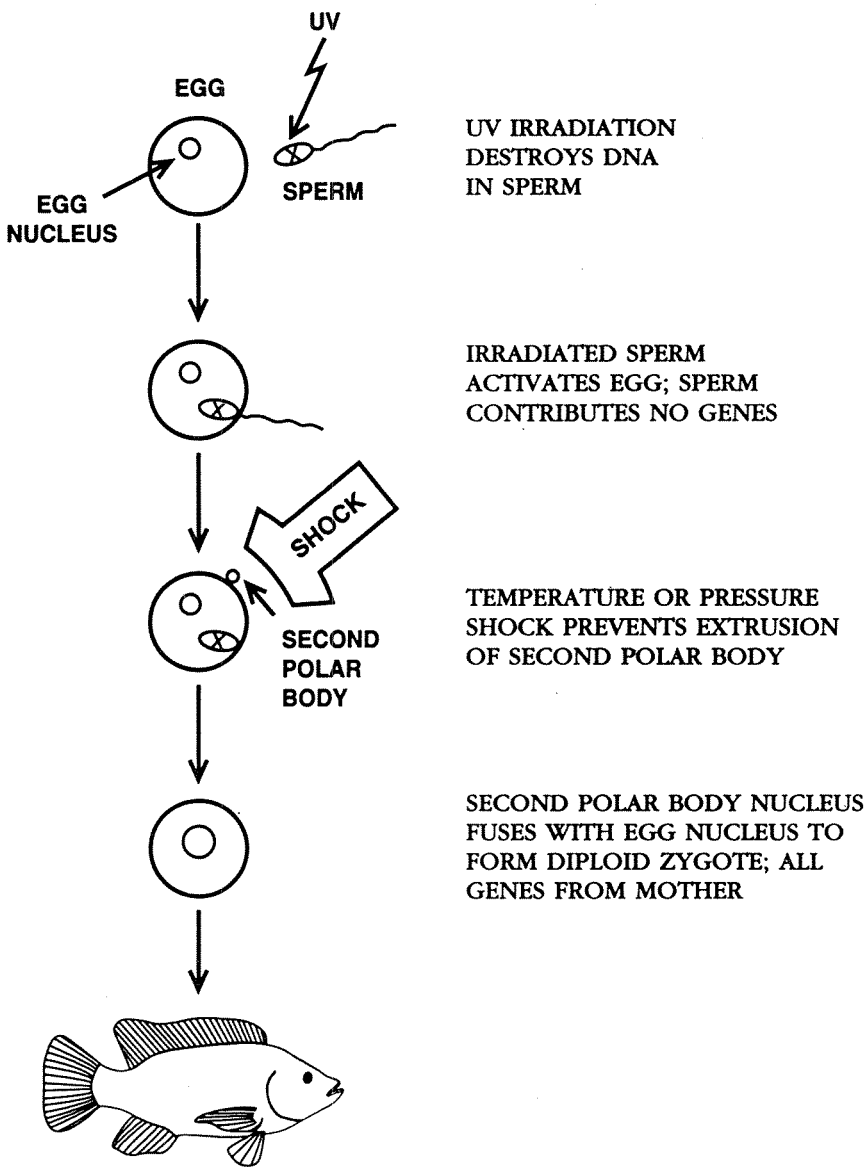


Figure 31. A schematic flow chart of meiotic gynogenesis. This type of gynogenesis differs from mitotic gynogenesis in that the egg "fertilizes" itself when a shock prevents the haploid second polar body from leaving the activated egg following the equational meiotic nuclear division. The haploid second polar body nucleus fuses with the egg haploid nucleus to produce a gynogenetic diploid zygote.

To further complicate matters, It is impossible to accurately predict the amount of inbreeding that will be produced by subsequent generations of meiotic gynogenesis, because the inbreeding will be a function of crossing over frequencies. Crossing over tends to occur at particular locations along each chromosome, so heterozygous loci tend to remain heterozygous for many generations. Because heterozygous loci will tend to remain heterozygous, full-sib mating will eventually produce greater levels of inbreeding than meiotic gynogenesis. Additionally, the inbreeding that is produced each generation will be different for each species and each population due to differences in crossing over frequencies.

The only way to quantify the inbreeding produced by meiotic gynogenesis is to examine the mothers and their offspring electrophoretically (a brief description of electrophoresis is presented in Chapter 8). Heterozygous loci in diploid meiotic gynogens that are produced from mothers that are heterozygous at those loci are heterozygous because of crossing over. The percentage of such loci that are heterozygous in the offspring can be used to determine crossing over frequency; once this is ascertained, it can be used to determine inbreeding for each meiotic gynogen. Studies with fish have shown that one generation of meiotic gynogenesis produced F = 55% to 79% (F = 79% is approximately that produced by seven generations of full-sib matings).

Although meiotic gynogenesis produces less inbreeding than mitotic gynogenesis, it can be used to quickly produce highly inbred fish. However, each meiotic gynogen will have a different level of inbreeding.

CONCLUSION

Most aquaculturists have been told that inbreeding causes problems and that it should be avoided at all costs. This is true only if inbreeding is undesired, because inbreeding is a powerful and valuable breeding technique that animal and plant breeders have used for years to produce superior brood stock and to produce superior plants and animals for grow-out. Any farmer or hatchery manager who wants to manage the genetic aspects of his population should know when and how to use inbreeding to improve productivity and profits, as well as when it should be avoided so that inbreeding depression does not ruin productivity and profits.

Inbreeding is used to create new breeds that breed true to form. Linebreeding can be used to bring a population up to the level exhibited by a superior animal and to increase a superior animal's contribution to his descendants. The use of herd bulls is a type of inbreeding that can be used to quickly improve a population. The creation of inbred families can improve the results of between-family selection when a breeder wants to improve a phenotype that has a low h^2. Finally, the creation of inbred lines is a way to increase hybrid vigour in crossbreeding programmes and to produce outstanding F_1 hybrids for grow-out.

A farmer can use inbred lines to improve selection and crossbreeding programmes only if he knows how to produce inbred lines efficiently. This means that he must know how to conduct regular systems of inbreeding so that he knows what matings must be made, generation after generation, in order to produce predictable levels of inbreeding. Several types of regular inbreeding programmes can be used to produce inbred lines. Parent-offspring and full-sib inbreeding programmes are the simplest and cheapest, and both quickly produce high levels of inbreeding. Full-sib inbreeding programmes are usually used because it produces large amounts of inbreeding, and it is rather easy to make this type of mating generation after generation. Half-sib and double first cousin inbreeding programmes are slightly more complicated and somewhat more expensive. Although they ultimately produce the same amount of inbreeding as parent-offspring and full-sib inbreeding programmes, they produce it at a slower rate.

Chromosomal manipulation can be used to quickly produce highly inbred lines of fish. One generation of mitotic gynogenesis or mitotic androgenesis will produce fish that are 100% homozygous and 100% inbred. A second generation of chromosome set manipulation is needed to produce 100% inbred fish that are capable of reproducing. One generation of meiotic gynogenesis will produce fish that have large, but unknown, levels of inbreeding; the amount of inbreeding produced by meiotic gynogenesis is variable and depends on crossing over frequencies. The use of chromosomal manipulation to produce inbred lines should be done only by scientists at large agribusinesses or at research stations.

CHAPTER 7.

PREVENTING INBREEDING DEPRESSION AND LOSS OF GENETIC VARIANCE IN HATCHERY POPULATIONS

Although inbreeding is a valuable breeding technique and one that can be combined with selection to improve response when h^2 is small or with crossbreeding to produce animals and plants that farmers grow for market, unplanned and uncontrolled inbreeding often leads to inbreeding depression-decreases in growth rate, fecundity, and survival. The problems that are produced by uncontrolled inbreeding are usually accompanied by loss of alleles via genetic drift.

When a farmer or hatchery manager chooses a management programme, either he elects to improve productivity by utilizing selection, crossbreeding, inbreeding, or a combination of these programmes; or he decides to forego genetic improvements and will not use any breeding programme. If a farmer uses a breeding programme, it is inevitable that he will produce inbred fish, and the level of inbreeding will increase over time. Additionally, genetic drift will alter gene frequencies. However, inbreeding and genetic drift are not as important under these circumstances as they are when no breeding programme will be utilized. The reasons why inbreeding and genetic drift are not as important under these circumstances, as well as methods used to minimize inbreeding during selection, will be discussed in Chapter 8.

When no breeding programme is used to improve the population or to produce genetically improved fish, the only genetic goals should be: to prevent or minimize inbreeding, as well as to prevent genetic drift from reducing genetic variance and to minimize the loss of alleles. When a breeding programme is used, genetic changes are planned and desired because they will lead to improved growth rate, etc. When no breeding programme is conducted, genetic changes are unplanned and random, and those caused by inbreeding and genetic drift can ruin the population.

Consequently, when a farmer or hatchery manager does not incorporate a breeding programme into his management plan, it is advisable to try and prevent inbreeding from reaching levels that depress productivity and profits and to prevent genetic drift from robbing the population of potentially valuable alleles.

This chapter will describe ways to prevent inbreeding from accumulating to levels that cause inbreeding depression when farmers and hatchery managers do not use selection or other breeding programmes, both when fish can be marked and identified and when fish are not marked. Since most farmers and hatchery managers do not mark fish, this usually means that N_e must be managed to minimize inbreeding. The management of N_e to prevent genetic drift from robbing the population of valuable alleles will also be described.

Managing N_e to prevent inbreeding- and genetic drift-related problems means managing N_e at a pre-determined size every generation. Maintaining N_e at a constant size is difficult, because N_e can decline due to diseases, poor spawning season, etc. If N_e declines, the population goes through what is called a "bottleneck." Bottlenecks accelerate the accumulation of inbreeding and magnify genetic drift. Preventing bottlenecks, especially during acquisition of the population, may be the most important aspect of brood stock management.

Finally, breeding techniques and brood stock management plans that can be used to increase N_e or that can be used to produce the same results with smaller N_e's will be described.

Recommended minimum N_e's that must be maintained by fish farmers or hatchery managers to prevent inbreeding from accumulating to levels that produce inbreeding depression and to prevent genetic drift from robbing the population of genetic variance will be discussed in Chapter 8.

PREVENTING INBREEDING DEPRESSION

The first step in managing a hatchery population to prevent inbreeding depression is to decide what level of inbreeding causes problems. Unfortunately, there is no answer to this seemingly simple question.

There have been relatively few inbreeding studies with fish. Some of those that have been conducted were done on hatchery fish which were probably already inbred. Even if a major study were to be conducted for an important species, such as common carp, Cyprinus carpio, or Nile tilapia, the inbreeding values that cause inbreeding depression and the level at which inbreeding depression become economically significant might not be the same for all hatchery populations of that species. Studies with other animals have shown that any level of inbreeding can cause inbreeding depression, but the level at which it becomes significant varies from study to study and is often different for different phenotypes. Consequently, no universally undesirable value of F will ever exist for fish. It will be different for different species and for different populations within a species, and it will also be different for different phenotypes.

Because no universally undesirable value of F will ever exist, farmers and hatchery managers have to make intelligent "guesstimates" about what levels of inbreeding they want to avoid. The inbreeding study with rainbow trout that was illustrated in Figures 6 and 7 (pages 18 and 19) showed that inbreeding depression became a serious problem when $F \geq 18\%$. However, F = 12.5% also produced some inbreeding depression. The effects of $F < 12.5\%$ were not evaluated, so the effects of mild levels are not known. Most inbreeding studies with fish have examined the effects of $F \geq 25\%$ (F = 25% is one generation of brother-sister matings).

Because there have been so few inbreeding studies in fish, because the effects of mild levels have not been investigated, and because no universally undesirable value of F is likely to be found, farmers and hatchery managers must decide what level of risk they are willing to accept. Those who want little risk should try to prevent inbreeding from exceeding 5%. Those who are willing to accept more risk should try to prevent inbreeding from exceeding 10%. In this manual, "risk" is defined as potential problems due to inbreeding and genetic drift; low levels of risk (smaller levels of inbreeding are desired) require more management, while high levels of risk (larger levels of inbreeding are acceptable) require less management.

These inbreeding values are not carved in stone. They are simply suggested values for populations where there is no selection to improve growth rate or other production phenotypes. Farmers and hatchery managers can substitute any value they choose. Small levels of inbreeding are more difficult and more costly to prevent, but efforts to prevent small levels will produce populations with fewer genetic problems. Large levels require less management and are less expensive, but choosing such levels may mean that the population has to be replaced when inbreeding depression rears its ugly head.

If fish are marked

If each fish receives an individual mark, tag, or brand, pedigrees can be developed, which means that individual inbreeding values can be determined. If all fish are marked, inbreeding can be prevented in small populations. The way this is accomplished is: pedigrees are created, and relatives are not allowed to mate. One way to help ensure this is to construct a table of covariance values for all brood fish, as was described in Chapter 3 (Table 1; page 35). If the Cov_{BI} value for two brood fish is 0.0, those fish are allowed to mate, because the offspring that will be produced will have F = 0%.

If a farmer wants to allow some inbreeding but wants to keep it below a certain level, he can use the table of covariance values to determine the matings that will produce inbreeding values within his desired range (0% to the level that has been chosen). To do this, the farmer allows all matings where $Cov_{BI} \leq 2$ (maximum desired level of inbreeding). For example, if a farmer wants to keep $F \leq 5\%$, he allows all matings where Cov_{BI} ranges from 0.0 to 0.10, and prevents those where $Cov_{BI} > 0.10$.

If a farmer marks his fish and maintains pedigrees, he can use the information in Figure 23 (page 57) to determine which matings will enable him to prevent undesired levels of inbreeding. Some types of consanguineous matings produce moderate amounts of inbreeding, while others produce virtually no inbreeding. If very low levels of inbreeding are desired, a farmer can restrict consanguineous matings to those

less related than first cousins. If second cousins are mated generation after generation, inbreeding will never exceed 2%. For practical purposes, a regular inbreeding programme of second cousin matings is an effective way to prevent inbreeding depression. Unfortunately, a regular inbreeding programme of second cousin matings is fairly complicated.

If fish are not marked

If fish are not marked, individual inbreeding values cannot be determined, so a farmer must try to prevent the average inbreeding value from exceeding his desired level. This means that the farmer must manage his population's N_e.

Two important decisions must be made before a farmer or hatchery manager can manage N_e to prevent inbreeding from exceeding the desired maximum level: The first is to decide what the maximum inbreeding value will be. As was described earlier in this chapter, F = 5% is proposed for conservative farmers and hatchery managers who want to take little risk, while F = 10% is proposed for those who are willing to accept more risk. However, each farmer or hatchery manager is free to choose his level of risk and to customize the maximum level of inbreeding for his population.

The second decision a farmer or hatchery manager must make is to determine the number of generations that will be incorporated into the work plan before exceeding the level of inbreeding that has been chosen. In this exercise, a generation is the replacement of brood fish by their offspring, and generations are not allowed to overlap (the mixing of generations).

Once these two decisions have been made, two simple procedures are used to determine the N_e that is needed to achieve a farmer's goal:

Step 1: Once the desired maximum level of inbreeding and the number of generations that will be incorporated into the work plan have been determined, the amount of inbreeding per generation that is needed to produce the maximum level in that number of generations must be determined. The only sensible way N_e can be managed to achieve such a goal is to maintain a constant N_e, which will produce a constant amount of inbreeding. The reasons for this are: the math needed to determine the N_e's is far simpler, and it is far easier to try and maintain a constant N_e than a fluctuating one. The formula needed to determine this value is:

$$\text{F/generation} = \frac{\text{total F desired}}{\text{number of generations}}$$

Step 2: Once the inbreeding per generation has been determined, the N_e that is needed to produce that level of inbreeding can be calculated by using the following formula:

$$F = \frac{1}{2N_e}$$

For example, if a farmer decides that he wants to keep inbreeding from exceeding 5% and he does not want inbreeding to reach 5% until he produces the 10th generation, the N_e that is needed can be determined as follows:

Step 1. Calculate the inbreeding per generation that will produce F = 5% (0.05) after 10 generations:

$$\text{F/generation} = \frac{\text{total F desired}}{\text{number of generations}}$$

$$\text{F/generation} = \frac{0.05}{10}$$

$$\text{F/generation} = 0.005$$

Thus, if F = 0.005/generation, it will reach 5% (0.05) when the 10th generation is produced.

Step 2. Calculate the N_e per generation that will produce F = 0.005/generation:

$$F = \frac{1}{2N_e}$$

$$0.005 = \frac{1}{2N_e}$$

$$0.005(2) = \frac{1}{N_e}$$

$$N_e = \frac{1}{0.005(2)}$$

$$N_e = 100$$

The farmer can achieve his goal of preventing inbreeding from exceeding 5% until he produces the 10th generation if he maintains an N_e = 100 for all 10 generations. If N_e = 100 for nine of the 10 generations but drops below 100 for a single generation, he will not be able to achieve his goal.

It is important to note that this procedure determines a minimum N_e, not the number brood fish that must spawned. The two are usually not the same. The only time they are the same is if a 1:1 sex ratio is used, if spawning success is 100%, and if each mating produces viable offspring. In this occurs, a farmer simply divides N_e by 2 to determine the number of males and females that must be spawned. In this example, a farmer would have to spawn 50 males and 50 females every generation to achieve his goal.

If the farmer uses a skewed sex ratio (usually more females than males), he will need more than 100 brood fish. The exact number he would need will be determined by the rarer sex.

If reproductive success is not 100%, a farmer must save and maintain more brood fish than the N_e that he is trying to achieve. The number of brood fish that must be maintained is determined by the typical spawning success rate and by the percentage of matings that produce viable offspring.

For example, if a farmer wants an $N_e = 100$, if 75% of the fish spawn, if 95% of those that spawn produce viable offspring, and if survival from the time brood fish are set aside until they are spawned is 70%, the farmer determines the number of brood fish that he needs in the following manner:

Step 1. Determine how many males need to spawned. $N_e = 100$; it will be assumed that the sex ratio will be equal (1:1), so 50 males and 50 females are needed.

Step 2. Determine how many fish are needed if some matings produce no viable offspring. In this example, 95% of the males produce viable offspring, so the number of males that are needed is:

$$\text{Number of males} = \frac{\text{number desired}}{\text{percent producing viable offspring}}$$

$$\text{Number of males} = \frac{50}{0.95}$$

$$\text{Number of males} = 52.63$$

Since the sex ratio in this example is 1:1, the number of males and number of females that are needed are the same; consequently, the number of females needed = 52.63.

Step 3. Determine how many male brood fish are needed if spawning success is <100%. Spawning success is 75%, and 52.63 males will spawn, so the number of male brood fish that will be needed is:

$$\text{Number of males} = \frac{\text{number that spawn}}{\text{spawning success}}$$

$$\text{Number of males} = \frac{52.63}{0.75}$$

$$\text{Number of males} = 70.17$$

Since the number of males and females are equal, the number of females needed = 70.17.

