



Conservation genetics of freshwater fish

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Genetic markers have helped to resolve many difficult taxonomic problems and map patterns of diversity within and among remnant populations of threatened and endangered species. Knowledge of historical patterns of gene flow can help to manage dispersal among anthropogenically fragmented populations. Genetic considerations are used in the design of captive breeding programmes that avoid inbreeding depression and artificial selection that may impact on Darwinian fitness. Case studies from endangered populations of topminnows from North American deserts are used to illustrate a variety of methods used in conservation genetic studies. Several merits of studying putatively neutral, molecular markers *v.* adaptive phenotypic traits are discussed.

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INTRODUCTION

Fish habitats are destroyed as a consequence of many factors. Headwater regions of streams have been altered by deforestation and watershed erosion, and siltation has destroyed the breeding habitats of many species that require clear oxygen-rich waters. Agricultural run-off, pesticides, fertilizers, sewage, and chemical pollutants add additional stresses to remnant fish populations. Impoundments for water retention and electrical generation create barriers to the natural dispersal pathways of migratory fishes, and eliminate opportunities for gene flow among populations of primary freshwater species. Canalization and diversion of streams have eliminated riparian zones and destroyed aquatic ecosystems that maintain water quality, nutrient recycling, and contribute to the nurture of fish populations.

Introduced exotic species provide the *coup de grace* for many native fishes. Construction of dams and canals provides artificial lacustrine and riverine habitats that often are stocked with non-native game fish and commercially important species. Introduced carp and tilapia flourish in impoundments and fishponds and compete with native fish for food and nesting sites. When these exotic species escape, they often reproduce in surrounding streams and quickly replace native fishes. Similarly, *Gambusia* have been introduced widely for mosquito control and have devastated natural populations of small fishes throughout the world (Meffe, 1985). Introduced species pose an additional threat if they transmit exotic diseases to native fishes (Leberg & Vrijenhoek, 1994). The potential impact of exotic helminths, bacteria, and viruses should be assessed before government agencies become involved in the wholesale transport and release of exotic fishes.

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Practices attributed to commercial fishing and hatcheries have altered native fish populations. Gillnet fisheries in Lake Victoria have contributed to the decline of endemic tilapias and greatly changed the composition of native fish communities (Fryer, 1972). In addition to the broad scale introduction of tilapia and carp throughout the world, sport and commercial fisheries based on translocated native species have contributed to the genetic adulteration of fish stocks. Genes from released sheepshead minnows *Cyprinodon variegatus* Lacépède 1803 used as bait for sport fishing have introgressed into native pupfish *C. pecosensis* Echelle and Echelle, 1978 populations (Echelle & Conner, 1989). Genes from hatchery-reared rainbow trout *Oncorhynchus mykiss* (Walbaum, 1792) have introgressed into many cutthroat trout *O. clarki* (Richardson, 1836) populations, and rainbow trout have completely replaced cutthroats in many streams on western slopes of the Rocky Mountains in the U.S.A. (Allendorf & Leary, 1988). Native Apache *O. apache* (Miller, 1972) and Gila trout *O. gilae* (Miller, 1950) have also been replaced by hatchery reared rainbow trout, or altered by introgressive hybridization (Loudenslager *et al.*, 1986). Competitive replacement and genetic swamping of native species is a serious problem contributing to homogenization of the world's freshwater fish fauna. Although released species may serve the nutritional and entertainment needs of many people, they alter local fish communities and may destabilize aquatic ecosystems.

CONTRIBUTIONS OF FISH CONSERVATION GENETICS

Fish biologists are often asked to design and implement plans to protect and preserve threatened and endangered species. Can genetic information help in this effort? Genetics should not be viewed as a substitute for sound ecological, demographic, and systematic studies. Nor is genetics a substitute for comprehensive knowledge of the aquatic landscapes needed to preserve and restore habitats for native species. However, conservation genetics should not be equated merely with surveys of heterozygosity (see e.g. Lande, 1988; Caro & Laurenson, 1994), an unfortunate caricature that fails to portray the contributions that genetic studies have made to:

- (1) resolving problems with taxonomically difficult groups;
- (2) the design of captive breeding programmes;
- (3) understanding natural breeding systems;
- (4) detecting diversity within and among geographical populations;
- (5) managing gene flow; and
- (6) understanding factors contributing to fitness.

