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Use of DNA Microsatellite Polymorphism to Analyze Genetic Correlations between Hatchery and Natural Fitness

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Abstract.—The presence of a hatchery-rearing stage in the life cycle of a fish will inevitably select for improved hatchery performance (domestication selection) even when the hatchery broodstock is collected every generation from the wild. This phenomenon poses a difficulty for enhancement programs, because the correlation between hatchery fitness and fitness in nature is usually negative. Intensity of domestication selection, genetic variance and covariance components, and the effect of domestication on fitness in the natural environment can be estimated using DNA microsatellite polymorphism. The procedures in use at the Marine Gene Probe Laboratory are briefly described. The most effective procedures employ single-locus microsatellite repeat polymorphisms analyzed by polymerase chain reaction. The procedure is illustrated with an experiment on Atlantic cod *Gadus morhua* that reveals the intensity of domestication selection in the first laboratory generation of this fish. A newly developed, maximum-likelihood procedure for detecting sib, parent–offspring, and more distant relationships in fish populations is illustrated with a hatchery population of rainbow trout *Oncorhynchus mykiss*. When applied to microsatellite polymorphism data, the procedure generates pedigree information that allows estimation of the magnitude of domestication selection, and the predicted indirect effects on natural fitness, during routine hatchery operation. The reduction in natural fitness expected in hatchery-release programs can be mitigated by identifying, and then rejecting, wild-caught potential breeders that are recognized as having hatchery parents or grandparents. An alternative, more interventionist, strategy is to deliberately choose such animals as broodstock, with the aim of coadapting the population to both the wild and hatchery environments.

Technological development in the European and North American aquaculture industry is aimed at increasing the yield and the yield-to-cost ratios of intensively managed systems. Cultivated fish and shellfish can be expected to adapt genetically (that is, evolve) in their increasingly artificial environments, whether or not there is a deliberate program of genetic broodstock improvement. It is generally recognized that adaptation to intensive cultivation is likely to result in a decrease in performance under less intensively managed artisanal conditions, and an even larger decrease in performance under natural conditions. The evidence for deterioration of natural fitness comes mainly from salmonids (Vincent 1960; Moyle 1969; Reisenbichler and McIntyre 1977; Fraser 1981; Keller and Plosila 1981; Chilcote et al. 1986; Leider et al. 1990; Hindar et al. 1992).

This paper focuses on the genetic changes that may occur when hatcheries produce fry or fingerlings that are released into environments to augment natural populations. The genetic changes of concern are the directional effects of hatchery adaptation (domestication selection) on fitness in nature. This a different problem than that created by random changes in allele frequencies in hatchery populations with small effective population sizes (Allendorf and Phelps 1980; Danzmann et al. 1989; Hedgecock and Sly 1990; Gaffney et al. 1992).

Tools are now available to study directly the tradeoff between domestication selection in hatcheries and natural selection in the wild (Queller et al. 1993). We illustrate some of the ways in which DNA microsatellite pedigrees are beginning to be used in the Marine Gene Probe Laboratory (MGPL) to estimate variances and genetic correlations between components of fitness in natural and hatchery environments. The procedures allow estimation of the magnitude of the domestication tradeoff and the implementation of various strategies for avoiding, minimizing, or (for the adventurous) using the effects of

domestication selection in hatchery release programs for augmenting natural stocks.

Negative Correlations Between Natural Fitness and Performance in Hatcheries

Genotype–environment (G–E) interaction refers to the situation in which the relative performance of genotypes, strains, stocks, or breeds varies among environments. The biological properties of wild and cultivated stocks will exhibit G–E interaction when fitness in hatcheries and fitness in nature involve traits that are different but genetically correlated and heritable.

Breeds of fish adapted to high-input aquaculture tend to do less well than do other breeds when tested in less intensively managed environments, and vice versa (Fraser 1981; Doyle and Talbot 1986; Chilcote et al. 1986; Hjort and Achreck 1982). Genotype–environment interactions involving different types of domestic environment have been demonstrated in common carp *Cyprinus carpio* (Moav et al. 1975, 1976b; Wohlfarth et al. 1986) and catfish *Ictalurus* spp. (Dunham et al. 1990). The adaptation of fish other than common carp to the diversity of artificial environments is just beginning and represents a period of rapid evolution under intense environmental stress (Kohane and Parsons 1988; Suboski and Templeton 1989).