Step 4. Determine how many fish must be saved and set aside if survival is <100%. Survival from the time fish are set aside until they are spawned is 70%; 70.17 males are needed, so the number that must be set aside is:

$$\text{Number set aside} = \frac{\text{number of males needed at spawning}}{\text{survival}}$$

$$\text{Number set aside} = \frac{70.17}{0.70}$$

$$\text{Number set aside} = 100.24$$

Since the number of males and females are equal, the number of females needed = 100.24.

Because you cannot have two-tenths of a brood fish, the number is rounded up to 101. Consequently, in this example, the farmer would need to save 101 male and 101 female brood fish to produce an N_e of 100. The same numbers of males and females are needed in this example, because a 1:1 sex ratio was used and each male and each female were mated only once. If a skewed sex ratio is used or if multiple matings are used, the arithmetic becomes more complicated, but the process that was just described can be used to determine how many brood fish must be used.

Effective breeding numbers that are needed to keep inbreeding from exceeding 1-15% for 1-100 generations are listed in Table 4. These numbers were calculated as described in this section. When calculating the N_e that was needed to achieve a desired level of inbreeding, the number that was determined was often not a whole number. When this occurred, the number was rounded to the next higher whole number. This was done because you cannot have a fraction of a fish, and rounding to the next higher whole number ensured that F would not exceed the desired level. For example, an N_e of 83.3 was rounded up to 84. If the number were rounded down, it would fall below the N_e that was calculated, which would mean that the goal could not be achieved.

PREVENTING GENETIC DRIFT

As was mentioned earlier, uncontrolled inbreeding and genetic drift are twin evils that occur in closed hatchery populations. Because they are conjoined twins and because both are determined by the population's N_e, it is important to know how to manage and control both. In many cases, managing the population to prevent one of these problems will prevent the other. In cases where this does not occur, a slight alteration in management goals (the desired N_e) will prevent both problems from adversely affecting productivity and profits.

It is not possible to prevent genetic drift. Genetic drift is simply random changes in gene frequency that occur because of sampling error, and the only way to prevent sampling error is to have an infinitely large population which is impossible. In aquaculture, sampling error is choice of brood fish that are allowed to spawn and produce the next generation or the acquisition of fish from another fish farm or the wild. Genetic drift causes problems when the random changes in gene frequency cause some alleles to be lost (the frequency goes to 0.0 or 0%). Once lost, these alleles can be regained only via mutation (which is rare) or by the acquisition of new brood fish. Rare alleles (frequencies <0.01) will be lost more easily than common ones, but common ones can also be lost. The probability of losing an allele is related to its frequency and the population's N_e (Table 2; page 47).

Consequently, a hatchery manager who wants to manage his hatchery population in order to prevent unwanted inbreeding from ruining productivity or profits must also manage the population to prevent genetic drift from robbing the population of genetic variance (loss of alleles).

Managing a population to control genetic drift means that a farmer or hatchery manager has to decide how much genetic drift is acceptable. Since genetic drift cannot be stopped, the question has to be modified. For management purposes, the question becomes: How large does N_e have to be to prevent the loss of alleles?

To answer this question, two decisions must be made. The first is: How valuable are the rare alleles; i.e., what is the frequency of the alleles that will be saved? The second is: What guarantee of saving these alleles is desired? The reason these decisions must be made is because the only way the loss of alleles can be prevented and the only way a 100% guarantee can be given that alleles were not lost via genetic drift is to have an infinitely large population. Hatchery populations are usually small, so the only way to manage them and prevent genetic drift from causing problems is to make a compromise between what is ideal (no changes in gene frequency and no loss of alleles) and what is achievable (determined by the above decisions).

Population biologists generally assume that if a gene has more than one allele and if the frequency of two alleles are ≥ 0.01, the gene is polymorphic, which means that for management purposes two or more alleles exist for that gene in the population. Alleles whose frequencies are <0.01 do not contribute to polymorphism. This does not mean they are unimportant; they do contribute to genetic variance, but they are often considered too rare to manage for practical biological conservation.

Consequently, a farmer or hatchery manager who wants to preserve as much genetic variance as possible and wants to accept little risk would choose to save alleles whose frequencies are 0.01. Farmers who feel that rare alleles are not that important for farming purposes would choose to save more common alleles. They might choose to save alleles whose frequencies are, say, 0.05. Alleles that are rarer than 0.05 are probably not that important in fish farming. If they were, domestication selection would have increased their frequency dramatically.

The frequency that is chosen is determined by the farmer's or hatchery manager's goals. There is no single correct value. However, hatchery managers who are raising fish that will be stocked in lakes and rivers should try and preserve as much genetic variation as possible and should try and prevent any changes in the genetic make-up of the population. This means saving alleles whose frequencies are 0.01-0.001. Farmers who raise food fish do not need to be as zealous. If they wish to conserve the population's genetic variance, they only need to save alleles whose frequencies are 0.05-0.01; however, many fish farmers should not even worry about the effects of genetic drift.

As was mentioned earlier, it is not possible to manage an N_e to produce a 100% guarantee of saving an allele, so practical workable guarantees must be chosen. In biology, two values are routinely used: 95% and 99%. These values come from statistics: when biologists evaluate experiments statistically, they typically use 0.01 and 0.05 as probability levels. The guarantee of saving an allele is: 1.0 - the probability of losing the allele.

A final decision must be made before the N_e can be managed to prevent the loss of rare alleles: How many generations will be incorporated into the management plan; i.e., how many generations will be produced until the guarantee of keeping the alleles is lowered to the desired level?

Once these decisions have been made, the N_e that is needed to prevent genetic drift from robbing the population of rare alleles is determined by using the following formula:

$$P = (1.0 - q)^{2N_e}$$

where: P is the probability of losing the allele in a single random sample (the fish that mate to produce the next generation or the transfer of fish from one hatchery to another); and q is the frequency of the rare allele that is to be saved ($q = 0.01$ or 0.05 or whatever value is chosen).

Table 4. Effective breeding numbers needed per generation to produce various levels of inbreeding after 1-100 generations. Effective breeding numbers were rounded up.

	Maximum level of inbreeding desired														
No. generations	1%	2%	3%	4%	5%	6%	7%	8%	9%	10%	11%	12%	13%	14%	15%
1	50	25	17	13	10	9	8	7	6	5	5	5	4	4	4
2	100	50	34	25	20	17	15	13	12	10	10	9	8	8	7
3	150	75	50	38	30	25	22	19	17	15	14	14	12	11	10
4	200	100	67	50	40	34	29	25	23	20	19	17	16	15	14
5	250	125	84	63	50	42	36	32	28	25	23	21	20	18	17
6	300	150	100	75	60	50	43	38	34	30	28	25	24	22	20
7	350	175	117	88	70	59	50	43	39	35	32	30	27	25	24
8	400	200	134	100	80	67	58	50	45	40	37	34	31	29	27
9	450	225	150	113	90	75	65	57	50	45	41	38	35	33	30
10	500	250	167	125	100	84	72	63	56	50	46	42	39	36	34
15	750	375	250	188	150	125	108	94	84	75	69	63	58	54	50
20	1000	500	334	250	200	167	143	125	112	100	91	84	77	72	67
25	1250	625	417	313	250	209	179	157	139	125	114	105	97	90	84
30	1500	750	500	375	300	250	215	188	167	150	137	125	116	108	100
35	1750	875	584	438	350	292	250	219	195	175	160	146	135	125	117
40	2000	1000	667	500	400	334	286	250	223	200	182	167	154	143	134
45	2250	1125	750	563	450	375	322	282	250	225	205	186	174	161	150
50	2500	1250	834	625	500	417	358	313	278	250	228	209	193	179	167
55	2750	1375	917	688	550	459	393	344	306	275	250	230	212	197	184
60	3000	1500	1000	750	600	500	429	375	334	300	273	250	231	215	200
65	3250	1625	1084	813	650	542	465	407	362	325	296	271	250	233	217
70	3500	1750	1167	875	700	584	500	438	389	350	319	292	270	250	234
75	3750	1875	1250	938	750	625	536	469	417	375	341	313	289	268	250
80	4000	2000	1334	1000	800	667	572	500	445	400	346	334	308	286	267
85	4250	2125	1417	1063	850	709	608	532	473	425	387	355	327	304	284
90	4500	2250	1500	1125	900	750	643	563	500	450	410	375	347	322	300
95	4750	2375	1584	1188	950	792	679	594	528	475	432	396	366	340	317
100	5000	2500	1667	1250	1000	834	715	625	556	500	455	417	385	358	334

Table 2 (page 47) lists the N_e's that are needed to produce various probabilities of losing alleles whose frequencies are 0.001-0.5 after a single generation of genetic drift. Table 2 shows that the probability of losing an allele is inversely related to its frequency: the smaller the frequency, the more likely it is that the allele will be lost via genetic drift. For example, if the frequency of an allele is 0.5, an N_e of only 10 is needed to produce a probability of 1×10^{-6} (0.000001) of losing the allele after a single generation of drift; but if the frequency of an allele is 0.001, an N_e of 6,880 is required to produce that level of probability. In Table 2, no probability is listed for each allelic frequency, once the probability of losing it reaches 1×10^{-6}. At that level of probability, the odds of losing the allele via drift approaches zero (the guarantee of saving it becomes 99.9999%).

However, proper brood stock management requires medium- to long-range planning, which means a farmer or hatchery manager must know how large the N_e must be to prevent the loss of alleles after 3 or 5 or 25 generations, depending on management goals. Minimum constant N_e's that are needed to produce 95% and 99% guarantees of keeping alleles whose frequencies are 0.1, 0.05, 0.01, 0.005, and 0.001 are listed in Table 5. The method that was used to calculate the values in Table 5 is outlined in Table 6.

The values listed in Table 5 show that fairly small N_e's are needed to save alleles whose frequencies are ≥ 0.05, while fairly large N_e's are required to save alleles whose frequencies are ≤ 0.005. This is why most farmers and hatchery managers should try and save alleles whose frequencies are 0.01-0.05. However, hatchery managers and farmers who have large farms, who culture a species which requires thousands of brood fish, or who spawn wild fish could try and save alleles whose frequencies are ≤ 0.01. The frequency of the allele that a farmer or hatchery manager wants to save is determined by the species that is cultured, the size of the farm, and the management goals.

The 95% and 99% guarantees apply for all alleles of a given frequency (e.g., 0.01 or 0.05). This means if 100 alleles have that frequency, 95% or 99% will be saved, but 5% or 1% will be lost.

Table 5. Effective breeding numbers needed per generation for 1-100 generations to produce 95% and 99% guarantees of saving alleles whose frequencies (f) are 0.1-0.001. The guarantee of saving an allele is: 1.0 and - probability of losing it. Effective breeding numbers were rounded up.

No.	f = 0.1		f = 0.05		f = 0.01		f = 0.005		f = 0.001	
generations	95%	99%	95%	99%	95%	99%	95%	99%	95%	99%
1	15	22	30	45	150	230	299	460	1498	2302
2	18	26	36	52	183	264	367	529	1838	2647
3	20	28	40	56	203	284	407	569	2038	2847
4	21	29	43	59	218	298	436	598	2181	2993
5	22	30	45	61	229	309	458	620	2292	3104
6	23	31	47	63	238	319	476	638	2382	3195
7	24	32	48	64	245	326	491	654	2459	3272
8	24	32	50	66	252	333	505	667	2526	3339
9	25	33	51	67	258	339	516	679	2584	3398
10	26	33	52	68	263	344	527	689	2637	3450
15	27	35	56	72	283	364	567	730	2839	3653
20	29	37	59	75	297	378	596	758	2983	3797
25	30	38	61	77	308	390	618	780	3094	3908
30	31	38	63	78	318	399	636	799	3185	3999
35	31	39	64	80	325	406	651	814	3262	4076
40	32	40	65	81	332	413	665	827	3329	4143
45	33	40	67	82	338	419	677	839	3388	4202
50	33	41	68	83	343	424	687	850	3440	4255
55	34	41	69	84	348	429	697	859	3488	4302
60	34	42	69	85	352	433	705	868	3531	4346
65	34	42	70	86	356	437	713	876	3571	4386
70	35	42	71	87	360	441	721	883	3608	4423
75	35	43	72	87	363	444	727	890	3643	4457
80	35	43	72	88	366	447	734	896	3675	4489
85	36	43	73	88	369	450	740	903	3705	4520
90	36	44	73	89	372	453	746	908	3734	4548
95	36	44	74	90	375	456	751	914	3761	4575
100	36	44	74	90	377	458	756	919	3786	4601

Table 6. Method used to determine effective breeding numbers that are listed in Table 5.

Given: You want to save alleles whose frequencies are 0.01 (q), and you want a 95% guarantee of saving the allele (P = 0.05) after 10 generations (when the 10th generation is produced). What constant N_e is needed to achieve this goal?

Step 1: Calculate the guarantee per generation that is needed to produce a 95% guarantee after 10 generations:

$0.95 = (\text{guarantee/generation})^{10}$

$\text{guarantee/generation} = (0.95)^{1/10}$

$\text{guarantee/generation} = 0.994883803$

$(0.95)^{1/10}$ can be determined by using the "y^x" button on a hand-held calculator.

Step 2: Calculate the probability of losing the allele per generation:

Probability of losing the allele/generation = 1.0 - guarantee of saving it /generation

Probability of losing the allele/generation = 1.0 - 0.994883803

Probability of losing the allele/generation = 0.005116196882

Step 3: Calculate the N_e that is needed to produce a P = 0.005116196882 when q = 0.01 (the frequencies of the alleles you are trying to save):

$$P = (1.0 - q)^{2N_e}$$

$$0.005116196882 = (1.0 - 0.01)^{2N_e}$$

To determine N_e, the formula must be converted to logarithms. This can be done by using the "log" button on a hand-held calculator.

$$\log 0.005116196882 = \log(0.99)^{2N_e}$$

$$\log 0.005116196882 = (2N_e)(\log 0.99)$$

$$\frac{\log 0.005116196882}{\log 0.99} = 2N_e$$

$$\frac{-2.291052751}{-0.004364805402} = 2N_e$$

$$524.89 = 2N_e$$

$$\frac{524.89}{2} = N_e$$

$$262.45 = N_e$$

N_e is rounded to the next higher whole number, so an N_e of 263 is needed every generation in order to produce a 95% guarantee of saving an allele whose frequency is 0.01 when the 10th generation is produced.

BOTTLENECKS

All of the recommendations that were made in the previous section were based on maintaining minimum constant N_e's. Unfortunately, it is difficult to maintain a constant N_e generation after generation. Anyone who has ever managed a population of fish knows all too well that it is difficult to maintain a steady-state population. Many factors conspire to occasionally reduce population size. Sudden drastic decreases in population size are called "bottlenecks." The genetic effects of bottlenecks can be devastating and can have long-term repercussions.

As was described in Chapter 4, the mean N_e over a series of generations is the harmonic mean, not the simple arithmetic mean. Consequently, the generation with the smallest N_e has a disproportionate influence on the average value. This means that a bottleneck can dramatically lower mean N_e, which in turn will dramatically increase inbreeding and genetic drift.

For example, if a farmer wants to maintain a constant N_e of 100 for 10 generations but experiences a bottleneck of 20 in generation 6, the mean N_e that he produced is:

$$\frac{1}{N_{e\cdot mean}} = \frac{1}{t}\left(\frac{1}{N_{e1}} + \frac{1}{N_{e2}} + ... + \frac{1}{N_{et}}\right)$$

$$\frac{1}{N_{e\cdot mean}} = \frac{1}{10}\left(\frac{1}{100} + \frac{1}{100} + \frac{1}{100} + \frac{1}{100} + \frac{1}{100} + \frac{1}{20} + \frac{1}{100} + \frac{1}{100} + \frac{1}{100} + \frac{1}{100}\right)$$

$$\frac{1}{N_{e\cdot mean}} = 0.1(0.01 + 0.01 + 0.01 + 0.01 + 0.01 + 0.05 + 0.01 + 0.01 + 0.01 + 0.01)$$

$$\frac{1}{N_{e\cdot mean}} = 0.014$$

$N_{e\ mean}$ can be determined by using the "1/x" button on a hand-held calculator.

$N_{e\ mean}$=71.4

The arithmetic average for this series of N_e's is 92, so mean N_e is 22% smaller than would be expected. If there were two bottlenecks of 20, mean N_e would have dropped to only 55.6.

Bottlenecks can have severe and long-lasting effects on inbreeding, because once inbreeding occurs, it lowers future N_e's, as was described in Chapter 4. The impact that bottlenecks have on average inbreeding depends on the size of the bottleneck and how many there are. A population which is poorly managed and which experiences multiple bottlenecks where the population is reduced to relatively few males and/or females will be quite inbred. On the other hand, a well-managed population that experiences a single bottleneck will be far less affected. A single bottleneck will produce an immediate increase in inbreeding and the average inbreeding may increase for a generation or two (the amount depends on the size of the bottleneck), but if the population is properly managed to minimize inbreeding before and after the bottleneck, the average inbreeding will plateau a few generations after the bottleneck, and the damage due to inbreeding might not be severe.

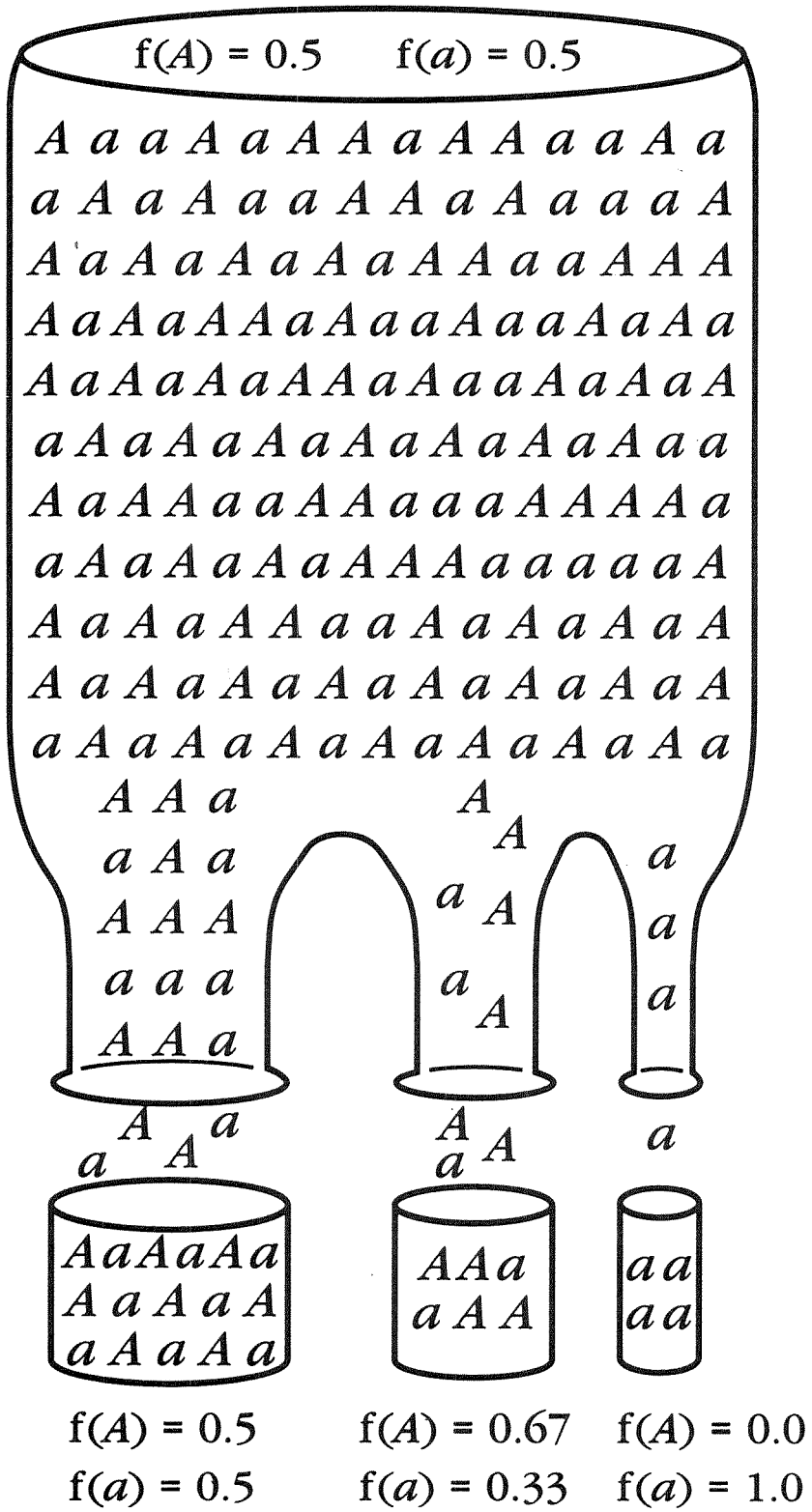


Figure 32. Effects of bottlenecks on gene frequency. Since genetic drift and the probability of losing alleles are inversely related to effective breeding number (N_e), the size of a bottleneck determines the amount of genetic change. The frequencies of the A and a alleles in the population are both 0.5. As the bottleneck (N_e) becomes smaller, genetic drift alters gene frequency until at an extreme, the frequency of an allele goes to zero.