Case studies of a variety of plants and animals are justly documented in a recent volume by Avise & Hamrick (1995). Although many of the examples used in this paper derive from our studies of topminnows (Poeciliidae) from Arizona (U.S.A.) and Sonora (Mexico), the information may be applicable broadly to studies of tropical species around the world.

Resolving taxonomic controversies

Defining evolutionarily significant units is a primary goal of conservation programmes (Waples, 1992). Since not all the endangered populations can be saved, one must know how individual populations relate to their species as a whole and determine relevant boundaries in the geo-political context that

governs species conservation efforts. Often, specific populations or species may receive management priority based on their degree of diversity, evolutionary uniqueness, or charismatic value with respect to other populations (Woodruff, 1989; Crozier & Kusmierski, 1994). However, these goals may be difficult to achieve with many poorly known fish taxa from tropical and sub-tropical regions of the world. It is particularly vexing for highly diverse groups such as the cichlids in East African Great Lakes, minnows in Lake Lanao (Philippines), or atherinids in Lake Chapala (Mexico). The haplochromine species flocks that occupy African lakes have resisted traditional taxonomic efforts. Many of the morphological characteristics that define these fish may have arisen in parallel, making cladistic approaches difficult (Eccles & Trewavas, 1989), and other defining traits may be phenotypically plastic (Hoogerhoud *et al.*, 1983; Hoogerhoud, 1984). Allozymes, which tend to be relatively invariant between these closely related morphotypes, provide little taxonomic resolution of these species flocks (see papers in Echelle & Kornfield, 1984). Very promising studies of mtDNA have started to sort out evolutionary lineages of Lake Malawi haplochromines (Moran *et al.*, 1994) and the broader phylogeny of African cichlids is beginning to emerge (Meyer, 1993). Studies with microsatellite DNAs have begun to unravel the complex mating systems of haplochromines (Parker & Kornfield, 1996), an important step to understanding factors involved in maintaining the genetic integrity of these cichlids.

Captive breeding

Fish are bred in captivity for: conservation and rescue; stocking and supplementation of sport and commercial fishing; domestication and selective breeding of food and ornamental varieties; experimental research organisms; and exhibition and education (Frankel & Soulé, 1981). Captive breeding often encounters fundamental problems that can be avoided with careful genetic planning and monitoring. A goal of some programmes is to breed selectively varieties that grow faster and survive better under crowded conditions and artificial diets. Domesticated stocks tend to be phenotypically more uniform and behaviourally predictable than wild stocks. They may be desirable for hatcheries, fish hobbyists and public display, but they are not natural and may represent poor starting materials for restocking or supplementation of endangered populations.

Inadvertent selection is also a problem in domesticated stocks. For example, I recently visited a trout hatchery where juveniles learned to follow the shadow and vibration of a feed-truck as it passed between the raceways. This behaviour may be adaptive in the hatchery, but I doubt if it contributes to the fitness of these fish when released into natural streams. With the hundreds of millions of hatchery trout released into natural streams each year, it is hard to believe that mutations favouring such domesticated behaviour have not already had a significant impact on wild populations (Meffe, 1992).

Captive breeding programmes that protect fish from environmental fluctuations and threats may speed the loss of variation that is needed to deal with natural stresses. Predators and diseases may constitute a major cause of fluctuating fitness in natural populations, and the loss of genetic diversity during domestication may cripple behavioural and immunological responses of fish to these enemies. Disease response in vertebrates is mediated through an immune

surveillance system that relies on genotypic diversity. Loss of diversity at major histocompatibility complex (MHC) loci may narrow immunological responses to pathogens. Consequently, the use of molecular markers to determine MHC diversity has been suggested as a means to assess the genetic quality of captive stocks of animals (Hughes, 1991); however, management of captive stocks by this criterion alone is ill-advised. Many independent gene loci are involved in the immunological, physiological, and behavioural responses to disease (Vrijenhoek & Leberg, 1991), and breeding for maximum MHC diversity will accelerate the loss of variation at these other loci (Miller & Hedrick, 1992).