In general, the direct response of each stock to selection in its own environment will be greater than its correlated response in other environments. Selection in each environment separately, given sufficient genetic variance, will therefore eventually produce strong G–E interaction (i.e., inferior performance of each stock in the alternative environment). This begins to happen when a population is transferred from the wild into a hatchery. The rate of divergence between the wild stock, undergoing natural selection, and the hatchery stock, undergoing domestication selection, will depend on the intensity of selection in each environment and the genetic variances and correlations of traits within and between environments (Rosielle and Hamblin 1981).

Domestication Selection in the First Hatchery Generation

The presence of a hatchery-rearing stage in the life cycle of a fish will select for improved hatchery performance, even when the hatchery broodstock is collected every year from the wild. This domestication selection occurs because the young produced from wild parents suffer some mortality before being released. (It is safe to assume that 100% survival in the hatchery is rare.) The intensity of first-generation domestication selection depends on the proportion of fertilized eggs that make it to release and on the proportion of the total hatchery mortality that is selective (i.e., a function of the animal's phenotype).

The progressive loss of natural fitness caused by domestication selection can be minimized by recruiting fresh broodstock every year, as is recommended, for example, by NASCO (1991). New broodstock will not eliminate the problem, however. If released animals constitute a significant proportion of the total breeding population then genetic changes induced by hatchery selection will accumulate through time. This hatchery contribution continues until domestication is balanced by natural counter-selection at some lower level of natural fitness, or until the augmented population becomes extinct.

The existence of nonrandom, selective mortality has not been easy to demonstrate. Selection differentials in the hatchery are not well-defined selection criteria (e.g., measurements of weight, color, or shape) that are used in artificial selection. Domestication selection is a form of uncontrolled natural selection in an artificial environment. The usual way to recognize its presence is by its result—behavioral or other phenotypic changes plus enhanced fitness in the hatchery and diminished fitness in nature. The action of domestication selection has usually been determined by inference, after several generations of domestication in the hatchery, not observed directly as a process.

Use of Microsatellite DNA Polymorphism to Demonstrate First-Generation Domestication Selection

An example of the use of microsatellites for the direct detection of selective mortality in the first domestic generation of a fish is provided by an experimental rearing program at Dalhousie University, Halifax, for Atlantic cod *Gadus morhua* (Lochmann et al., in press).

Figure 1 indicates the allelic diversity present at several loci in the parental, wild population of Atlantic cod based on a DNA microsatellite pedigree analysis developed at the MGPL. Figure 1 also shows the power that these loci provide for distinguishing populations (Bruford and Wayne 1993). The primer sequences for some of the Atlantic cod loci used in the experiment have been published (Wright 1993; Brooker et al., in press). The allelic

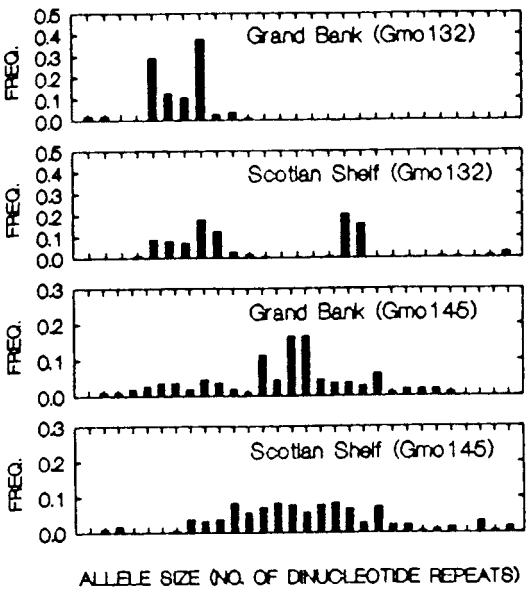

FIGURE 1.—Allelic distributions of two microsatellite loci (*Gmo132* and *Gmo145*) of Atlantic cod at two locations off the Canadian Atlantic coast. Grand Bank is located southeast of Newfoundland and Scotian Shelf east of Nova Scotia. The x-axis is allele size measured in number of dinucleotide repeat units (each tick represents an increase of two base pairs from an arbitrary origin). The y-axis is relative allele frequency.