The effects that bottlenecks can have on genetic drift are far more devastating. The probability of losing an allele is inversely related to N_e, so the odds of losing the allele increase as N_e decreases. Bottlenecks increase the probability dramatically. The effect that a bottleneck can have on gene frequency is illustrated in Figure 32.

For example, if a farmer is trying to maintain a constant N_e of 344 for 10 generations in order to produce a guarantee of 99% for keeping an allele whose frequency is 0.01 (Table 5) and there is a bottleneck of 25 in generation 8, the probability of losing the allele when the 10th generation is produced is determined as follows:

Step 1. The probability of losing the allele each generation must be determined.

Step 2. The guarantee of keeping the allele each generation must be determined (1.0 - probability of losing the allele).

The probabilities of losing the allele and the guarantees of keeping the allele for all 10 generations are:

N_e	Step 1: Probability of losing allele ($P = (1.0 - q)^{2N_e}$)	Step 2: Guarantee of keeping allele ($1.0 - P$)
344	$P = (0.99)^{2(344)} = 0.0009931477$	0.999006852
344	$P = (0.99)^{2(344)} = 0.0009931477$	0.999006852
344	$P = (0.99)^{2(344)} = 0.0009931477$	0.999006852
344	$P = (0.99)^{2(344)} = 0.0009931477$	0.999006852
344	$P = (0.99)^{2(344)} = 0.0009931477$	0.999006852
344	$P = (0.99)^{2(344)} = 0.0009931477$	0.999006852
344	$P = (0.99)^{2(344)} = 0.0009931477$	0.999006852
25	$P = (0.99)^{2(25)} = 0.605006067$	0.394993932
344	$P = (0.99)^{2(344)} = 0.0009931477$	0.999006852
344	$P = (0.99)^{2(344)} = 0.0009931477$	0.999006852

Step 3. The guarantee of keeping the allele for 10 generations must be determined. It is the product of the guarantee of keeping the allele for each generation; i.e., the guarantees for each generation are multiplied to determine the guarantee after the 10th generation is produced:

Guarantee after 10 generations = (0.999006852)(0.999006852) (0.999006852)(0.999006852) (0.999006852)(0.999006852)(0.999006852)(0.394993932) (0.999006852)(0.999006852)

Guarantee after 10 generations = 0.3915

Step 4. The probability of losing the allele after 10 generations must be determined. It is 1.0 - the guarantee of keeping the allele:

Probability of losing allele = 1.0 - Guarantee of keeping allele

Probability of losing allele = 1.0 - 0.3915

Probability of losing allele = 0.6085

The bottleneck of 25 at generation 8 increased the probability of losing the allele in the 10th generation from 0.01 (which was the goal) to 0.6085.

The overall effect of a single bottleneck on genetic drift is far more severe than it is on average inbreeding, and its occurrence can prevent a farmer or hatchery manager from achieving his goals, even if he has done an outstanding job for many generations. This is because once an allele is lost, it can be recovered only by mutation or the introduction of new brood stock. Consequently, prevention of bottlenecks should be the major brood stock management goal if the genetic goal is to minimize the detrimental effects of inbreeding and genetic drift.

TECHNIQUES THAT CAN BE USED TO MANAGE EFFECTIVE BREEDING NUMBER

Since inbreeding and genetic drift are controlled by the population's N_e, it should be obvious that managing N_e is the most effective way to control or to prevent these twin problems from ruining productivity and profits. Minimum constant N_e's need to be maintained to prevent inbreeding from exceeding desired levels or to produce desired guarantees of keeping alleles with certain frequencies.

However, there are some management techniques that can be used to "artificially" increase N_e or to manage the brood fish to "stretch" generations. These techniques lengthen the time frame before inbreeding and genetic drift cause problems. By incorporating some of these management techniques, smaller numbers of brood fish can be used; smaller N_e's can be used; or the brood fish that are spawned can be managed more effectively to prevent genetic degradation of a population.

Stretching generations

The simplest and least expensive technique that can be used to increase the time frame until inbreeding- and genetic drift-related problems cause trouble is to stretch the generation interval. This is not always possible, since some fish die when they spawn. But when it is possible, this technique is very effective. In this manual, a generation is defined as the replacement of brood fish by their offspring. Generations are not allowed to overlap. The definition of "generation" does not contain a specific time frame in terms of years. So if the normal time it takes for a fish to go from egg to brood fish and then for that fish to spawn and be discarded so that it can be replaced by its offspring is 2 years, the generation interval for that species is 2 years. If the time interval for another species is 4 years, the generation interval for that species is 4 years.

But these time intervals can be stretched if brood fish are allowed to spawn for an extra year or two before they are replaced by their offspring. The longer a generation can be stretched, the longer it will take for inbreeding- and genetic drift-related problems to become evident.

For example, let's assume that the generation interval for the species a farmer cultures is 2 years. Let's also assume that he wants to keep inbreeding below 5% for 10 generations (20 years). To do this, he needs an N_e of 100 per generation (Table 4). However, if he can stretch the generation interval so that it doubles to 4 years, he can use an N_e of only 50 to produce the same amount of inbreeding (5%) at the end of 20 years, because he will produce only five generations during this time span. Consequently, a farmer can maintain and spawn fewer fish by stretching the generations.

The major constraint with this type of brood stock management is survival. If survival of brood fish declines during this "extra" period, N_e of the population will decline. However, if survival of brood fish is not adversely affected by the extra stretched interval, this technique is an easy way of controlling inbreeding. One way of compensating for mortalities is to maintain additional brood fish and to use them to replace those that die.

Pedigreed mating

Most farmers or hatchery managers use what is called "random mating" when they spawn their fish. Random mating is a breeding protocol where fish are mated without regard to phenotypic value. The only time random mating is not used is when selective breeding or crossbreeding programmes are implemented to improve growth rate or other phenotypes.

The N_e of a population can be "artificially" increased by using a breeding programme called "pedigreed mating." Pedigreed mating differs from random mating in that each female leaves one daughter and each male leaves one son to be used as brood fish in the following generation. In actuality, each male and each female can leave more than one descendant, but all fish must leave the same number. This means that a male that spawns with five females will leave the same number of sons as a male that spawns with only one female. They will leave unequal numbers of daughters, but they will leave the same number of sons. The sons and daughters that become brood fish in the following generation must be chosen randomly from each family. No fish can be chosen because it has a specific phenotype or is a certain weight; if that is done, some form of selection is occurring.

When pedigreed mating is used, the N_e of the population increases because the genetic variance is artificially increased by ensuring that each brood fish is represented in the next generation. When pedigreed mating is used, N_e is determined by using the following formula:

$$N_e = \frac{16(\text{number of males})(\text{number of females})}{3(\text{number of males}) + \text{number of females}}$$

if there are more males,

or

$$N_e = \frac{16(\text{number of males})(\text{number of females})}{3(\text{number of females}) + \text{number of males}}$$

If there are more females.

If equal numbers of males and females are spawned, either formula can be used.

For example, if a farmer spawns 50 females and 30 males using random mating, his N_e is:

$$N_e = \frac{4(\text{number of males})(\text{number of females})}{\text{number of females} + \text{number of males}}$$

$$N_e = \frac{4(30)(50)}{50 + 30}$$

$$N_e = 75$$

If he uses pedigreed mating, the N_e increases to:

$$N_e = \frac{16(\text{number of males})(\text{number of females})}{3(\text{number of females}) + \text{number of males}}$$

$$N_e = \frac{16(30)(50)}{3(50) + 30}$$

$$N_e = 133.3$$

Pedigreed mating increased N_e by 77.8% in the above example, and the inbreeding that would be produced would decline from 0.67% to 0.38%.

The only difficulty in using pedigreed mating is that you have to be able to mark the fish or be able to grow each family in an isolated pond or tank so that individual families can be identified.

Altering spawning techniques

There are several practical ways that spawning can be modified in order to increase N_e and thus reduce inbreeding and the effects of genetic drift.

Increasing the spawning population — One way to reduce inbreeding and the effects of genetic drift is to spawn more fish than are needed. Most farmers and hatchery managers have been trained to be efficient, and this means spawning the fewest number of fish that will enable them to meet production goals. Farmers in particular want to be efficient so that they spend less money raising their crop. Furthermore, one way hatchery managers at public hatcheries are evaluated is cost per fingerling. But this philosophy is at odds with the control of inbreeding and genetic drift.

This problem is especially acute in aquaculture, because the fecundity of some species of fish is so great that it is often possible to spawn one or two females and males and produce the number of fingerlings that are needed for grow-out. But the ability to spawn relatively few fish must be moderated if inbreeding and genetic drift are to be controlled.

The best way to manage a population is to determine what N_e is needed to prevent inbreeding- and genetic drift-related problems under the time frame that has been chosen and then spawn the number of brood fish that will enable the farmer to produce that N_e. The farmer should spawn many more fish than he normally would and simply keep a small and equal random sample of eggs or fingerlings from each spawn. He can sell the surplus eggs or fish to other farmers. If he cannot sell the surplus fish, they should be discarded. This might seem wasteful, but this practice improves the genetics of the population.

Spawn a more equal sex ratio — An easy way to increase N_e and thus reduce the rate of inbreeding and genetic drift is to spawn a more equal sex ratio. Most farmers and hatchery managers use skewed sex ratios when they spawn their fish. This is done because one male can usually be used to fertilize eggs from several females. This enables aquaculturists to use and maintain fewer males, which lowers production costs.

This may be a great idea in terms of efficiency, but it is a bad idea if you want to manage and control the genetic quality of the population. When a skewed sex ratio is used, the rarer sex has a disproportionate influence on the size of N_e.

The impact that a skewed sex ratio has on inbreeding can be seen in the following formula:

$$F=\frac{1}{8(\text{numer of females})}+\frac{1}{8\,(\text{number of males})}$$

where: number of males and number of females are the numbers that produce viable offspring. The effect that a skewed sex ratio has on inbreeding is more pronounced when the total breeding population is small. For example, if a farmer spawns 100 females and 10 males he produces the following amount of inbreeding:

$$F=\frac{1}{8(100)}+\frac{1}{8(10)}$$

$$F=0.01375$$

If he moderates the sex ratio and uses 100 females and 40 males the inbreeding becomes:

$$F=\frac{1}{8(100)}+\frac{1}{8(40)}$$

$$F=0.004375$$

The addition of 30 males reduced inbreeding per generation from 1.38% to 0.44%.

The impact that skewed sex ratios have on inbreeding even means that smaller populations can produce less inbreeding. For example, a population with 50 males and 50 females produces less inbreeding than one with 20 males and 200 females (0.5% vs 0.69%) even though it is less than half as large.

The inbreeding that is produced by various combinations of males and females is shown in Table 7. The inbreeding values in that table show that if the number of one sex is held constant, increasing the other sex produces diminishing returns; i.e., the reduction in inbreeding becomes less and less despite substantial increases in the commoner sex. For example, if a farmer spawns 10 males and 100 females, F = 1.38%; if he spawns 10 males and 250 females, F = 1.30%. Table 7 clearly shows that the best way to reduce inbreeding is to increase the number of males and females and to spawn a sex ratio that is as close to 1:1 as possible.

Table 7. Percent inbreeding that is produced each generation in a population with random mating among various numbers of males and females. Inbreeding values were rounded to the nearest hundredth.

No. females	No. males																					
	1	2	3	4	5	6	7	8	9	10	20	25	50	75	100	125	150	175	200	225	250	∞
1	25.00	18.75	16.67	15.62	15.00	14.58	14.29	14.06	13.89	13.75	13.12	13.00	12.75	12.67	12.62	12.60	12.58	12.57	12.56	12.56	12.55	12.50
2	18.75	12.50	10.42	9.38	8.75	8.33	8.04	7.81	7.64	7.50	6.88	6.75	6.50	6.42	6.38	6.35	6.33	6.32	6.31	6.31	6.30	6.25
3	16.67	10.42	8.33	7.29	6.67	6.25	5.95	5.73	5.56	5.42	4.79	4.67	4.42	4.33	4.29	4.27	4.25	4.24	4.23	4.22	4.22	4.17
4	15.62	9.38	7.29	6.25	5.62	5.21	4.91	4.69	4.51	4.38	3.75	3.62	3.38	3.29	3.25	3.22	3.21	3.20	3.19	3.18	3.18	3.12
5	15.00	8.75	6.67	5.62	5.00	4.58	4.29	4.06	3.89	3.75	3.12	3.00	2.75	2.67	2.62	2.60	2.58	2.57	2.56	2.56	2.55	2.50
6	14.58	8.33	6.25	5.21	4.58	4.17	3.87	3.65	3.47	3.33	2.71	2.58	2.33	2.25	2.21	2.18	2.17	2.15	2.15	2.14	2.13	2.08
7	14.29	8.04	5.95	4.91	4.29	3.87	3.57	3.35	3.17	3.04	2.41	2.29	2.04	1.95	1.91	1.89	1.87	1.86	1.85	1.84	1.84	1.79
8	14.06	7.81	5.73	4.69	4.06	3.65	3.35	3.12	2.95	2.81	2.19	2.06	1.81	1.73	1.69	1.66	1.65	1.63	1.62	1.62	1.61	1.56
9	13.89	7.64	5.56	4.51	3.89	3.47	3.17	2.95	2.78	2.64	2.01	1.89	1.64	1.56	1.51	1.49	1.47	1.46	1.45	1.44	1.44	1.39
10	13.75	7.50	5.42	4.38	3.75	3.33	3.04	2.81	2.64	2.50	1.88	1.75	1.50	1.42	1.38	1.35	1.33	1.32	1.31	1.31	1.30	1.25
20	13.12	6.88	4.79	3.75	3.12	2.71	2.41	2.19	2.01	1.88	1.25	1.12	0.88	0.79	0.75	0.72	0.71	0.70	0.69	0.68	0.68	0.62
25	13.00	6.75	4.67	3.62	3.00	2.58	2.29	2.06	1.89	1.75	1.12	1.00	0.75	0.67	0.62	0.60	0.58	0.57	0.56	0.56	0.55	0.50
50	12.75	6.50	4.42	3.38	2.75	2.33	2.04	1.81	1.64	1.50	0.88	0.75	0.50	0.42	0.38	0.35	0.33	0.32	0.31	0.31	0.30	0.25
75	12.67	6.42	4.33	3.29	2.67	2.25	1.95	1.73	1.56	1.42	0.79	0.67	0.42	0.33	0.29	0.27	0.25	0.24	0.23	0.22	0.22	0.17
100	12.62	6.38	4.29	3.25	2.62	2.21	1.91	1.69	1.51	1.38	0.75	0.62	0.38	0.29	0.25	0.22	0.21	0.20	0.19	0.18	0.18	0.12
125	12.60	6.35	4.27	3.22	2.60	2.18	1.89	1.66	1.49	1.35	0.72	0.60	0.35	0.27	0.22	0.20	0.18	0.17	0.16	0.16	0.15	0.10
150	12.58	6.33	4.25	3.21	2.58	2.17	1.87	1.65	1.47	1.33	0.71	0.58	0.33	0.25	0.21	0.18	0.17	0.15	0.15	0.14	0.13	0.08
175	12.57	6.32	4.24	3.20	2.57	2.15	1.86	1.63	1.46	1.32	0.70	0.57	0.32	0.24	0.20	0.17	0.15	0.14	0.13	0.13	0.12	0.07
200	12.56	6.31	4.23	3.19	2.56	2.15	1.85	1.62	1.45	1.31	0.69	0.56	0.31	0.23	0.19	0.16	0.15	0.13	0.12	0.12	0.11	0.06
225	12.56	6.31	4.22	3.18	2.56	2.14	1.84	1.62	1.44	1.31	0.68	0.56	0.31	0.22	0.18	0.16	0.14	0.13	0.12	0.11	0.11	0.06
250	12.55	6.30	4.22	3.18	2.55	2.13	1.84	1.61	1.44	1.30	0.68	0.55	0.30	0.22	0.18	0.15	0.13	0.12	0.11	0.11	0.10	0.05
∞	12.50	6.25	4.17	3.12	2.50	2.08	1.79	1.56	1.39	1.25	0.62	0.50	0.25	0.17	0.12	0.10	0.08	0.07	0.06	0.06	0.05	0.00

Source: Tave, D. 1990. Effective breeding number and broodstock management: I. How to minimize inbreeding. Pages 27-38 in R.O. Smitherman and D. Tave, eds. Proceedings Auburn Symposium on Fisheries and Aquaculture. Alabama Agricultural Experiment Station, Auburn University, Alabama, USA.

Paradoxically, increasing the number of the commoner sex may prevent the loss of rare alleles via genetic drift, simply because by spawning more fish a farmer increases the likelihood that one of the brood fish will carry a rare allele. However, increasing the number of the rarer sex will also increase the likelihood of choosing a brood fish with a rare allele, and this approach is better because it will increase N_e significantly.