Completely avoiding inadvertent selection and the loss of genetic diversity may be impossible in captive populations, but the problem can be forestalled with the periodic introduction of genes from wild stocks. Nevertheless, *in situ* breeding programmes are more desirable than captive breeding that remove fish from natural sources of physical and biotic stress. If captive propagation is a last resort for an endangered species, breeders should avoid population bottlenecks when founding a new stock. Theoretical and empirical studies involving fish show that small founding populations quickly lose most rare alleles (Allendorf, 1986; Leberg, 1991). Careful genetic management of the founder population can allay these losses (Lacy, 1989), and multi-locus molecular markers (e.g. allozymes, mitochondrial DNA, MHC, microsatellite DNAs, etc.) can be used to monitor the retention of diversity. Nonetheless, breeding designs based on careful pedigree analysis may be better at maintaining total genetic diversity than management based on a limited number molecular markers (Hedrick & Miller, 1992).

Loss of diversity and inbreeding depression are expected consequences of a poorly managed breeding programme. Depressed performance, survival and reproduction have been associated with inbreeding in captive bred animals (Ralls & Ballou, 1983) and hatchery stocks of fish (Gall, 1987). The inbreeding coefficient of an individual, F , is an estimate of its homozygosity due to alleles that are identical by descent. This coefficient can be estimated directly from pedigrees, but when such information is not available, an estimate can be made from records of the effective number of breeding adults (N_e) in each generation, and sometimes from genotypic frequencies in natural populations. However, N_e is typically much smaller than the observed number of breeding adults. Gall (1987) provides a helpful discussion of factors affecting N_e as it applies to fisheries. The breeding system (e.g. sex-ratio biases, harem formation, inbreeding, high reproductive variance among individuals, etc.) and fluctuations in population size over time can reduce N_e to a fraction of the actual population size. For example, if sperm from a single male are used to inseminate the eggs of 100 females, N_e is roughly equivalent to a population of only four adults, not 101 adults. All the progeny of such a generation would be half-sibs because they share the same father. Inbreeding proceeds rapidly with highly skewed sex ratios. To maximize N_e , breeders should attempt to balance male and female contributions to the next generation. Similarly, excessive contribution by a few highly fecund females will substantially reduce N_e . Fluctuation in the numbers of breeding adults between generations also depresses N_e . The average effective size is the harmonic mean of the N_e in each generation. Imagine a founding stock of 10 fish (five males+five females) used to start a colony that is

subsequently maintained with 1000 breeding adults. After 10 generations the mean of N_e is only 91 fish, and after 100 generations is about 500 fish. The founding size of a captive stock should be as large as possible, as most diversity will be lost at founding and not regained. Crow & Denniston (1988) provide a comprehensive treatment of demographic factors shaping N_e .

Determination of N_e requires accurate estimates of demographic and behavioural factors that rarely are known for natural fish populations. However indirect statistical methods exist for estimating N_e from temporal changes in gene frequencies (Waples, 1989) and linkage disequilibrium (Bartley *et al.*, 1992). Unfortunately, such estimates are associated with very large errors, due to the limited number of loci and sample sizes used to estimate genotypical frequencies (Richards & Leberg, 1997). Nevertheless, these indirect methods typically reveal that N_e is substantially smaller than the census number of adults. For example, Bartley *et al.* (1992), estimated that a mass strip-spawning involving 2000 adult rainbow trout was genetically equivalent to 89 individuals (CI 44.7–265.8). Similarly, a mass strip-spawning involving 10 000 adult Chinook salmon *O. tshawytscha* (Walbaum) was equivalent to only 133 individuals (CI 67.5–354.7). Artificial spawning through hormone induction and stripping of gametes typically mixes eggs and sperm from individuals that may not be equally ripe. Because of the high variance in viability and fertility among batches of gametes, a few males and females often produce most of the next generation. Despite their inaccuracy, indirect estimates of N_e can be used to monitor the effectiveness of hatchery breeding protocols. The estimation error can be reduced by examining larger samples of fish and more genetic markers. Molecular methods such as microsatellite DNAs and amplified fragment length polymorphisms (AFLPs) offer numerous markers that may improve the accuracy of such estimates.