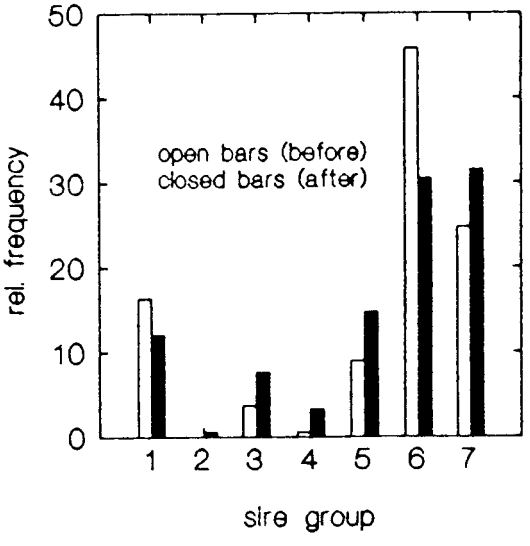

FIGURE 2.—Relative survival of seven groups of half sibs (seven males mated to one female). Mortality occurred before or after the point of "no-return," when larvae must begin to self feed.

diversity is so high in fish caught off the east coast of Canada (45 or more alleles at some microsatellite loci) that each animal is essentially unique when as few as 3 or 4 loci are scored.

In a rearing experiment, a number of wild males and females were spawned and the eggs pooled. After approximately 20 d the larvae hatched and were provided with lower-than-optimal food levels. During a 2-d period beginning about 8 d after hatching, the Atlantic cod larvae decreased in size as the yolk sac was absorbed and the larvae attempted to feed for the first time. DNA identification of larvae produced by different sires revealed that this critical 2-d period in the life of the larvae was profoundly selective: offspring of some male parents survived the transition to self feeding much better than did others. Figure 2 shows the relative survival of the offspring of seven different sires mated to the same female. Because only one female was involved, there is no between-group variance caused by genetic maternal effects and egg quality. The relative survival of offspring of the same sires mated to other females maintained the same rank order, however.

The conclusion is that mortality was strongly selective in the laboratory, even at this very early larval stage. The coefficient of variance of sire-group survival was approximately 60%. This variance is not merely phenotypic opportunity for selection (Downhower et al. 1987), it is selection (directly observed genetic variance in survival).

Estimating the Tradeoff Between Domestication and Natural Selection

The measurement of first-generation selection gives only one-half of the answer to whether or not selective mortality in the hatchery might diminish the natural fitness of released animals. It is also necessary to estimate the correlated changes in natural fitness in the natural environment. Here, too, individual pedigrees based on microsatellite polymorphisms make the appropriate measurements possible.

Genetic correlation is calculated as the correlation between the value of a trait (e.g., growth) in one environment and the value of the same trait, observed in family members in another environment. When the trait is an all-or-nothing component of fitness, such as mating or survival, the correlation of family mean liabilities can be calculated (Falconer 1981). The procedure for observing and predicting such changes requires the identification

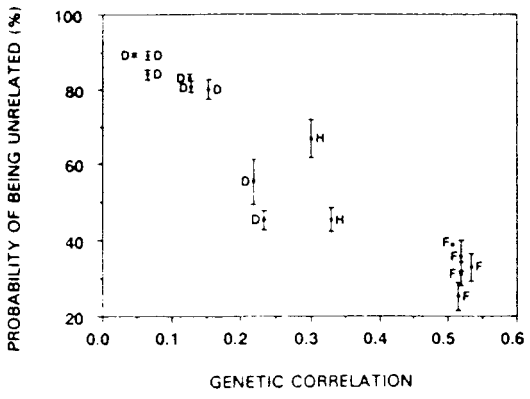

FIGURE 3.—Relationship between the mean probability of being unrelated, as calculated from microsatellite data, and the expected genetic correlations. Comparisons are between full-sib fish (F), half sibs (H), and fish more distantly related through grandparents (D).

of the family membership of individuals released from a hatchery into the wild and subsequently recaptured. It also requires the identification of individuals from the same family reared in the hatchery. Several levels of genetic relationship (e.g., full sibs, parent–offspring, and half-sibs) can be used.