If the number of brood fish cannot be increased at a hatchery or farm, the only ways N_e can be increased are to switch to pedigreed mating or to moderate the sex ratio so it is closer to 1:1. The improvements in moderating the sex ratio or in switching to pedigreed mating can be assessed by evaluating the effective breeding efficiency (N_b) of the proposed changes. The N_b is the ratio of N_e to the size of the breeding population (N):

$$N_b = \frac{N_e}{N}$$

The N_b's for random and pedigreed matings over all possible sex ratios are shown in Figure 33. The curves for both types of mating clearly show that sex ratios that are in the 40% male:60% female to 60% male:40% female range are far more effective in maximizing N_e, within the constraints of a fixed population size, than are sex ratios that are more skewed than 60:40.

For example, if a farmer uses random mating, spawns 100 fish, and wants to use a skewed sex ratio, the following sex ratios would produce the following N_b's:

Sex ratio (female:male)	N_b
90:10	36%
80:20	64%
70:30	84%

The farmer can then use these N_b's to decide what sex ratio is best in terms of spawning efficiency, fingerling production, and prevention of inbreeding. The N_b's show that he can moderate the skewness of his sex ratio slightly, yet still use a sex ratio which is quite skewed (70:30) and produce far less inbreeding. The inbreeding that is produced by a 70:30 sex ratio is 57% less than that produced by a 90:10 sex ratio. Figure 33 shows that some sex ratios do not have to be moderated to help control inbreeding; a 55:45 sex ratio has an N_b of 99%, so moderating that ratio closer to 50:50 will produce little improvement in terms of inbreeding control.

Figure 33 also shows the benefits that can be derived by switching from random mating to pedigreed mating. If pedigreed mating is used, a farmer can use a skewed sex ratio and still produce a large N_b. For example, if pedigreed mating is used, a 79:21 sex ratio produces an N_b of 102%, which is larger than the N_b that can be produced by a 50:50 sex ratio (100%) with random mating, provided N remains constant. Tremendous gains, in terms of N_b, can be made if a farmer switches from random to pedigreed mating and brings the sex ratio closer to 50:50.

Fertilization techniques — A final approach to managing N_e is to alter certain hatchery practices if gametes are stripped. When eggs and sperm are stripped, many farmers and hatchery managers pool eggs from several females and then fertilize the eggs either by using pooled milt or by adding milt from several males in a sequential manner. Both techniques will lower N_e from what you think it is. The reasons are: One, if milt is pooled and added, there is competition among the sperm, due to a difference in potency. Some males produce more vigorous sperm, and these sperm cells will fertilize a disproportionate number of eggs. In some cases, most of the eggs will be fertilized by sperm from a single male. Second, if milt is added in a sequential manner, the sperm from the first male will fertilize most of the eggs, while that from the last few males will fertilize comparatively few eggs.

The solutions to both problems are quite simple. Fertilize eggs from each female with sperm from a single male. If you want to have sperm from several males fertilize eggs from each female, subdivide each egg mass and fertilize each lot separately. If this is done, N_e can be accurately determined, because you know which brood fish produced offspring.

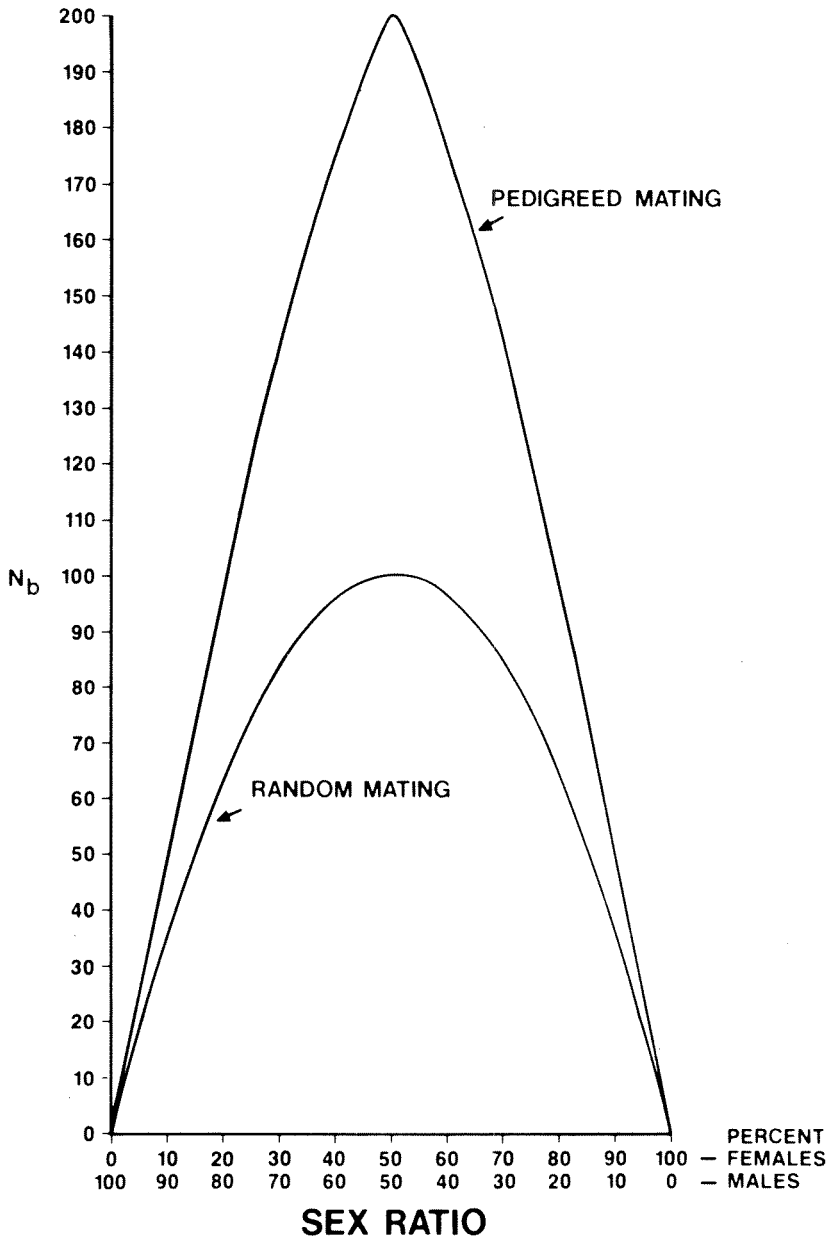


Figure 33. Effective breeding efficiency (N_b) over all possible sex ratios for both random mating and pedigreed mating. Effective breeding efficiency is a measure of the efficiency of the sex ratio or mating system in maximizing N_e within the constraints of a fixed population size (N).

Source: Tave, D. 1984. Effective breeding efficiency: An index to quantify the effects that different breeding programs and sex ratios have on inbreeding and genetic drift. Progressive Fish-Culturist 46:262-268.

CONCLUSION

Unless a farmer is going to conduct a selective breeding programme or use inbreeding to improve the results of selection or crossbreeding, a population should be managed genetically to prevent unwanted inbreeding from causing inbreeding depression and to prevent genetic drift from robbing the population of alleles and genetic variance. If fish can be marked, inbreeding depression can be prevented by creating pedigrees and by preventing consanguineous matings or by preventing matings between relatives more closely related than second cousins.

Marking fish and preventing consanguineous matings will not prevent genetic drift. Managing a population to minimize the effects of genetic drift can be be accomplished only by managing N_e.

When fish are not marked (which will be the case for most hatchery populations), the only way to prevent unwanted inbreeding from accumulating and to prevent the ravages of genetic drift is to manage the population's N_e. When a farmer is not using a breeding programme to improve a population, managing a population's N_e is probably the most important aspect of brood stock management. A population's N_e is one of the most important pieces of information about the population, because N_e is inversely related to both inbreeding and genetic drift. Consequently, managing N_e is a key aspect of fish husbandry.

There is no universal N_e that can be used to manage every population. It must be customized for each population. This chapter outlined the techniques and methods that must be used to determine the N_e that is needed. That number can be determined by answering a series of questions: One, what level of inbreeding will cause problems? Two, what is the frequency of the rare alleles that a farmer wants to save, and what guarantee does he want that the alleles have been saved? Three, how many generations does the farmer want to incorporate into the work plan before that level of inbreeding has been reached and when the guarantee will end?

The levels of inbreeding that cause problems in hatchery populations are not known, so appropriate levels must be determined by a "guesstimate." Two values were proposed: 5% and 10%. The value chosen depends on how important the population is and what the goals are. Populations that are being cultured for food can use either 5% or 10%. Populations that are being cultured for stocking programmes must use 5%; if possible, lower levels should be used for these populations.

The frequency of the rare alleles that should be saved depends on the population. If the fish are being farmed, the frequency should be 0.05 for most farmers who wish to manage their populations genetically, and 0.01 for those who have the ability and desire to conserve as much genetic variance as possible. If the population is being cultured for stocking programmes the frequency should be no greater than 0.01; if possible, the frequency should be 0.005-0.001. These frequencies are not absolute but are presented as guideline values.

When managing a population's N_e, the major goal is to maintain N_e at a constant level every generation. If N_e drops below the desired value for a single generation, the genetic goals cannot be achieved. Maintaining N_e at the desired level generation after generation may be the most difficult aspect of brood stock management, because N_e can decline for a variety of reasons. Sudden and drastic decreases in N_e are called bottlenecks, and they can cause permanent and irreversible genetic damage.

Finally, there are a number of management techniques that can be used to increase N_e or that can be used to produce the same level of inbreeding but with a smaller number of brood fish: pedigreed mating; stretching generations; bringing the sex ratio closer to a 1:1 ratio; and altering some fertilization techniques when gametes are stripped.

Recommended N_e's, based on the information generated in this chapter, that will enable a farmer or hatchery manager to prevent inbreeding- and genetic drift-related problems are presented in Chapter 8.

CHAPTER 8.

RECOMMENDATIONS

Scientists and extension agents feel most comfortable when they can give a farmer or hatchery manager an unambiguous piece of advice to solve or prevent a problem. Farmers and hatchery managers feel more comfortable when they receive unambiguous advice, because this means that there is only one way of doing something, and if they follow the advice they will solve or prevent the problem and become successful. To this end, a number of publications have recommended that aquaculturists or hatchery managers should maintain a specific N_e in order to prevent inbreeding - or genetic drift-related problems. These recommendations have ranged from 50 to 1,000; the majority suggest either 500 or 1,000.

Unfortunately, there is no single N_e and there is no simple set of genetic guidelines that can be prescribed for every farmer and every hatchery manager. Unlike disease prevention and treatment, the recommendations and protocols that are needed to prevent genetic problems vary from farmer to farmer, from farm to farm, and from programme to programme.

The reason why recommendations must be site-specific is because each farmer and each hatchery manager have different goals. Those for farmers with small farms will differ from those for farmers with medium to large farms. Those for fingerling producers will be different from those for farmers who simply grow fish for market. Finally, those for hatchery managers who manage populations that are being cultured to restock lakes and rivers will be radically different from all others, because the goals and plans for these programmes are to maintain and restore damaged natural fish stocks, not produce fish for market.

Proper management of a population, whatever the goal may be, begins with acquisition of the stock. The acquisition of a hatchery population is difficult and expensive, and this is where many genetic problems occur. The acquisition is of the utmost importance, and it may be the single most important aspect of brood stock management because this act determines the maximum amount of genetic variance and also determines how much inbreeding will be produced via future matings. What is acquired determines the population's genetic potential. In most cases, brood stock management after the population has been acquired is done just to keep the population from getting worse, through preservation of what was acquired by minimizing inbreeding and genetic drift. The first part of this chapter describes genetic aspects of acquiring a population and provides recommended N_e's that should be used during the acquisition process.

This second portion of this chapter synthesizes the information that was developed in Chapters 4, 5, and 7 to produce a series of recommendations on how large N_e must be to prevent inbreeding and genetic drift from causing genetic problems in hatchery populations when no selective breeding programme will be conducted. Recommendations will be made for populations at small, medium-sized, and large farms that produce fish for food and also for hatcheries that produce fish that will be stocked in fisheries programmes.

Except where noted, most recommendations in this section assume that farmers and hatchery managers cannot mark their fish and follow individual pedigrees. The only way to prevent inbreeding and genetic drift from causing genetic problems when these conditions exist is to manage a population's N_e.

Recommended N_e's were developed using the following assumptions: First, subsistence-level farmers should ignore the problem. Second, the size of the farm and the farmer's degree of sophistication determine how much management is needed; i.e., how much genetic risk the farmer should assume. Farmers who produce fingerlings for the local market should assume a low degree of genetic risk, which means that they should try and control inbreeding and genetic drift. Those who produce fish for themselves and some for the local market and who raise the fish using extensive management can assume more genetic risk; i.e., they can allow inbreeding to accumulate to fairly high levels and can ignore genetic drift. Third, the type of aquaculture programme determines how much genetic risk should be assumed. If the fish are being cultured to restock a lake or river, a very low level of genetic risk should be assumed, which means that a greater degree of genetic management is required than would be necessary if the fish were being raised for food. The only management options that make sense for programmes that raise fish for fisheries programmes are to accept as little genetic risk as possible and to minimize both inbreeding and genetic drift. A major goal in this type of fish culture is

to conserve genotypic and genetic variance, and this can be accomplished only by minimizing inbreeding and genetic drift.

The final section discusses inbreeding and its management during a selective breeding programme. When a farmer conducts a selective breeding programme, his primary objective is to alter, not conserve, gene and genotypic frequencies in order to improve the population. Inbreeding is inevitable during a selective breeding programme, because each act of selection creates a bottleneck, and when select brood fish mate, a good percentage of the matings are consanguineous. Because of this, inbreeding is not the concern that it is in the absence of selection, when the only genetic goal is to conserve the status quo.

While inbreeding is not a major concern during selection, you do not want inbreeding to accumulate to levels where selection is being conducted simply to counteract inbreeding depression. Ways to minimize inbreeding during a selective breeding programme, as well as breeding techniques that can be used to produce genetically improved fish with 0% inbreeding for grow-out are described.

Genetic drift will also occur during a selective breeding programme. In fact, genetic drift can actually occur at a faster rate under these circumstances, because comparatively fewer fish are spawned than would be the case if random mating were used. Selection and preventing genetic drift are diametrically opposed breeding programmes. The goal of selection is to change gene frequencies and thus improve productivity, not conserve the status quo. Additionally, the major effects of genetic drift will be on genes that are not under direct or indirect selection. Consequently, prevention of genetic drift is not a major concern during a selective breeding programme.

ACQUISITION OF A POPULATION

The acquisition of a population is often the most important step in the management of the population. Mistakes that are made during acquisition can create permanent genetic damage, which can render meaningless the efforts to preserve the genetic quality of the population. You cannot manage and conserve what you do not have.

Before acquiring a population, a farmer or hatchery manager must ask the following questions: What is the purpose of the fish that will be acquired, and what do I want to do with them? For example, if a farmer wants to produce fast-growing fish for the local food market, he might want to purchase fish from another farmer or from a fingerling supplier who has a healthy, fast-growing strain. Conversely, he could acquire wild fish and create his own fast-growing strain. Both approaches will work; one will provide faster returns, while the other will enable the farmer to direct selection from the beginning and to create his own improved strain.

Hatchery managers who are raising fish that will be used to rebuild depleted populations in lakes and rivers should acquire fish from the lakes and rivers that will be stocked. It makes little sense to try and save an ailing population by stocking an exotic strain. This approach will often doom that population, especially if it is unique and small. If the stocked population hybridizes with the natural population, the stocking programme will cause the genetically unique population to disappear. If the stocked population out-competes the natural population for food or for spawning sites, the natural population will go extinct. It is counterproductive to raise a single strain and to use that strain for all stocking programmes. The stocks that are raised and stocked must be customized for the bodies of water where they are to be stocked.

The most important aspect of acquiring a population and the one that will have the greatest effect throughout its culture history is the genetic size of the acquired population. The N_e that is obtained will determine how much genetic variance exists and it will thus determine future inbreeding. If the N_e of the acquired population is small, the foundation generation will be a bottleneck, and this creates what is called the "founder effect." The effect that this has on inbreeding can be dramatic, but it often plateaus after a few generations. However, the long-term effects on genetic variance can be disastrous. A bottleneck that occurs in the foundation generation can permanently rob the population of many alleles that will be needed if the fish are to be stocked in the wild or if future selective breeding programmes are to be conducted.

This problem even occurs unintentionally, due to the fecundity of fish and the inability to identify individuals. I once saw someone ship 2,000 full-sibs (a single family produced by one mother and one father) to fill a request for a foundation population. The recipient thought he was receiving a population with a potential N_e of 2,000. The N_e of the fish that were received might have been 2,000, but the N_e that produced the foundation population was only 2. That foundation population had little genetic variance, and all first-generation offspring would have had F = 25% (more if the two parents were related). Although this example is extreme, many hatchery stocks have been started with small foundation populations that were not that much larger. Even when better efforts are taken to obtain as large an N_e as possible, the fish that are sampled are often produced by only a handful of brood fish.

The history of a stock should be examined before it is acquired. Obviously, the production history of the fish needs to be determined: growth rate, survival, fecundity, disease history, ease of spawning, etc. If possible, this information should be determined for every year that the fish have been at the hatchery. A progressive decrease in yield, survival, or fecundity might be clinical signs of inbreeding depression.

One other bit of information is equally important: the N_e for every generation. Even if N_e's are unknown, data sheets that list the number of fish that spawned will allow you to determine approximate N_e's for every generation.

Good stocks should not be discarded simply because the N_e's are smaller than desired. Background information about the strain's N_e's is simply another tool that will enable you to better evaluate the strain and to determine if the fruits of a farmer's labour are sweet or bitter.

One reason you want to determine the strain's history is so that you do not re-acquire the strain you already have. Many farmers acquire their foundation stock from a single good source and then rename the strain. A background check on the fish might reveal that all farmers in a region have the same strain, but it has a different alias on each farm.

If the foundation stock is going to come from a wild population, everything should be done in order to acquire a large N_e. It is often assumed that the most efficient way to do this is to randomly sample the population. This can work, but a population often assumes a non-random distribution, so a random sample will not accurately reflect the make-up of the population. If the population assumes a non-random distribution, use the natural history of the population and the way it is distributed in the lake or river to collect as large an N_e as possible. One way to accomplish this is to sample from many locations and to sample over time.

If the life history of the fish is well known and if the fish lay eggs masses, one way to collect a foundation stock with a large N_e is to obtain samples from many egg masses. If each egg mass is produced by a single male and female (or a known number of multiple males and females), the N_e of the fish that produced the foundation population can be determined. Egg masses should be collected over the entire spawning season, or the collection will be a form of selection that eliminates some genetic variance and reduces N_e from what it could have been. If egg masses are obtained, the families can be raised in individual tanks until they can be marked.

How large should the N_e of the foundation population be? Recommended N_e's that are presented in the next section can be used. However, more fish have to be collected than are needed to create the desired N_e, because of mortalities caused by transportation, disease, etc.

WHEN NO SELECTIVE BREEDING PROGRAM WILL BE CONDUCTED

When no selective breeding programme will be conducted, the only genetic goal of brood stock management is to prevent changes in the gene pool; this is accomplished by minimizing inbreeding and genetic drift. Such management will prevent genetic decreases in yield, etc. However, not all farmers need to be concerned about these problems or need to manage their populations to prevent them from occurring. Separate sets of guidelines are presented for different farm sizes and the goals that the farmers or hatchery managers might have for the populations that are being cultured.