Natural breeding systems

Attempts to maintain diversity in endangered fish populations must take into account their natural breeding systems. A mating system can often be described from analysis of genotypic frequencies encoded by molecular markers. If the mating system affects all genes equally (e.g. polyandry or inbreeding), any neutral markers will suffice as long as they are sufficiently polymorphic. Studies of assortative mating, however, require molecular markers that covary with the visible markers involved in mate selection (e.g. pigmentation patterns, size, sounds, movements, habitat choice, etc.) that may reside on one or a few chromosomes. For example, cichlids from Lake Malawi, mate assortatively for colourful pigmentation patterns, and may mate randomly with respect to molecular markers (G. Turner, pers. comm.).

Molecular markers can be used to determine some subtle aspects of a mating system. Allozymes were used to show that broods produced by the viviparous poeciliid fish, *Poeciliopsis monacha* are fathered by stored sperm from multiple males (Leslie & Vrijenhoek, 1977). Recently, highly variable microsatellite loci were used to study the mating system of the Gulf pipefish *Syngnathus scovelli* Everman & Kendall, 1896 (Jones & Avise, 1997). Pipefish males have a marsupium in which they carry developing embryos. By comparing DNA fingerprints of the males and their broods, the authors inferred that males rarely carry eggs produced by more than one female. However, some females are

successful in depositing their eggs with several males. These pipefish have a polyandrous mating system. In contrast, microsatellite studies revealed that the mouth-brooding cichlid *Pseudotropheus zebra* Boulenger, 1899 has a polygynandrous mating system (Parker & Kornfield, 1996). The genetic consequences of natural mating systems should be considered in the development of captive breeding plans.

Diversity within and among populations

Conservation biologists use a variety of tools to define appropriate management units of threatened and endangered species. For example, visible traits (e.g. stripes on tigers) often define geographical subspecies. It would be desirable to know whether locally adaptive life history and physiological differences covary with these visible traits, but these adaptive factors are rarely well defined and are difficult to study. On the other hand, selectively neutral markers can provide an independent picture of historical processes that shape the distribution of genetic diversity within and among remnant populations of threatened and endangered species. If neutral markers can delineate the boundaries of evolutionarily significant units, locally adaptive traits should also be enclosed within these boundaries.

To this end, total genetic diversity of a species (H_T) can be partitioned into two components, or $H_T = H_S + D_{ST}$, where H_S is the average gene diversity (i.e. heterozygosity) within, and D_{ST} the average variance in allelic frequencies between populations (Nei, 1975, pp. 149–154). Dividing each side of the equation by H_T , expresses the within- and between-population components as proportions of the total. The proportion D_{ST}/H_T is more commonly expressed as G_{ST} (also known as F_{ST} or θ , Wright, 1978; Weir & Cockerham, 1984).

For selectively neutral genes, F_{ST} is inversely proportional to the rate of interpopulational gene flow (discussed below). For example, humans are highly vagile, and only 7% of our genetic diversity is found in the differences between the major racial groups (Nei, 1975). In contrast, the Ord kangaroo rat *Dipodomys ordii* is less vagile, and 70% of its genetic diversity is found in the differences among local populations (Nei, 1975). A goal of conservation programmes should be to preserve dynamic processes (e.g. natural selection, gene flow, and genetic drift) that created the structure of diversity within and between constituent populations of an endangered species. These components of diversity may affect a species' ability to respond to new evolutionary challenges (Wright, 1978).