Before the advent of DNA microsatellite identification the procedures for estimating genetic correlation involved rearing sib groups separately until individuals were large enough to be tagged. This procedure is operationally complex and expensive. Perhaps the greatest difficulty with this approach is that the experimental environment, with its separate rearing stage, is neither natural nor completely representative of selection under normal hatchery practice.

Mathematical Aspects of DNA Pedigrees for Estimating the Correlated Response to Domestication Selection

A maximum-likelihood procedure has been developed (C. Herbinger, unpublished) that can assign individuals to sib groups (and other groupings including grandparents, parent–offspring, and first cousins) on the basis of microsatellite information, even when the parents are not known (Figure 3).

The illustrative data in Figure 3 come from an experimental population of rainbow trout *Oncorhynchus mykiss*, in which the expected genetic correlations among the individuals are known with some confidence and can be compared with the maximum-likelihood predictions. The full-sib relationships, denoted as F in Figure 3, are known exactly from first-order pedigrees. The other groupings are corrected for suspected second-order relationships among grandparents. For example, the distantly related individuals, denoted as D, fall into three groups that are suspected to have zero, one, or three grandparents in common.

The microsatellite pedigrees greatly simplify the estimation of genetic correlations of fitness across the hatchery and natural environment. The differential survival of sib groups in both environments can be studied with little or no modification of hatchery procedures. All that is required is samples collected from hatchery-origin fish before and after release. The sampling is nondestructive; a single scale contains enough DNA for the microsatellite analyses. The calculation of direct and correlated responses to selection (hatchery adaptation and changes in natural fitness, respectively) can be complicated when there are unknown genetic correlations (family relationships) in a population. Microsatellite pedigrees can provide required information.

Molecular Aspects of Microsatellite DNA Polymorphism

Protein polymorphisms (isozymes or allozymes) have been used in the past to mark or identify genotypes in aquaculture experiments (Moav et al. 1976a). The level of isozyme polymorphism available for marking is relatively low, however, so that even with special breeding arrangements no more than 2 or 3 different genotypes (families or subpopulations) can be distinguished in pooled populations. This number is too small for measurement of genetic variances and correlations although it is useful for other purposes (e.g., for breed comparison and genetic stock identification in natural populations) (Chilcote et al. 1986; Utter et al. 1989; Shaklee et al. 1990; Brodziak et al. 1992). Allozymes have also been used very successfully to detect differences in the reproductive success of hatchery and wild fish at the population level, but not the individual or family level (Leider et al. 1990).

We have adopted single-locus microsatellite polymorphisms for genetic pedigree analysis for several reasons: (1) the level of polymorphism is very high—with heterozygosities approaching 100% at some loci—which allows the identification of all families in commercial hatcheries; (2) the DNA sampling is noninjurious, which allows identified animals to be used subsequently as broodstock; and

(3) the procedure is straightforward and cost effective.

The laboratory practice and underlying molecular biology of DNA microsatellite polymorphism in fish has recently been comprehensively reviewed by Franck et al. (1991) and Wright (1993). The aquaculture genetics projects under way at the MGPL are currently based on two of the several possible types of DNA fingerprinting; several other procedures are currently under investigation in the MGPL.

Minisatellite polymorphisms analyzed by Southern blotting.—Minisatellites consist of tandemly repeated sequences of 9–65 base pairs (e.g., Jarman and Wells 1989). These tandem arrays of core sequence repeats, which may exist at several locations in the same genome (i.e., at several loci), vary in length among individuals. Because the number of length variants (alleles) segregating at each locus may be very large, this type of polymorphism has been termed hypervariable variable number tandem repeat (VNTR) polymorphism.