Small farms

Most farmers who own small fish farms (arbitrarily defined as farms <2 ha) should not be concerned about inbreeding or genetic drift. Many of these farmers are subsistence-level farmers, and yields and profits can be increased far more if these farmers learn how to use proper fertilization techniques, to manage water quality, to use supplemental feeding, etc. Improvements in these areas of management will be a more effective use of the farmer's time and financial resources. If the farmer increases yield by using better management, his family will have more food, and he may be able to sell surplus fish.

Not all farmers who own small fish farms are subsistence-level farmers. Some might be quite sophisticated and use state-of-the-art management. Those farmers might be able to incorporate inbreeding control and/or prevention into yearly management plans. Those who could benefit from this additional type of breeding management should follow the recommendations that are outlined in the next section on medium-sized farms.

Medium-sized farms

Farmers who own medium-sized fish farms (arbitrarily defined as those that have 2 ha of ponds) range from subsistence-level to sophisticated, so a wide range of management options is available. The recommendations depend on a farmer's type of management and his long-term goals. As before, subsistence-level farmers should not worry about genetic aspects of management. They need to improve the environmental aspects first (feeding, fertilization, etc.).

Most farmers who own medium-sized fish farms should be concerned with preventing only inbreeding problems, but some will also want to prevent genetic drift-related problems. Table 8 provides guideline minimum constant N_e's for fish farmers who own medium-sized fish farms. The N_e's presented in Tables 8, 9, and 10 were compiled from values given in Tables 4 and 5 (pages 79 and 81).

Four options are presented in Table 8. The first two are for farmers who are willing to accept a high level of "genetic risk." In this case, risk means that the farmers are willing to allow inbreeding to accumulate to 10% and want to try and save alleles whose frequencies are 0.05. It can be argued that these farmers should ignore genetic drift, and most should not worry about it; however, an examination of Table 4 shows that the N_e's that are needed to prevent inbreeding from exceeding 10% are quite small for the first 10 generations ($\leq$50), so the addition of a few extra brood fish to prevent mild levels of genetic drift is easily accomplished. In this case, the recommendation is to have either a 95% or 99% guarantee of keeping alleles whose frequencies are 0.05. The 0.05 frequency level was chosen because it is an order of magnitude more difficult to save alleles rarer than f = 0.05 (Table 5).

It is also likely that many alleles that are rarer than f = 0.05 are lost due to domestication selection. If rare alleles are valuable (they increase survival, make fish less excitable, improve reproductive success, or improve other phenotypes that are important at fish farms), domestication selection will increase the frequencies dramatically. Additionally, unless the farmer is using state-of-the-art management, improvements in the environmental aspects of management will have a far greater impact on yields and profits than improvements in the genetic aspects of management.

The recommended minimum N_e's for this type of farmer and this level of genetic risk range from 30 to 100 (Table 8), depending on the number of generations that the farmer wants to incorporate into his plan. Since most farmers will at most plan ahead for only a few generations, the recommended N_e's that are needed by most farmers are quite small; in many cases N_e will be $\leq$50. If a farmer only wants to prevent inbreeding from reaching certain levels, the N_e's listed in Table 4 can be used.

Table 8. Minimum constant effective breeding numbers (N_e's) needed to prevent inbreeding- and genetic drift-related problems on medium-sized fish farms. Minimum N_e's are presented for four options: Two are for farmers who are willing to accept a higher level of risk-who want to keep inbreeding from exceeding 10% and want either a 95% or 99% guarantee of keeping alleles whose frequencies are 0.05. The other two options are for farmers who want less risk-who want to keep inbreeding from exceeding 5% and want either a 99% guarantee of keeping alleles whose f = 0.05 or a 95% guarantee of keeping alleles whose f = 0.01.

No. generations	Keep F≤10%; 95% guarantee for alleles f = 0.05	Keep F≤10%; 99% guarantee for alleles f = 0.05	Keep F ≤5%; 99% guarantee for alleles f = 0.05	Keep F ≤5%; 95% guarantee for alleles f = 0.01
1	30	45	45	150
2	36	52	52	183
3	40	56	56	203
4	43	59	59	218
5	45	61	61	229
6	47	63	63	238
7	48	64	70	245
8	50	66	80	252
9	51	67	90	258
10	52	68	100	263
15	75	75	150	283
20	100	100	200	297

More sophisticated farmers will want to accept less genetic risk, which is defined as preventing inbreeding from exceeding 5% and producing either a 99% guarantee of keeping alleles whose frequencies are 0.05 or a 95% guarantee of keeping alleles whose frequencies are 0.01. Farmers who are using intensive management and who want to have relatively few genetic problems should try and save alleles whose frequencies are 0.01, because this will virtually guarantee that alleles that are more common than 0.01 will be saved. The recommended minimum N_e's that are needed for this level of genetic risk range from 45 to 297, depending on the number of generations that are incorporated into the management plan. An N_e of 61 will prevent inbreeding from exceeding 5% and will produce a 99% guarantee of keeping alleles whose frequencies are 0.05 for five generations, while an N_e of 229 will prevent inbreeding from exceeding 5% and will produce a 95% guarantee of keeping alleles whose frequencies are 0.01 for five generations.

Many farmers will be able to incorporate these recommended N_e's into their management plans without incurring additional expense, because a 2-ha farm requires dozens to hundreds of brood fish in order to produce enough fingerlings for stocking. For example, a tilapia farmer will need 50 to >200 brood fish in order to stock his grow-out ponds.

Large farms

Farmers who own large fish farms (arbitrarily defined as those that have >2 ha of ponds) generally use intensive levels of management because such farms produce fish for local, regional, and national markets. Some farmers will use low levels of technology, but by and large, such farmers will be more likely to use state-of-the-art management.

Farmers who use extensive management can accept a higher level of risk (less genetic management) than those who use more intensive management. Table 9 provides recommended N_e's for large farms. The higher-level risk option means preventing inbreeding from exceeding 10% and providing 95% and 99% guarantees of keeping alleles whose frequencies are 0.05. Recommended N_e's range from 30 to 250, depending on the number of generations that will be incorporated into the work plan.

Table 9. Minimum constant effective breeding numbers (N_e's) needed to prevent inbreeding- and genetic drift-related problems on large fish farms. Minimum N_e's are presented for four options: Two are for farmers who are willing to accept a higher level of risk-who want to keep inbreeding from exceeding 10% and want either a 95% guarantee of keeping alleles whose f = 0.05 or a 99% guarantee of keeping alleles whose f = 0.05. The other two options are for farmers who want less risk-who want to keep inbreeding from exceeding 5% and want either a 95% guarantee of keeping alleles whose f = 0.01 or a 99% guarantee of keeping alleles whose f = 0.01.

No. generations	Keep F≤10%; 95% guarantee for alleles f = 0.05	Keep F≤10%; 99% guarantee for alleles f = 0.05	Keep F≤5%; 95% guarantee for alleles f = 0.01	Keep F≤5%; 99% guarantee for alleles f = 0.01
1	30	45	150	230
2	36	52	183	264
3	40	56	203	284
4	43	59	218	298
5	45	61	229	309
6	47	63	238	319
7	48	64	245	326
8	50	66	252	333
9	51	67	258	339
10	52	68	263	344
15	75	75	283	364
20	100	100	297	378
25	125	125	308	390
30	150	150	318	399
35	175	175	350	406
40	200	200	400	413
45	225	225	450	450
50	250	250	500	500

Farmers who use more intensive levels of management to produce food or who are fingerling producers should accept less risk (more genetic management). In this case, the farmer should try and prevent inbreeding from exceeding 5% and should try and produce 95% or 99% guarantees of keeping alleles whose frequencies are 0.01. Recommended constant N_e's for this option range from 150 to 500, depending on the number of generations that are incorporated into the work plan.

As was the case with medium-sized farms, most farmers who own or manage large farms should be able to incorporate these recommendations into hatchery management plans. For example, the N_e's needed for the higher risk option for five generations are 45 and 61, while those for the lesser risk option are 229 and 309. A constant N_e of 344 will keep inbreeding from exceeding 5% and will produce a guarantee of keeping alleles whose frequencies are 0.01 for 10 generations. It should be relatively easy to incorporate this type of genetic management into yearly work plans on large farms, because the N_e's listed in Table 9 should be no larger than those that most farmers use to achieve fingerling production quotas.

Public hatcheries

Hatchery managers who produce fish that will be stocked in lakes and rivers in order to restore damaged fisheries have an entirely different management goal. The goal in this type of aquaculture is to restore an ailing fisheries by stocking a self-reproducing population. Consequently, the genetic goal is to maintain as much genetic diversity as possible. The hatchery manager does not want to alter phenotypic or genotypic frequencies by selection, domestication, inbreeding, or genetic drift.

Table 10. Minimum constant effective breeding numbers (N_e's) needed to prevent inbreeding- and genetic drift-related problems in hatchery stocks that are used for fisheries management projects to restock lakes and rivers. Minimum N_e's are presented for four options: moderate risk, which is to keep inbreeding from exceeding 5% and to produce a 99% guarantee of keeping alleles whose f = 0.01; low risk, which is to keep inbreeding from exceeding 5% and to produce a 99% guarantee of keeping alleles whose f = 0.005; little risk, which is to keep inbreeding from exceeding 1% and to produce a 99% guarantee of keeping alleles whose f = 0.005; and no risk, which is to keep inbreeding from exceeding 1% and to produce a 99% guarantee of keeping alleles whose f = 0.001.

No. generations	Keep F≤5%; 99% guarantee for alleles f = 0.01	Keep F≤5%; 99% guarantee for alleles f = 0.005	Keep F≤1%; 99% guarantee for alleles f = 0.005	Keep F≤1%; 99% guarantee for alleles f = 0.001
10	344	689	689	3450
15	364	730	730	3653
20	378	758	1000	3797
25	390	780	1250	3908
30	399	799	1500	3999
35	406	814	1750	4076
40	413	827	2000	4143
45	450	839	2250	4202
50	500	850	2500	4255
55	550	859	2750	4302
60	600	868	3000	4346
65	650	876	3250	4386
70	700	883	3500	4423
75	750	890	3750	4457
80	800	896	4000	4489
85	850	903	4250	4520
90	900	908	4500	4548
95	950	950	4750	4750
100	1000	1000	5000	5000

The reason why the genetic goal is to maintain as much genetic diversity as possible and not alter it during the fish culture phase of the operation is that no one knows what alleles or allelic combinations are important for survival in the wild. Also, no one can predict which alleles will become important in the future. Environments change, and it is difficult to predict what they will be like in the future and what alleles will be important.

This means the genetic goal is to prevent or minimize inbreeding and genetic drift. Table 10 contains recommended N_e's for four management options. The management options presented in Table 10 go from the 10th to the 100th generation. The reason why no options are given for generations 1-10 is because this type of management requires long-term planning. If short-term options are unavailable, hatchery managers and fishery department officials are not tempted to take shortcuts and go for short-term benefits at the expense of the long-term success of the programme. The number of generations that should be incorporated into the programme will depend on the generation interval. Fish with generation intervals ≤1 year will require more than those with generation intervals of 3-4 years, simply because the smaller the generation interval, the more quickly inbreeding can accumulate during a specific time period. For example, if you want to manage a population for 12 years, a species with a 4-year generation interval requires only three generations of management; a species with a 1-year generation interval requires 12 generations of management. A minimum of 10 years should be incorporated into the work plan, but if possible, 25 generations should be considered a minimum. Conservation biology (stocking to restore a fisheries is a type of conservation biology) requires long-term planning if it is to succeed.

In this type of aquaculture, the definitions of risk are different than they are for fish farming. Here, moderate and low risk means preventing inbreeding from exceeding 5% and trying to produce 99% guarantees of keeping alleles whose frequencies are either 0.01 (moderate risk) or 0.005 (low risk), respectively. Effective breeding numbers for these options range from 344 to 1,000, depending on the number of generations.

A third option is to accept little risk, which means preventing inbreeding from exceeding 1% and producing a 99% guarantee of keeping alleles whose frequencies are 0.005. Effective breeding numbers for this option range from 689 to 5,000, depending on the number of generations.

The best option, in terms of genetic conservation, is to accept no genetic risk-to prevent inbreeding from exceeding 1% and to produce a 99% guarantee of keeping alleles whose frequencies are 0.001. This option requires N_e's that range from 3,450 to 5,000, depending on the number of generations.

Obviously, there will be a compromise between what is best, in terms of genetic conservation (the no risk option), and what is practical, in terms of the budget and available manpower. Effective breeding numbers needed for the first two options are not unreasonably large. For example, N_e's needed to achieve the genetic goals for 25 and 50 generations are 390 and 500, respectively, for the moderate risk option and 780 and 850, respectively, for the low risk option. Although these options are not as effective as the others, both should produce populations that have minimal genetic damage, although the effects of 5% inbreeding on survival in the wild are not known. One study found that inbreeding in Atlantic salmon reduced the return rate of stocked fish.

The third option (little risk) is an improvement on the second (moderate risk), in that inbreeding will never exceed 1%. Effective breeding numbers needed to achieve the genetic goals for 25 and 50 generations are 1,250 and 2,500, respectively. While the N_e's required for this level of genetic risk might be larger than the number of brood fish that can be maintained or spawned at some hatcheries, such N_e's are realistic for many hatcheries that routinely spawn several thousand fish.

The no risk option will prevent inbreeding from exceeding 1% and will produce a virtual guarantee of keeping polymorphic genes in the polymorphic state. Effective breeding numbers needed to produce the genetic goals for this management option are 3,908 for 25 generations and 4,255 for 50 generations. Most hatcheries will not be able to use this option, simply because they will not be able to hold or spawn this many fish. However, if this option can be used, it will produce a population of fish that has a better chance of surviving to become a self-reproducing population. If this occurs, the hatchery aspect of the project will have succeeded and can be ended.

An option that can be used to prevent hatchery-induced inbreeding or genetic drift problems is to wild-source new brood fish every two to three generations. This option is not always available, especially if the target lake or river has been damaged by pollution or by over-fishing; also, the option is expensive and requires additional skilled labour. But if this option is incorporated into the work plan, it might be a way to prevent domestication selection from altering the gene pool, and it is a way of preventing inbreeding and can minimize genetic drift. Even if the population cannot be replaced every few years, the same effect might be achieved if 10-25% of the brood fish are replaced on a yearly basis.

Some aspects of management might have to be altered in order to achieve the desired N_e. The sex ratio should be 1:1 or as close to that as possible. If possible, more matings should be made than are needed, and only an equal portion of each egg mass should be used. If gametes are stripped, eggs and sperm should not be pooled before fertilization. After fertilization, families should be raised in individual tanks so that mortalities can be determined and the effects on N_e can be quantified. If each brood fish is allowed to spawn only once and if it mates only with one other fish, N_e will be easy to determine.

In addition, the hatchery manager must be able to practice what is called "no selection." No selection is the absence of any type of selection that will alter the population's gene pool. Matings should be random, and no fish should be chosen or excluded for any reason. This means fish must be spawned over the entire spawning season, not when it is convenient for the hatchery manager. Fish that mature at the beginning and at the end of the season must also be spawned. The number of matings that are made during a week should be determined

by the percentage of fish that ripen during that period. There is a tendency to spawn only those fish that are ready during the middle of the spawning season, because it is an efficient use of labour. However, this is a form of selection, and it will lower N_e and will change gene frequencies. Studies have shown that spawning time can be radically altered by selective breeding.

If possible, fish should be tagged so that pedigrees can be maintained. This will enable hatchery managers to determine N_e with greater confidence. New tagging techniques have been developed that are easy, reliable, and inexpensive.

Finally, there must be some way to assess the effect of the management programme on inbreeding and genetic drift. Effective breeding number can be determined, and this will enable hatchery managers to predict what the effects on inbreeding and genetic drift will be; however, this is not empirical proof that the management programme has produced the desired results. Studies have found that genetic drift occurs despite efforts to prevent it.

The effects of the management programme on the population's gene pool can be determined by using electrophoresis. Electrophoresis is a biochemical technique that enables geneticists to determine protein phenotypes and their genotypes. This technology has become the most important genetic tool for population biologists, because it enables them to study a population's genes over time and space.

Electrophoresis is a technique that examines proteins that are taken from eye, liver, heart, blood, or muscle. The tissue is ground and turned into a liquid. Small amounts of the liquid are then inserted into sheets of starch or other material called "gels." An electric current is run through the gels, and this causes the proteins to migrate through the gel. The distance the proteins move is determined by their size and chemical composition. After a predetermined time, the electric current is stopped, and the gel is stained to reveal specific protein phenotypes and their genotypes. The way protein phenotypes and genotypes are determined is illustrated in Figure 34.

These evaluations should be done with what are called "neutral" genes (genes where different protein phenotypes are of equal value; i.e., both work equally well, and neither phenotype provides a fish with an increase in fitness). If non-neutral genes are used, the effects of selection could be confounded with inbreeding or genetic drift.

If this is done every generation, gene frequencies can be determined, and the frequencies over time can be compared. This will provide a good estimate of genetic drift. Additionally, this technique can be used to determine inbreeding. The percentage of homozygotes can be determined each generation. Since inbreeding increases homozygosity, any increase that is measured will approximate the amount of inbreeding.

If the population is examined electrophoretically every generation, information on gene frequencies and linkage disequilibrium can be used to assess the population's N_e, which will provide a good estimate of inbreeding. Effective breeding numbers that are determined using this information provide valuable information about the population, because N_e is often smaller than that which is determined by counting the fish that spawn. If N_e is determined indirectly from electrophoretic data, differential reproduction and survival are taken into account, and this provides a more accurate estimate of N_e.

The only drawback to electrophoresis is the cost. A well-equipped lab costs up to US$100,000, and it can cost several dollars to examine each fish. In addition, these tests require highly trained personnel. Many universities and research businesses have electrophoresis labs and market these services, which means a hatchery does not need to have its own genetics lab in order to examine its population electrophoretically. Even though these tests can be expensive, if the prevention of inbreeding and the problems associated with genetic drift are a crucial aspect of brood stock management, this technique is a valuable tool that will reveal whether the management programme is succeeding. And it is the only way to quantify the effects of management on the population's gene pool.