For river-dwelling fish, the partitioning of diversity can be extended hierarchically to reflect the complexities of stream-order $H_T = H_S + D_{SR} + D_R$, where H_S is the average heterozygosity within local colonies, D_{SR} is the variance between tributaries within rivers, and D_{RT} is the variance between different rivers. Again, the D values are inversely proportional to historical rates of dispersal at each level. Echelle (1991) reviewed studies of hierarchical structure in river-dwelling fishes, and three examples are summarized here (Table I). The rainbow trout *O. mykiss*, a migratory species, has 85% of its diversity within local populations, and 15% between. The Yellowstone cutthroat trout, a non-migratory species, has 67.6% of its diversity within populations and 32.4% between. In contrast, the small topminnow *Poeciliopsis occidentalis* Baird & Girard, 1853 has only 21.3%

TABLE I. Hierarchical partitioning of genetic diversity in three river-dwelling fish of the North American west, estimated from equation (2)

Species	H_T	Percentage total genetic diversity			Reference
		Within populations	Within rivers	Between rivers	
<i>O. mykiss</i>	0.069	85.0	7.7	7.3	Ryman (1983)
<i>O. clarki lewisi</i>	0.029	67.6	15.7	16.7	Allendorf & Leary (1988)
<i>P. occidentalis</i>	0.107	21.3	25.5	53.2	Vrijenhoek <i>et al.</i> (1985)

of its diversity within populations and 78% between. For *P. occidentalis*, most diversity exists in the differences between populations in different rivers, and between partially isolated populations within the desert streams of Sonora, Mexico.

Habitat destruction and impoundments increase the geographical fragmentation of many species. Reduced dispersal and gene flow will eventually shift the relative proportions of within- and between-population components of diversity. Genetic drift will cause a progressive loss of the heterozygosity within isolated colonies and a concomitant increase in the differences among them. The total diversity (H_T) does not necessarily decline as a consequence of drift alone, but metapopulation processes (local extinctions and recolonizations) can interact with drift and dispersal to reduce total diversity. Local fish populations may go extinct in drought years and habitats may be recolonized from more permanent populations during wet years. Depending on the pattern of recolonization (from a few sources or many) diversity may decline rapidly within and between populations. If local extinction and recolonization events are frequent, the metapopulation will coalesce rapidly, and differences within and between populations will be lost as H_T approaches zero (Maruyama & Kimura, 1980; Gilpin, 1991). Such events may explain the differences in H_T seen in some *Gambusia* populations from arid regions *v.* less arid regions (Echelle *et al.*, 1989).

Managing dispersal and gene flow

A goal of species preservation plans should be to restore gene flow in anthropogenically fragmented populations. Manual translocations and fish ladders around dams are examples of methods that can accomplish this goal, but rates of transport should be based on a knowledge of historical rates of gene flow. When comprehensive genetic analyses reveal no substantive divergence between populations, and geographical evidence indicates recent fragmentation, translocations might be warranted as a means of supplementing local population sizes and avoiding drift and inbreeding. Conversely, when populations are separated geographically and differ genetically, it is best to keep native fish populations within their respective basins. Two models for managing gene flow are considered below (Meffe & Vrijenhoek, 1988).

The Death Valley model. During pluvial times, pupfish of the Death Valley system were distributed throughout an interconnected system of rivers and lakes

near the present borders of southern California and Nevada. Natural drying during the past 10 000 years left remnant populations that now are completely isolated. Genetic drift and local adaptation during the post-pluvial period has resulted in remarkable divergence of morphology, physiology, behaviour, and life-history traits—a level that rivals the classical example of Darwin's finches (Soltz & Hirshfield, 1981). Genetic diversity in the *Cyprinodon* complex can be represented as $H_T = H_S + D_{ST}$, and surveys of molecular variation in these fish revealed that most diversity exists between the isolated populations (Turner, 1974; Echelle & Dowling, 1992).

Although it might be possible to reconstruct historical patterns of connectivity among currently isolated pupfish populations, such studies would have little bearing on the management of these fish. Translocation of pupfish between isolated springs and marshes would be unnatural, and inappropriate, because their current isolation is a consequence of natural climatic processes rather than anthropogenic disturbance. Hybridization between these isolated populations could alter traits that define the present strains, and may disrupt local adaptations. Conservation agencies involved with these fish strive to maintain ecologically and genetically healthy populations within their natural locales.