When DNA extracted from the target fish is cut with restriction enzymes, subjected to Southern blotting, and probed with the core sequence, many VNTR loci can be revealed simultaneously. The result is a multilocus satellite pattern that resembles a bar code and which, because of the hypervariability of the loci, uniquely identifies individuals (Jeffreys et al. 1988). The alleles at each locus are inherited in strictly Mendelian fashion (except for a high mutation rate), so in principle the multilocus microsatellites can be used to identify the target fishes' parents (e.g., for pedigree analysis). In practice, the number of loci is often so large that alleles from different loci overlap, which introduces some ambiguities into the analysis (Lynch 1988).

The problem of overlapping alleles from different loci on a Southern blot can be overcome by using VNTR probes that hybridize to only one locus (i.e., to the unique sequence DNA that flanks or lies near the repeated core VNTR sequence) (Wong et al. 1986; Taggart and Ferguson 1990). This was accomplished in the MGPL for Atlantic salmon *Salmo salar* by Bentzen et al. (1991).

Polymerase chain reaction (PCR) procedure.—This procedure provides a simpler and less ambiguous alternative to Southern blotting, when the objective is pedigree analysis of large numbers of fish. The PCR procedure involves the synthesis of pairs of oligonucleotides that are complementary to unique sequence DNA lying adjacent to each end of the polymorphic VNTR locus. The intervening VNTR sequence is amplified by PCR and electrophoresed on acrylamide sequencing gels (Jeffreys et al. 1988). The current preference in the MGPL is to use VNTR sequences called microsatellites, which are comparatively small (1–4 base pair repeats, rather than 6–95 base pair sequence repeats that characterize minisatellites). High-resolution sequencing gels are required to avoid confounding alleles that have nearly the same length.

Discussion

One of the strategies for minimizing the effect of domestication on the natural fitness of enhanced stocks involves maintaining open, natural populations by recruiting hatchery broodstock from the wild in every generation (NASCO 1991). Unfortunately, this strategy will not completely avoid domestication selection. The microsatellite identification experiment with Atlantic cod described in this paper demonstrates that intense selective mortality can be detected in the earliest larval stages of offspring of animals from the wild. If this proves to be true for fish that are regularly stocked in enhancement programs, such as rainbow trout and Atlantic salmon, then stocking programs that spawn only wild broodstock will fail to produce offspring that are free from hatchery effects.

Fortunately, mathematical procedures and DNA pedigree techniques have been developed that can limit the accumulation of domestication selective effects in stocked populations beyond the first generation. Effecting some limit will require us to identify wild fish that have parents or grandparents of hatchery origin and then avoid spawning them. The sensitivity of the procedures that are now available seems adequate for identifying these fish.

An interesting alternative strategy for minimizing the accumulation of domestication effects is theoretically available now that DNA microsatellites have simplified the detection of pedigree relationships. Rather than trying to avoid the spawning of hatchery fish, or wild fish with recent hatchery ancestors, one could use fitness in the wild as a criterion for selection in the hatchery. There are at least two ways that this might be attempted, both based on the identification of the relatives of fish that have survived, thrived, and ultimately been captured in the wild.

In closed domestic populations, without regular addition of wild breeders, the hatchery parents or hatchery sibs of the successful wild fish can be chosen for spawning. This represents a type of progeny testing or sib selection. Because domestication selection would proceed as usual in the hatchery, the hoped for beneficial outcome would be simulta-

neous genetic adaptation to the hatchery and natural environments. The reduction in negative correlations between fitnesses in the two environments would be expected if the appropriate genetic variance exists in the population.

An alternative approach could be used in open populations where some or all of the broodstock is recruited from the wild every year. In this case, fish that have hatchery origins or ancestors could be deliberately chosen as broodstock rather than avoided. Such fish do carry a higher proportion of genes that have been successful in surviving (and reproducing) in both environments.

We recognize that these alternative genetic management strategies are more interventionist than those usually suggested (Moav et al. 1978). They certainly are in direct opposition to the idea that genetic changes in natural populations should be kept to a minimum (Allendorf and Ryman 1987; Nelson and Soule 1987). On the other hand, they may imply a more willing acceptance of the hatchery as an permanent, essential component of the reproductive environment of endangered fish populations.

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