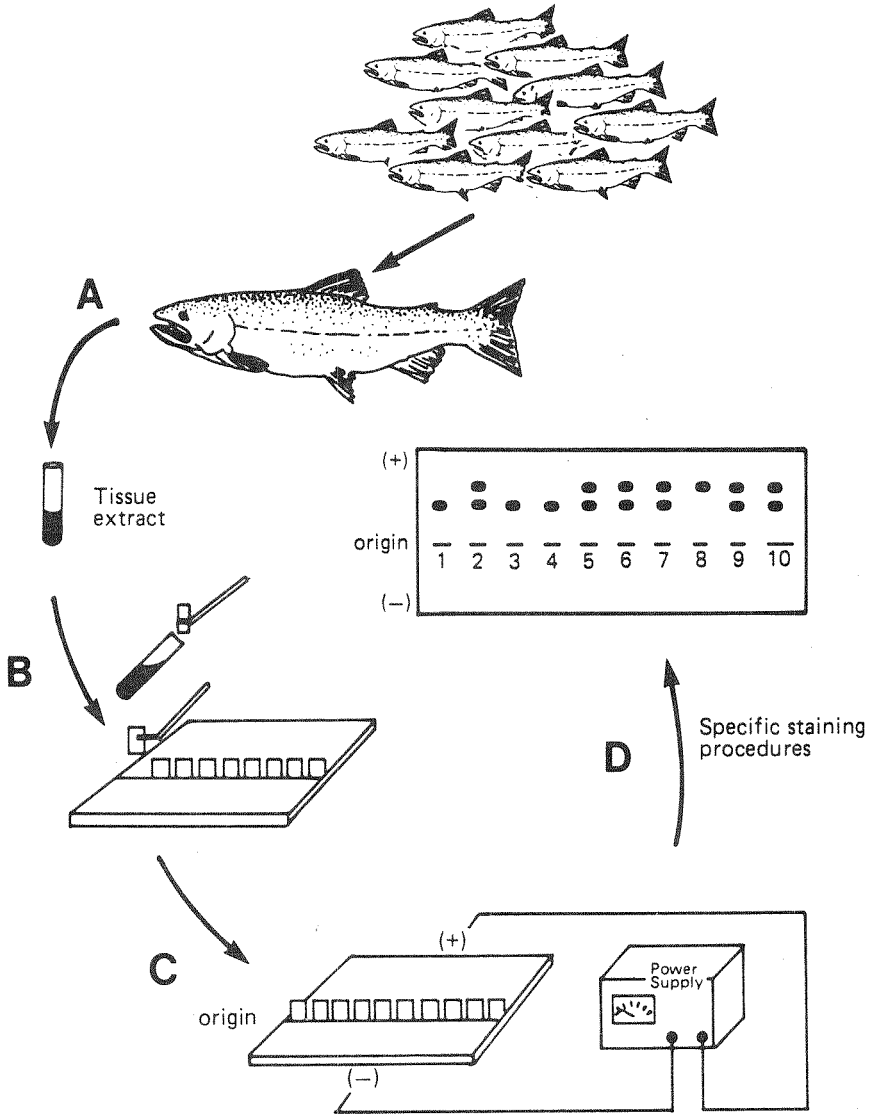


Figure 34. Schematic diagram of how protein phenotypes and genotypes are determined electrophoretically. Protein from various tissues are taken from fish (A), and extracts are placed in gels (B). Electricity is run though the gels (C) to cause the protein to migrate. The gels are stained to reveal the protein phenotypes (D). The stained gel in this figure reveals the phenotypes for 10 fish. The phenotypes are the bands that are revealed by the stain. In this case, there are three possible phenotypes. Heterozygotes have two bands and the two homozygotes each have a single band; consequently, the gene that produces these phenotypes has two alleles, and each allele produces a band. The frequency of each allele can be determined by adding the number of alleles that are revealed by the phenotypes (bands) and dividing that by the total number of alleles. If only one band is present, it is counted twice, because that fish is a homozygote (two copies of the same allele). Since there are 10 fish, there are 20 total alleles. If the top allele is the D allele and the bottom is the D' allele, the frequency of the D allele is 8/20 or 0.4, and the frequency of the D' allele is 12/20 or 0.6.

Source: Utter, F., P. Aebersold, and G. Winans. 1988. Interpreting genetic variation detected by electrophoresis. Pages 21-45 in N. Ryman and F. Utter, eds. Population Genetics & Fishery Management. Washington Sea Grant Program, University of Washington Press, Seattle, Washington, USA. Reprinted by permission from Washington Sea Grant Program.

Customizing recommendations

The recommended N_e's presented in Tables 8, 9, and 10 were created from a combination of published information and educated guesswork. The effects that these N_e's have on inbreeding and genetic drift in hatchery populations have not been tested empirically. It is unlikely that they ever will be evaluated, because such studies would be difficult and expensive; furthermore, such studies would take many generations-in some cases, >25 generations-simply to determine if a population behaved as expected.

Because of this and because N_e's must be customized for each farmer or hatchery manager and for each farm or hatchery, many fish culturists will want to create their own set of recommendations and determine a constant N_e that best suits their needs. This can be accomplished by using the N_e's presented in Tables 4 and 5 (pages 79 and 81). Fish culturists who only want to prevent inbreeding depression can use the N_e's in Table 4, while those who wish to prevent both inbreeding depression and the ravages of genetic drift need to use both tables.

The information in Tables 4 and 5 can be used in two ways: One, a farmer or hatchery manager can use the tables to determine the constant N_e that is needed to keep inbreeding from exceeding a predetermined level and which is needed to produce a certain guarantee of keeping alleles of a given frequency for a predetermined number of generations. Two, a farmer or hatchery manager can use the tables to determine how much inbreeding and what guarantee of keeping alleles a specific constant N_e will produce over various numbers of generations.

For example, if a farmer decides he wants to keep inbreeding from exceeding 6% and he wants to produce a 95% guarantee of keeping alleles whose frequencies are 0.05 for eight generations, he will find that the N_e from Table 4 is 67, while that from Table 5 is 50. Since he wants to achieve both goals, he needs to use the larger N_e, which means he needs a constant N_e of 67 to achieve his goals.

Conversely, if a hatchery manager knows that he can maintain a constant N_e of 1,250 but would have difficulty in maintaining one larger than that, he can can use Tables 4 and 5 to determine how effective this N_e would be in minimizing inbreeding and the effects of genetic drift. Table 4 reveals that a constant N_e of 1,250 will produce: F = 1% at generation 25; F = 2% at generation 50; F = 3% at generation 75; F = 4% at generation 100. Table 5 shows that this N_e will produce a >99% guarantee of keeping alleles whose frequencies are 0.005 for 100 generations. This information reveals that a constant N_e of 1,250 will do an excellent job of preventing inbreeding and genetic drift from adversely affecting the population, provided bottlenecks have not already crippled it genetically.

The ability to customize N_e's is important. If a farmer or hatchery manager is given a set of guidelines and is told that he must maintain an N_e which he feels is impossible, impractical, or prohibitively expensive, he will simply ignore the advice and feel that the genetic aspects of brood stock management are more trouble than they are worth. On the other hand, if a farmer or hatchery manager can customize his N_e and knows what that N_e can accomplish genetically, he is more likely to incorporate genetics into brood stock management.

WHEN SELECTIVE BREEDING PROGRAMS WILL BE CONDUCTED

Selection and prevention of inbreeding and genetic drift are, for practical purposes, incompatible genetic goals. The way to minimize inbreeding is to maintain a large N_e or to prevent consanguineous matings. Selection is a breeding programme that reduces the population's N_e by culling (eliminating) fish that fall below a cut-off value (minimal acceptable phenotypic value) and that allows only select brood fish (those whose phenotype[s] meet or exceed the cut-off value) to mate. When you mate the best with the best, you often mate relatives, which produces inbreeding. This has led some to call selection a form of directed inbreeding. All populations that have been improved by selection are inbred to a degree; some are extremely inbred.

Even though some inbreeding is inevitable, there are some techniques that can be used to moderate inbreeding so that it does not counteract selection. There is no point in conducting a selective breeding programme simply to offset inbreeding depression. The idea is to minimize inbreeding-but not at the expense of the selective breeding programme.

Genetic drift will also occur during a selective breeding programme. Selection and the conservation of genetic variance are opposite types of genetic management. Selection is designed and conducted to change gene frequencies and eliminate many alleles-that is its purpose. The goal is to change the frequencies of the alleles at the loci that control the phenotypes that are under selection. But selection will also change other gene frequencies because of linkage groups (groups of genes are "linked" when they are located on the same chromosome) and because of indirect selection (selection for one phenotype that affects another; e.g., selection for length also selects for weight and food conversion). Genetic drift is not the concern that it is when no selection is being conducted, because the major effects of genetic drift will be on genes that are not affected by the selective breeding programme. Consequently, farmers and hatchery managers should not be overly concerned about genetic drift when they conduct a selective breeding programme.

Individual selection

There are two basic types of selection. The simplest is individual (also called "mass") selection, where selection is based on individual phenotypic values. Fish that meet or exceed the cut-off value are saved, and the others are culled. Family relationships are totally ignored; an individual is selected or culled based on its own merit.

Inbreeding is inevitable when conducting individual selection. Because family relationships are ignored during the selection process, a majority of the select brood fish can come from only a few families, particularly if one or two matings produce outstanding offspring. When this occurs, the population goes through a severe bottleneck which generates inbreeding. Figure 35 shows how a bottleneck can be produced during individual selection and why inbreeding occurs.

Inbreeding is also produced because family relationships are usually ignored during the mating process when select brood fish are spawned. This is inevitable if fish are not marked. If family relationships are ignored when matings are made, relatives will be mated; among these matings will be brother-sister and half-sib matings, which produce considerable inbreeding.

Even though inbreeding cannot be prevented when conducting individual selection and when the fish are not marked, the rate of inbreeding might be minimized by maintaining N_e at a predetermined size. The N_e's in Table 4 can be used as guidelines; however, inbreeding will be greater than that indicated in Table 4. The N_e's in Table 4 are those that will keep inbreeding from exceeding undesired levels when there is no selection and when random mating occurs. When a selective breeding programme is being conducted, matings are not random; they are what is called "assortative matings" (the best are mated with the best). Additionally, each act of selection creates a mini-bottleneck, which can retroactively lower the N_e of the previous generation.

One way to prevent or reduce inbreeding is to mark fish and avoid consanguineous matings when select brood fish mate. This might decrease the genetic gain, but it will prevent inbreeding. If this mating programme is used, the population must be sufficiently large so that adequate numbers of select brood fish can be spawned without consanguineous matings. If relatives are not allowed to mate, the number of potential matings that can be made among the select brood fish will decrease dramatically.

After a few generations, it might not be possible to prevent consanguineous matings, but if brother-sister matings or half-sib matings can be avoided, large accumulations of inbreeding can be prevented. If only cousins are allowed to mate, relatively little inbreeding will accumulate in the select population. Five generations of first cousin matings will produce less inbreeding than a single generation of brother-sister matings. If only second cousins are allowed to mate, inbreeding will never exceed 2%.

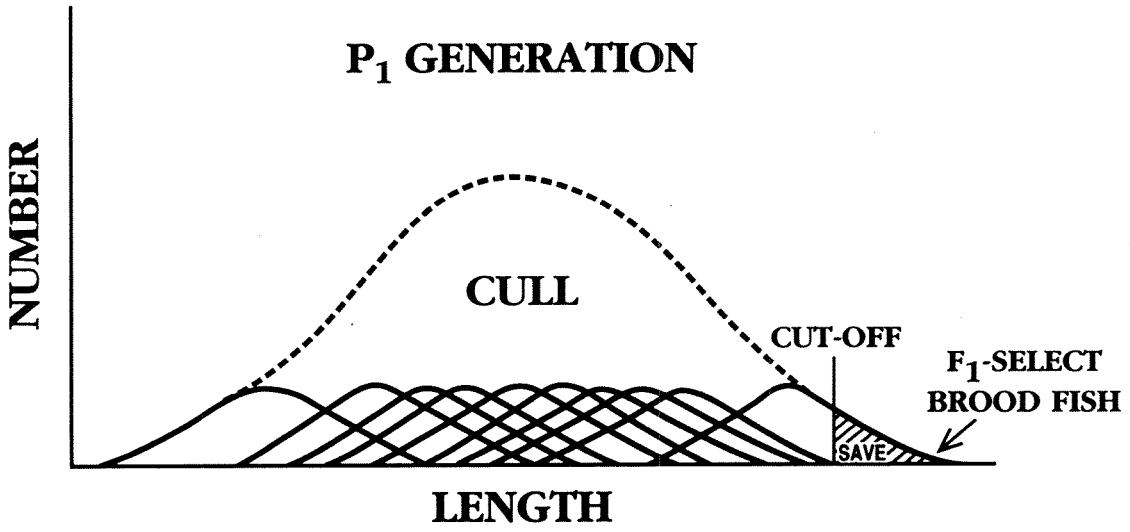


Figure 35. This graph shows why inbreeding occurs during individual selection. The size distribution of a population and the size distributions of the 10 families that make up the population are shown. The placement of the cut-off value produced a population of select brood fish in which every individual came from a single family. The selection process retroactively lowered the N_e of the P_1 generation to 2. When the F_1-select brood fish are spawned, all fish in the F_1-select generation will have F = 25%. This example is a bit extreme, but it illustrates why inbreeding is inevitable when individual selection is conducted.

After: Newkirk, G.F. 1979. A discussion of possible sources of inbreeding in hatchery stock and associated problems. Proceedings of the World Mariculture Society 10:93-100.

A second approach that can be used to moderate inbreeding is to divide the population undergoing selection into cohorts (sub-populations). Dividing a population into cohorts is a breeding technique that can be used to improve the efficiency of selection. For example, a population can be divided into cohorts based on spawning date to neutralize the effect of age (number of growing days and water temperature during those days) on size; this prevents these environmental sources of variance for growth from becoming confounded with heritable differences during selection. This approach can also be used to minimize inbreeding.

If a population is divided into a number of cohorts, selection is conducted independently in each cohort, and select brood fish from each cohort are mixed before they are spawned. By conducting selection in this manner, the number of parents that produce select brood fish will increase, which reduces the inbreeding that will be produced. No further selection is allowed after select brood fish are mixed, because a second round of selection could eliminate one or more cohorts, which would negate some of the effort that has been expended, and it would also increase the rate of inbreeding.

If select brood fish from each cohort are marked or are held in separate ponds or tanks until they can be spawned, a breeding programme called "rotational mating" can be used to prevent inbreeding for a number of generations (Figure 36). The number of inbreeding-free generations of selection that can be produced depends on two factors: the number of cohorts and how cohorts are produced after the first generation of selection.

During the first generation of rotational mating, F_1-select brood fish are mated as follows: females from cohort 1 are mated to males from cohort 2, females from cohort 2 are mated to males from cohort 3, etc. Each mating combination is used to produce an F_1-select generation cohort. A second generation of selection occurs in each F_1-generation cohort, and when F_2-select brood fish are mated, the mating pattern is staggered: females from the first cohort are mated to males from the third cohort, etc. Each mating combination is used to produce an F_2-generation cohort. A third generation of selection occurs in each F_2-generation cohort, and when F_3-select brood fish are mated, the mating pattern is staggered: females from the first cohort and mated to males from the fifth cohort, etc. The number of cohorts needed to prevent inbreeding after the first generation of selection doubles for each additional generation:

Number of cohorts	Select generation when inbreeding will be produced. Select brood fish that produced these fish will have F = 0%
2	F_2
4	F_3
8	F_4
16	F_5
32	F_6

If mating combinations cannot be used to produce the cohorts as described above as and as illustrated in Figure 36 after the first generation of selection, new cohorts must be created from a pooled population of select brood fish every generation. If this approach is used, rotational mating will prevent inbreeding in the F_1-select generation, but it will only minimize it thereafter. After the first generation of selection, rotational mating can be used to prevent brother-sister matings, which will slow the rate at which inbreeding is produced.

A major liability of this method of preventing or minimizing inbreeding is the cost. It is more expensive to produce and maintain a number of cohorts over successive generations than to conduct selection in a population as a whole, and the cost of the programme escalates as the number of cohorts increases.

A second liability is the fact that if the cohorts aren't needed to control a major source of V_E (e.g., spawning date) and to prevent it from being confounded with V_A during selection, dividing a population into cohorts will decrease response to selection. Response to selection is greater in a single large population than in one of equal size that is divided into subpopulations.

The use of cohorts prevents inbreeding for a limited number of generations. Eventually, inbreeding will be produced. Once inbreeding is produced it usually accumulates rather rapidly; eventually the level of inbreeding will approach that which would have occurred without the use of cohorts. However, the use of cohorts can be quite useful in preventing inbreeding for four to six generations and in minimizing inbreeding for several generations thereafter.

If the production of inbreeding-free fish for grow-out is a top priority, crossbreeding can be combined with selection to produce fish with no inbreeding generation after generation. In this breeding programme, selection is conducted simultaneously in two strains that were previously shown to produce outstanding F_1 hybrids; selection in the two strains can be for either the same phenotype or for different traits. After the first generation of selection, some of the select brood fish are hybridized to produce F_1 hybrids for grow-out or for sale to other farmers. The F_1 hybrids will have F = 0%, since the parents are not related. Other select brood fish from both strains undergo a second generation of selection. This process is continued every generation (Figure 37). All F_1 hybrids are sold, and none is allowed to reproduce. If all F_1 hybrids are sold and if the two strains are kept genetically isolated from each other, F_1 hybrids with F = 0% can be produced generation after generation.

INDIVIDUAL SELECTION WITH 8 COHORTS AND ROTATIONAL MATING

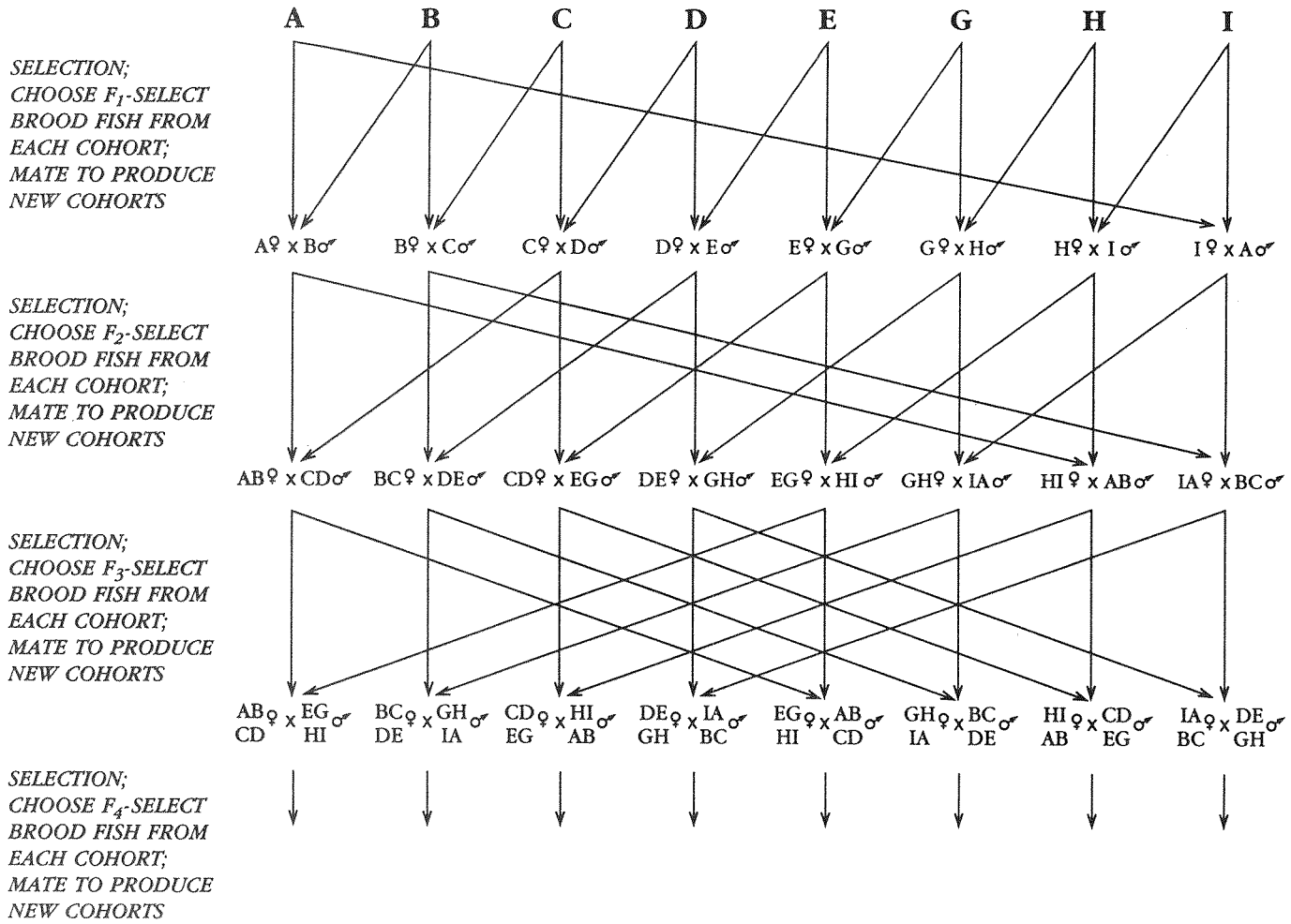


Figure 36. Schematic diagram of rotational crossing, a mating programme that can be used to prevent or minimize inbreeding during individual selection when the population is broken into cohorts. In this figure, there are eight cohorts (A-I). After the first generation of selection, F_1-select brood fish from each cohort are mated as follows: Females from cohort A are mated to males from cohort B; females from cohort B are mated to males from cohort C, etc. Each of these mating combinations forms a new cohort. A second generation of selection occurs, and F_2-select brood fish are mated as follows: females from the first cohort (A♀ X B♂) are mated to males from the third cohort (C♀ X D♂), etc. After the third generation of selection, F_3-select brood fish are mated as follows: females from the first cohort (AB♀ X CD♂) are mated to males from the fifth cohort (EG♀ X HI♂), etc. The rotational mating programme outlined in this figure for eight cohorts produced no inbreeding during this selective breeding programme. Inbreeding will occur when the F_4-select brood fish, which have no inbreeding, spawn and produce the F_4-select generation. Once inbreeding cannot be prevented, it can be minimized if pedigrees are followed, full-sib matings are prevented, and half-sib matings are minimized.

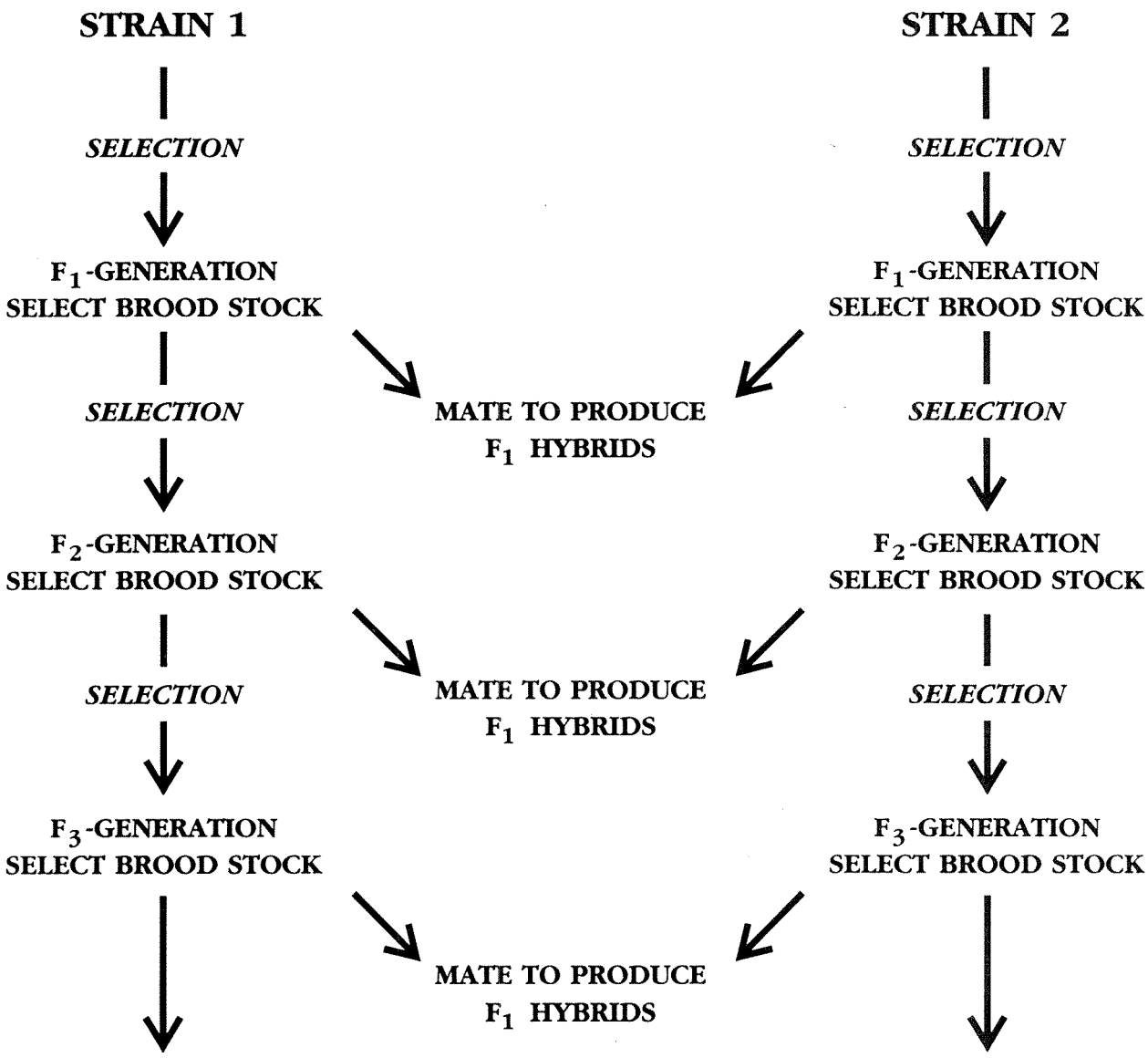


Figure 37. Schematic diagram of how hybridization can be combined with selection to produce fish that have F = 0% for grow-out. The F_1 hybrids that are continually produced in this breeding programme will always have F = 0% if the hybrids are not allowed to mate and if the two strains are kept genetically pure. This breeding programme enables a breeder to combine improvements from selection and hybridization. Since F_1 hybrids cannot breed true, this type of breeding programme is used to prevent farmers from using the animals or seed for brood stock, and it also ensures that customers must purchase new animals or seed every year.

By combining selection and hybridization, a farmer can produce fish that are genetically superior (as a result of selection) and that exhibit hybrid vigour (as a result of crossbreeding), and as a bonus, he can advertise the fact that the fish have no inbreeding. This combined breeding programme also enables a breeder to protect his investment. By selling hybrid fingerlings, a fingerling farmer sells fish that will not breed true. Selling hybrids to farmers is a traditional way breeding companies protect their investment-farmers have to buy new seed or animals every year.

This type of breeding programme does not guarantee that inbreeding will not cause problems. Inbreeding will occur in both strains. If inbreeding reaches certain levels, it could effect fecundity, which will make the production of hybrid fingerlings more difficult and expensive. One way to prevent inbreeding-induced decreases in fecundity from affecting fingerling production is to select simultaneously in four strains and produce di-hybrids; the breeding programme needed to do this is similar to that illustrated in Figure 22 (page 55). This type of breeding programme is twice as expensive as that needed to produce F_1 hybrids, but this is how seed companies circumvent the problem of lowered fecundity in their select but highly inbred lines of corn (maize) and other plants.

It is often difficult to determine inbreeding in a select population when the fish are not marked. When it is difficult to measure inbreeding, the select population can be examined electrophoretically each generation, and the results can be compared to those which existed before selection and to those from a contemporary control population, in order to determine how much inbreeding has accumulated. This will not help prevent inbreeding, but it will enable a farmer or hatchery manager to measure it.

If inbreeding accumulates to levels that cause significant inbreeding depression, selection can be relaxed for a generation by mating the select fish to fish from another strain. This will temporarily reduce inbreeding to 0%. If the farmer imports only superior fish, this might have little effect on the phenotypes that are under selection.

Family selection

The second basic type of selection is family selection. There are two types of family selection: between-family selection, where whole families are selected or culled based on family means; within-family selection, where the best fish from each of a number of families are saved. Inbreeding is also inevitable with this type of selection. When between-family selection is used, relatively few families are saved. This retroactively lowers N_e of the previous generation to a very small number. If select brood fish are not marked and are mated at random, then because the select brood fish came from relatively few families, relatives will mate, and the inbreeding that will be produced can become quite large.

The easiest way to prevent inbreeding during between-family selection is to mark the select brood fish and to prevent consanguineous matings. This approach can prevent inbreeding for a number of generations and can minimize it thereafter by preventing brother-sister or half-sib matings.

When the fish cannot be marked, the only way to prevent inbreeding or to minimize it during between-family selection is to treat each of the select families as if it were a cohort and use rotational mating to produce successive generations. After the first generation of selection, between-family selection will choose the best families from each "cohort." The rotational mating program that is used is similar to that illustrated in Figure 36.

When within-family selection is used, if fish can be marked, it is fairly easy to prevent inbreeding for many generations. This type of selective breeding program can maintain N_e at a constant or at an increasing level. Because this type of selection does not put the population though a bottleneck every generation, it will be possible to prevent consanguineous matings for many generations, as long as a sufficient number of families are entered into this selective breeding programme.

Another way to prevent or minimize inbreeding is to use rotational mating. The mating programme that would be used is similar to that illustrated in Figure 36.

As was the case with individual selection, fish with no inbreeding can be produced for grow-out by hybridizing select brood fish from two select strains.

CONCLUSION

No universal prescription can be written for every farmer and hatchery manager that will cure or prevent problems associated with unwanted inbreeding or the effects of genetic drift. Recommendations must be customized, and depend on: the size of the farm; the goals the farmer or hatchery manager has; his level of expertise; the type of production management that he uses to raise fish; whether the fish can be marked; the presence or absence of a selective breeding programme; and the number of generations that are to be incorporated in the work plan before genetic trouble occurs.

Subsistence-level farmers who own small farms (<2 ha) do not need to worry about the genetic aspects of productivity. They need to become better managers and learn how to increase yields and profits by proper feeding, fertilization, etc.

Farmers who are more technologically advanced should be encouraged to incorporate some genetic management into brood stock management plans. Most farmers are not going to conduct a selective breeding programme; when this is the case, the only genetic goals will be to conserve some genetic variance and to minimize inbreeding. Since most farmers do not mark their fish, the only way to accomplish these goals is to manage the population's N_e at a predetermined level.

Farmers who own either small farms or medium-sized farms (2 ha), who are good managers, and who raise fish under extensive conditions can manage the genetics of the population. These farmers should accept a high level of genetic risk (minimal genetic management). They should try and keep inbreeding from exceeding 10% and try and produce either 95% or 99% guarantees of keeping alleles whose frequencies are 0.05. Most of these farmers should not worry about genetic drift, but the N_e's that are needed to protect the population from the ravages of genetic drift are about the same as those needed to prevent inbreeding problems. These goals will require N_e's of between 30 and 100 per generation, depending on the number of generations in the work plan; N_e's needed for five generations are only 45 and 61, respectively, so this type of management should be easy to incorporate into yearly work plans.

Farmers who own medium-sized farms and who raise their fish under intensive conditions should try to incorporate less genetic risk (more genetic management) into their brood stock management plan. They should try and prevent inbreeding from exceeding 5% and should try and produce either a 99% guarantee of keeping alleles whose frequencies are 0.05 or a 95% guarantee of keeping alleles whose frequencies are 0.01. Effective breeding numbers needed to achieve these goals range from 45 to 297, depending on the number of generations in the work plan; N_e's needed for five generations are 61 and 229, respectively. Even though these N_e's are larger, it should be possible for many farmers to incorporate them into yearly work plans without incurring additional costs. For example, these recommendations can easily be incorporated by tilapia farmers, because they need dozens to hundreds of brood fish in order to produce the fingerlings that are needed to stock the ponds. It might be more difficult for some carp farmers, but the N_e's that are suggested are not excessively large.

Farmers who own large farms (>2 ha) have a broad range of options. Those who use extensive farming methods should accept more genetic risk than those who use intensive farming technology or those who produce fingerlings for the local fish farming industry. Those who use extensive farming methods should try and keep inbreeding from exceeding 10% and should try and produce either 95% and 99% guarantees of keeping alleles whose frequencies are 0.05. This will require N_e's that range from 30 to 250, depending on the number of generations in the work plan; N_e's needed for five generations are only 45 and 61, respectively.

Farmers who use intensive farming or those who are fingerling producers should try to prevent inbreeding from exceeding 5% and should try and produce either 95% or 99% guarantees of keeping alleles whose frequencies are 0.01. Effective breeding numbers needed to achieve these goals range from 230 to 500, depending on the number of generations in the work plan; N_e's needed for five generations are 229 and 309, respectively.

Although these goals require larger N_e's, most farmers should be able to incorporate them into brood stock management plans because of the number of brood fish needed to produce the required fingerlings for stocking or sale.

Hatchery managers who operate public hatcheries designed to produce fish that will be stocked in lakes and rivers culture populations that require considerable genetic management. In fact, managing the population's gene pool so that there are few changes should be the top management goal, and only long-term planning should be incorporated; the minimum should be 25 generations, but if this is not possible, 10 generations should be used. In this context, considerable genetic risk is defined as trying to keep inbreeding from exceeding 5% and trying to produce a 99% guarantee of keeping alleles whose frequencies are 0.01. Effective breeding numbers needed for these goals range from 344 to 1,000, depending on the number of generations in the work plan. Moderate risk is defined as trying to prevent inbreeding from exceeding 5% and trying to produce a 99% guarantee of keeping alleles whose frequencies are 0.005. These goals will require N_e's that range from 689 to 1,000, depending on the number of generations in the work plan. Little risk is defined as preventing inbreeding from exceeding 1% and trying to produce a 99% guarantee of keeping alleles whose frequencies are 0.005. These goals will require N_e's that range from 689 to 5,000, depending on the number of generations in the work plan. In terms of genetic conservation, the best management option is to accept no genetic risk-to try and prevent inbreeding from exceeding 1%, and to try and produce a 99% guarantee of keeping alleles whose frequencies are 0.001. These goals will require N_e's that range from 3,450 to 5,000.

Although the "no risk" option is most desirable in terms of genetic management, it is probably an unrealistic goal at most hatcheries. The best options are compromises between what is best (the no risk option) and what is achievable in terms of the budget, labour, and facilities. Because of this, the low risk and little risk options are those that should be incorporated; N_e's needed to achieve these goals for 25 generations are 780 and 1,250, respectively.

Even though the N_e's presented in Tables 8, 9, and 10 can be used by most farmers and hatchery managers, some will want to customize N_e for their farms or hatcheries and for their budgets and will want to use a combination of genetic goals not listed in these tables. This can be accomplished by using the N_e's presented in Table 4 and 5.

A number of publications that have produced a single recommended N_e have suggested using either 500 or 1,000. The logic behind these values is that many population geneticists feel that when N_e is 500-1,000, the population behaves as if it were infinitely large. The values presented in Tables 9 and 10 show that a constant N_e of 500 will prevent inbreeding- and genetic drift-related problems in farmed populations and that an N_e of 1,000 can do a fairly effective job of conserving genetic variance and minimizing inbreeding in populations that are used for stocking programmes. However, the values presented in Tables 8, 9, and 10 also show that: an N_e of 500 is excessively large for most farmers, while an N_e of 1,000 might be too small for some hatchery managers. Although it might be easier to conduct an extension programme by recommending a single N_e that should be maintained by all farmers, N_e's should be customized, because many farmers can maintain an N_e as small as 45. If an excessively large N_e is recommended, many farmers will simply ignore the advice.

When managing a population's N_e, the most important aspect is to maintain a constant N_e and to prevent a bottleneck. A bottleneck will reduce the N_e of the population and will make it difficult, if not impossible, to achieve genetic goals. The time when most bottlenecks occur is during acquisition of the population. It is difficult to ship large numbers of fish, so there is a tendency to ship fish that have come from a handful of spawns; furthermore, mortalities that occur during and after transportation can drastically lower N_e. If N_e goes though a bottleneck during acquisition of a population, the population may be crippled genetically, and efforts to properly manage it are of little value.

Inbreeding and genetic drift are of great concern when a farmer or hatchery manager wants to conserve the population's genetic and genotypic variance and does not want to alter the population in any way, including via selection. However, when a farmer wants to improve growth or other phenotypes by using a selective breeding programme, the problems associated with inbreeding and genetic drift become less important. Selection produces inbreeding and alters gene frequencies.

Inbreeding will increase in a select population because each act of selection creates a bottleneck and because when the best are mated, the mating of relatives often occurs. Farmers who conduct individual selection can prevent inbreeding from counteracting selection by marking fish and preventing consanguineous matings. If consanguineous matings cannot be prevented, the accumulation of inbreeding can be lessened by preventing brother-sister or half-sib matings. If consanguineous matings are restricted to fish less related than first cousins, inbreeding will rarely be a problem.

If fish cannot be marked, inbreeding will occur. Minimum N_e's can be maintained to try and prevent inbreeding, but inbreeding will occur because each act of selection is a bottleneck. Inbreeding can be prevented by breaking the population into cohorts, conducting selection independently in each cohort, and mating select brood fish from the cohorts by rotational mating.

If family selection is used, inbreeding can be prevented by marking fish and preventing consanguineous matings. If fish are not marked, rotational mating can be used to prevent or minimize inbreeding.

If inbreeding depression become significant in a select line, selection can be relaxed for a generation by mating the select brood fish with outstanding fish from an unrelated strain.

Finally, a farmer can produce fish with no inbreeding for grow-out by hybridizing two unrelated select strains; this not only produces fish with F = 0% for grow-out, but it prevents customers from using the hybrids as brood fish, because F_1 hybrids do not breed true.

GLOSSARY

Additive gene action: The type of gene action whereby each of two alleles contributes equally to the production of qualitative phenotypes; neither allele is dominant. The heterozygous genotype produces a phenotype that is intermediate between those produced by the homozygous genotypes.

Additive genetic variance (V_A): The portion of phenotypic variance for a quantitative phenotype that is due to the additive effects of all alleles across all loci. This is the heritable component of variance, and it is exploited by selection. The proportionate amount of phenotypic variance due to additive genetic variance is called "heritability."

Allele: An alternate form of a gene.

Androgen: 1) A fish that has only a male parent; all genes in an androgen come from the father. 2) Anabolic steroid hormone that causes the production of male traits.

Ancestor: A fish that appears in a previous generation in a fish's pedigree. For inbreeding purposes, it is a relative that contributed genes to the individual, either directly or via another fish.

Autosome: A chromosome that is not a sex chromosome.

Between-family selection: A selective breeding programme for quantitative phenotypes where selection occurs at the family rather than at the individual level. In this type of selective breeding programme, whole families are either culled or saved, and that decision is based on family means.

Bottleneck: A severe restriction in a population's effective breeding number.

Centromere: The constricted region of a chromosome that holds sister chromatids together.

Character: A synonym for phenotype. See phenotype.

Chromatid: One copy of a homologue that is joined to the other by the centromere. The joined pair is called "sister chromatids."

Chromosomal manipulation: Biotechnical manipulation of eggs, sperm, or zygotes by temperature or pressure shocks either to alter chromosome set number or to create fish with only a single parent. This procedure can be used to create highly inbred fish that have only a mother (gynogens) or only a father (androgens).

Chromosome: The structure on which the genes are located. Chromosomes reside in the nucleus of each cell and, in most species, they occur in pairs. There are two types of chromosomes: autosomes and sex chromosomes.

Coefficient of inbreeding (F): The measure of inbreeding. It is the probability that the two alleles at a locus are identical by descent.

Cohort: A sub-population.

Common ancestor: A fish that is an ancestor of both parents. A common ancestor appears on both sides of a fish's pedigree. Parents that share a common ancestor produce inbred offspring.

Complete dominant gene action: A type of gene action whereby one allele is expressed more strongly than the other in the production of qualitative phenotypes: the allele that is expressed more strongly is called the "dominant allele," and the other is called the "recessive allele." A gene that exhibits complete dominance produces two qualitative phenotypes: a dominant phenotype and a recessive phenotype. The dominant allele always produces the dominant phenotype and suppresses phenotypic expression by the recessive allele in the heterozygous state; consequently, homozygous dominant and heterozygous genotypes both produce the dominant phenotype. The recessive phenotype is produced only when a fish is homozygous recessive.

Consanguineous mating: A "blood related" mating; i.e., a mating between relatives. A consanguineous mating produces inbred offspring.

Control population: A population of fish where no inbreeding occurs. The inbred population is compared to this population to quantify inbreeding depression.

Crossbreeding: A breeding programme where fish from different populations or species are mated to produce hybrids. Crossbreeding is used to exploit dominance genetic variance, and it can also be used to produce offspring with no inbreeding. The terms "crossbreeding" and "hybridization" are synonymous.

Crossing over: The exchange of chromosomal segments between homologues. This occurs during meiosis.

Cull: The removal of fish from the population during selection. Culled fish are not allowed to spawn.

Cut-off value: The minimum acceptable phenotypic value during selection for a quantitative phenotype. Fish that meet or exceed the cut-off value are saved; those that fall below the cut-off value are culled.

Diploid (2N): A fish or cell where chromosomes occur in pairs. Although there are naturally occurring triploid (3N) and tetraploid (4N) species, all fish are considered to be diploids is this manual.

Di-hybrid: A hybrid produced by mating two F_1 hybrids.

Domestication: The selection process by which the farm (the culture environment and the management programme) and the farmer alter the genetic and phenotypic make-up of a population. This unplanned, non-directed process usually produces faster growing, healthier, calmer, and less aggressive animals that are easier to raise.

Dominance genetic variance (V_D): The portion of phenotypic variance for a quantitative phenotype that is due to the interaction between the two alleles at all loci. This portion of genetic variance is not heritable because it is destroyed during meiosis. It is recreated in new and in different combinations every generation at fertilization. It is exploited by crossbreeding, and helps explain inbreeding depression.

Dominant allele: An allele that is expressed more strongly than its partner allele. When the mode of gene action is complete dominance, the dominant allele completely suppresses the recessive allele in the heterozygous state. When the mode of gene action is incomplete dominance, the dominant allele only partially suppresses the recessive allele in the heterozygous state.

Dominant gene action: The type of gene action whereby one allele is expressed more strongly than the other. There are two types: complete dominant gene action and incomplete dominant gene action.

Dominant phenotype: The qualitative phenotype produced by the dominant allele.

Effective breeding efficiency (N_b): The ratio of the effective breeding number (N_e) to the breeding population (N): $N_b = N_e/N$.

Effective Breeding Number (N_e): The breeding (genetic) size of a finite population. Inbreeding and genetic drift are inversely related to N_e.

Electrophoresis: A biochemical technique that determines protein phenotypes and genotypes.

Environmental variance (V_E): The portion of phenotypic variance for a quantitative phenotype that is due to environmental factors (e.g., spawning date, age of mother, temperature).

Epistasis: A type of gene action where one gene suppresses or alters the qualitative phenotypes produced by a second gene. This interaction usually results in the production of new qualitative phenotypes.

Epistatic genetic variance (V_I): The portion of phenotypic variance for a quantitative phenotype that is due to the interactions of alleles across loci. This portion of genetic variance is difficult to exploit, and most is non-heritable as a result of the reduction division during meiosis; consequently, it is usually ignored.

Equational division: The second meiotic division. The division of the replicated homologues (sister chromatids) that exist in the secondary gametocytes into the gametes. This is final step of meiosis.

Estrogen: Anabolic steroid hormone that causes the production of female traits.

F: The symbol for coefficient of inbreeding. It is a measure of the percent increase in homozygosity that has been created by inbreeding over the population average. For each gene, it is the probability that the two alleles are identical by descent.

F_1 hybrid: A hybrid produced by mating two unrelated groups.

F_1, F_2, etc.: The names of the generations that are produced during a breeding programme. The F_1 generation is the first generation of fish produced during a breeding programme; the F_1 generation is produced by the P_1 (parental) generation. The F_2 generation is produced by the F_1 generation, etc.

Family: A group of fish that have the same mother and father.

Family selection: A selective breeding programme for quantitative phenotypes where selection occurs at the family, rather than at the individual level. Selection is based on family means rather than on individual values. There are two types of family selection: between-family selection and within-family selection.

Founder effect: The loss of genetic variance that occurs when a population is started with a small effective breeding number.

Gamete: An egg or a sperm. Gametes are haploid (N).

Gene: The basic unit of inheritance. Genes are the chemical blueprints that determine the production of phenotypes. Genes are located on chromosomes.

Generation: The length of time it takes to replace brood fish with their offspring.

Genetic drift: Random changes in gene frequency caused by sampling error (shipment of fish from one station to another; brood stock selection). The ultimate effect of genetic drift is the loss of genetic variance. Genetic drift is inversely related to effective breeding number.

Genetic-environmental interaction variance (V_{G-E}): The portion of phenotypic variance for a quantitative phenotype that is due to the interactions between the fish's genes and the environment. It is due to genes that are expressed differently in different environments.

Genetic variance (V_G): The portion of phenotypic variance for a quantitative phenotype that is due to the genes. There are three sub-components of genetic variance: additive genetic variance (V_A), dominance genetic variance (V_D), and epistatic genetic variance (V_I).

Genome: A fish's entire genetic make-up.

Genotype: The genetic make-up of a fish at the locus (or loci) that produces a specific phenotype. Fish are either homozygous or heterozygous at each locus.

Gynogen: A fish that has only a female parent; all genes in a gynogen come from the mother.

h^2: The symbol for heritability.

Haploid (N): A fish or cell that contains only one chromosome (homologue) from each chromosome pair. Gametes are haploid.

Heritability (h^2): The portion of phenotypic variance for a quantitative phenotype in a given environment that is due to additive genetic variance ($h^2 = V_A/V_P$). Heritability describes the percentage of phenotypic variance that is heritable. Phenotypes with h^2's >0.25 can be improved efficiently by individual selection; those with h^2's <0.15 are difficult to improve by individual selection, and family selection is usually used. Inbreeding can be used to improve the results of family selection when h^2 <0.15.

Heritable: Something that is transmitted from a parent to its offspring.

Heterozygote: A fish with two different alleles at a locus.

Heterozygous: The genotype that occurs when the pair of alleles at a locus are not identical. Inbreeding decreases the percentage of heterozygous loci.

Heterozygous phenotype: The qualitative phenotype produced by the heterozygous genotype. A heterozygous phenotype can be produced when the mode of gene action is incomplete dominance or additive.

Herd bull: The only male in a population. A herd bull is mated with all females.

Homologues: The two chromosomes that combine to form a chromosome pair. One homologue comes from the father, while the other comes from the mother.

Homozygote: A fish with an identical pair of alleles at a locus.

Homozygous: The genotype that occurs when the pair of alleles at a locus are identical. Inbreeding increases the percentage of homozygous loci. The coefficient of inbreeding (F) is a measure of the increase in homozygosity that has occurred because relatives mated.

Homozygous phenotype: The qualitative phenotype produced by the homozygous genotype. When the mode of gene action is complete dominance, the recessive phenotype is the homozygous phenotype. When the mode of gene action is incomplete dominance or additive, there are two homozygous phenotypes.

Hybridization: A synonym for crossbreeding. See crossbreeding.

Inbreeding: The mating of relatives.

Inbreeding depression: Declines in growth rate, fecundity, etc. and an increase in the percentage of deformed/abnormal fish that occur because of inbreeding.

Incomplete dominant gene action: A type of gene action whereby one allele is expressed more strongly than the other during the production of qualitative phenotypes, but the dominant allele cannot suppress the recessive allele in the heterozygous state. Two copies of the dominant allele are needed to produce the dominant phenotype. Because the recessive allele is able to function in the heterozygous state, that genotype produces a phenotype (the heterozygous phenotype) that is marginally different from the dominant phenotype. The recessive allele produces a third phenotype (the recessive phenotype) when the fish is homozygous recessive.

Independent assortment: The segregation of homologues of each chromosome pair (and the genes on these chromosomes) into the secondary spermatocytes or into the secondary oocyte and the first polar body. The segregation of the homologues of each chromosome pair is independent of that which occurs in all other chromosome pairs. This process destroys all dominance genetic variance and most epistatic genetic variance.

Indirect selection: Changes that occur for one phenotype when selection is placed on another. A breeding programme that improves one phenotype by selecting for another. This occurs because the two phenotypes have a positive genetic correlation.

Individual selection: A selective breeding programme for quantitative phenotypes where selection is based on individual merit. An individual whose phenotypic value is equal to or exceeds the cut-off value is saved, while those whose phenotypic value falls below the cut-off value are culled. Family relationships are ignored. Individual selection is also called "mass selection."

Linebreeding: An inbreeding programme where an outstanding individual (usually a male) is mated with its descendants to increase its contribution to the gene pool.

Linkage: Genes are linked when they are located near each other on a chromosome. Linked genes tend to cross over together.

Locus (plural = loci): The location of a gene on a chromosome. The terms "gene" and "locus" are often used interchangeably, and they are used synonymously in this manual.

Mass selection: A synonym for individual selection. See Individual selection.

Mean: The arithmetic average.

Meiosis: The process by which the diploid (2N) complement of chromosomes is reduced to the haploid (N) state during gametogenesis. Heritable mutations, crossing over, and independent assortment occur during meiosis.

Meiotic gynogen: A gynogen that is created by chromosomal manipulation by: 1) fertilizing an egg with irradiated sperm; and 2) shocking the egg to prevent the second polar body from leaving. The haploid second polar body and haploid egg nucleus fuse to form a diploid gynogen; consequently, all genes come from the mother.

Mitotic androgen: An androgen that is created by chromosomal manipulation by: 1) fertilizing an irradiated egg; and 2) shocking the haploid zygote during first cleavage to prevent nuclear and cell division. Mitotic androgens are 100% homozygous and 100% inbred; additionally, all genes come from the father.

Mitotic gynogen: A gynogen that is created by chromosomal manipulation by: 1) fertilizing eggs with irradiated sperm; and 2) shocking the haploid zygote during first cleavage to prevent nuclear and cell division. Mitotic gynogens are 100% homozygous and 100% inbred; additionally, all genes come from the mother

Monomorphic: When there is only one allele at a given locus in a population.

Monosex population: A population composed only of males or only of females.

Mutation: A mistake that occurs during chromosomal replication. When a mutation occurs, a gene is incorrectly replicated during the creation of the new chromosome. This new allele may be capable of producing a different (mutant) phenotype. Many mutant alleles produce lethal or sub-viable phenotypes. The accumulation of recessive sub-viable and lethal mutations and their subsequent pairing and expression when relatives mate is a major factor in inbreeding depression.

Neutral gene: A gene where all phenotypes are equally fit; i.e., no phenotype and thus no genotype give a fish a selective advantage.

Nick: The production of superior hybrids.

Overdominance: When the heterozygous phenotype is superior to the two homozygous phenotypes. Inbreeding decreases the frequency of overdominance phenotypes, which helps explain inbreeding depression.

P_1: The parental generation; the fish that are used to start a breeding programme. The P_1 generation produces the F_1 generation.

Pedigree: An individual's family tree.

Pedigreed mating: A system of mating in which each male leaves one son and each female leaves one daughter for the following generation's brood stock. The choice of sons and daughters must be random.

Phenotype: The physical expression of a fish's genotype. There are two phenotypic categories: qualitative phenotypes, which are described; quantitative phenotypes, which are measured. The terms "phenotype," "trait," and "character" are used interchangeably.

Phenotypic variance (V_P): The total variance that is measured for a quantitative phenotype in a population. It is the sum of genetic variance, environmental variance, and genetic-environmental interaction variance.

Polymorphic: When there are two or more alleles at a given locus in a population. In population genetics, this means that there are at least two alleles whose frequencies are ≥ 0.01.

Population: A group of fish that have a common background.

Progeny testing: A breeding programme that is used to decipher a fish's genotype by examining its offspring's phenotypes.

Qualitative phenotypes: Phenotypes that are described: for example, colour.

Quantitative phenotypes: Phenotypes that are measured: for example, weight.

Random mating: A system of mating where fish are mated without regard to phenotype.

Recessive allele: An allele whose phenotype is expressed only when an individual has two copies of the allele (homozygous recessive). The pairing and expression of detrimental recessive alleles helps explain inbreeding depression.

Recessive phenotype: The qualitative phenotype produced by the recessive allele.

Reduction division: The first meiotic division. The separation (segregation) of replicated homologues of each chromosome pair (independent assortment) during the creation of the secondary spermatocytes or of the secondary oocyte and the first polar body during meiosis. This destroys all dominance genetic variance and most epistatic genetic variance.

Rotational mating: A mating programme where families or cohorts are mated in a staggered manner over successive generations to prevent inbreeding.

Select population: The population that is created by selection.

Selection: A breeding programme whereby a breeder saves only those individuals or families that meet or exceed predetermined phenotypic criteria for quantitative phenotypes or those individuals that exhibit the desired qualitative phenotype. Fish that do not meet these criteria are culled.

Sex chromosome: The chromosomes that determine sex. They can be morphologically different in males and females, but in most species of fish there is no obvious morphological difference.

Sex-reversed: Fish that are one sex phenotypically but the other genetically. Sex-reversed fish are created by feeding anabolic sex hormones to sexually undifferentiated fry.

Sibs: Brothers and sisters. Fish that have the same mother and father are full-sibs. Fish that have the same mother or the same father, but not both, are half-sibs.

Sister chromatids: A homologue and its replicated "twin"; they are joined at the centromere.

Standard deviation: The square root of the variance. The standard deviation is a value that describes how phenotypic values are disbursed about the mean. When combined with the mean, it is the best way to describe a quantitative phenotype.

Test fish: A fish whose genotype is known. Test fish are mated to fish whose genotypes are to be deciphered during a progeny test. Test fish are usually homozygous recessive.

Tetrad: A bundle of four chromosomes. Tetrads occur when the homologues of each chromosome pair replicate, become sister chromatids, and then pair-up during the early stages of meiosis. Crossing over occurs when the chromosomes exist as tetrads.

Tetraploid: A fish or cell where each chromosome occurs in sets of four.

Topcross: An F_1 hybrid that is produced by mating an inbred line to a non-inbred or random-bred line. This technique often produces outstanding F_1 hybrids.

Trait: A synonym for phenotype. See phenotype.

Triploid (3N): A fish or cell where each chromosome occurs in sets of three.

Variance: The average squared deviation of phenotypic values from the mean. It is a value that describes how phenotypes are disbursed about the mean. The square root of the variance is called the "standard deviation."

Within-family selection: A selective breeding programme for quantitative phenotypes, whereby selection occurs at the family rather than at the individual level. In within-family selection, each family is considered to be a sub-population, and selection occurs independently within each family.

Zygote: The cell that is created when a sperm fertilizes an egg. This is often called a "fertilized egg."

RECOMMENDED READING

Books

General textbooks on fish genetics

Kirpichnikov, V.S. 1981. Genetic Bases of Fish Selection. Springer-Verlag, New York, New York, USA.

This textbook, written in a highly technical manner, contains valuable information about most aspects of fish genetics. The bibliography contains over 2,000 references, and includes references for a umber of hard-to-obtain papers about inbreeding studies from Russia and Eastern Europe.

Tave, D. 1993. Genetics for Fish Hatchery Managers, 2nd ed. Van Nostrand Reinhold, New York, New York, USA.

This textbook is written for extension specialists, government hatchery personnel, and fish farmers. It contains sections on all aspects of fish breeding, including one about inbreeding, genetic drift, and management of effective breeding number. There is a bibliography of over 800 references.

Papers

Highly technical articles that describe experiments or studies about: inbreeding; genetic drift; the effect of inbreeding and genetic drift on selection; effects of genetic drift on various phenotypes; recommended effective breeding numbers; and ways to manage hatchery populations to prevent inbreeding- and genetic drift-related problems.

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This manual, written for extension workers, aquaculturists, and those who work with natural resource management programmes, primarily deals with the problems caused by unwanted inbreeding in cultured fish populations and describes management techniques that can be used to prevent or minimize inbreeding. The manual also describes how inbreeding can be used to improve captive populations of fish. The manual contains chapters on: basic genetics and the genetics of inbreeding; how to determine individual inbreeding values when pedigrees are known; how to determine the average inbreeding value in a population when pedigrees are not known; genetic drift, which is random changes in gene frequency; how inbreeding programmes can be used to improve cultured populations of food fish; how to prevent inbreeding depression and loss of genetic variance in farmed populations; and recommendations on how to manage cultured populations of fish to prevent unwanted inbreeding and genetic drift from depressing productivity, profits and survival. One of the most important aspects of managing a closed population of fish at a fish farm or fish culture station is the management of the population's effective breeding number, because inbreeding is inversely related to the effective breeding number. Techniques to determine and manage the effective breeding number are described, and recommended minimum effective breeding numbers are provided for a variety of farm sizes and fish culture goals. A number of culture techniques can affect inbreeding, and ways to modify them so that there is minimal impact on inbreeding are discussed. Finally, ways to minimize inbreeding during selective breeding programmes are described.

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