The stream-hierarchy model. Knowing the hydrographical organization of river systems could guide a managed dispersal programme, but sometimes geography is misleading. For example, the topminnows occupying downstream portions of the Río Mayo in Sonora, Mexico are genetically more similar to populations of the neighbouring Río Yaqui than to upstream populations in the Río Mayo (Vrijenhoek *et al.*, 1985). Perhaps recent stream captures occurred between lower regions of the Mayo and Yaqui, while the upper and lower regions of this river remained isolated. Such information is relevant to a management plan for this species (which is listed as endangered in Arizona, U.S.A.), because translocation of fish without a rational design could obliterate regional differences that may have adaptive value. A managed dispersal plan could be more realistically based on empirical estimates of gene flow among populations.

Several indirect methods exist for inferring rates of gene flow from geographical surveys of molecular variation (Slatkin & Barton, 1989; Slatkin & Maddison, 1990; Slatkin, 1993). The goal is to estimate Nm , the effective number of migrants exchanged between populations per generation. The pseudoparameter, Nm , is a product of the average size (N) and proportion of immigrants (m) in each population, which typically are unknown. Assuming an island model of population structure, Nm is inversely related to F_{ST} . The island model is a geographical null model of population structure, in which dispersal is unbiased with respect to the distance between habitat islands. A stepping-stone model may approximate dispersal of fish in rivers better. Stepping-stone dispersal occurs between neighbouring populations, and long-distance dispersal is assumed to be rare. Unlike the island model, stepping-stone dispersal produces a correlation of gene frequencies that decreases as the number of steps between populations increases (Kimura & Weiss, 1964). A similar decline results from isolation-by-distance among continuously distributed populations. Consequently, gene flow between populations is expected to decline with distance

TABLE II. Isolation-by-distance among *Poeciliopsis occidentalis* populations from Arizona (U.S.A.) and Sonora (Mexico)

Distance	Intercept	Slope	r^2	Mantel P
Straightline	1.531	- 1.071	0.179	<0.0001
Water-course	1.772	- 0.929	0.167	<0.0001

Intercepts and slopes of the regressions are given along with the coefficients of relationships (r^2). Because pair-wise estimates are not independent, the relationship between Nm and distance values was tested with Mantel's permutation test (Manly, 1991). The P values are one-sided probabilities from 5000 permutations that such a negative slope would occur by chance alone.

under stepping-stone dispersal or isolation-by-distance models of dispersal, but not under an island model. These models provide a foundation for different management criteria that might be applied to freshwater fishes in the North American deserts (Meffe & Vrijenhoek, 1988).

The complex hierarchical branching patterns of riverine systems and potential for isolation between rivers calls for models that can take such complexity into account. Slatkin (1993) developed a robust approach for estimating Nm between all possible pairs of populations, regardless of the underlying model of population structure. Pair-wise estimates of Nm can then be tested for correlations with various geographical models of dispersal. To illustrate this procedure, data from an allozyme survey of 21 *P. occidentalis* populations from Arizona, U.S.A. and Sonora, Mexico were used (Vrijenhoek *et al.*, 1985; Meffe & Vrijenhoek, 1988). The Nm values between all pairs of populations were estimated using Slatkin's (1993) DIST program. Geographical distances between populations were measured by two methods: distance along a water-course and through deltas between rivers; v. straight-line (shortest overland) distance between populations. The Nm values declined monotonically with distance regardless of the geographical model (Fig. 1, Table II), however, the explanatory power of straight-line distances was slightly greater than water-course distances. Although I doubt these fish can traverse the intervening desert, this curious result makes sense if inter-drainage dispersal occurs in part through stream capture. Although *Poeciliopsis* are relatively salt tolerant, inter-drainage dispersal through coastal deltas or estuarine regions may be unlikely.

The average rate of gene flow within rivers ($Nm=2.50$) can be interpreted as the genetic equivalent of five migrants in every two generations. As expected, this value was greater than the rate of gene flow between rivers (mean $Nm=1.14$), or about one migrant per generation. As previously noted, an exception occurs in the Río Mayo, where the average rate of gene flow between upper and lower portions of this river ($Nm=0.02$, or two migrants every 100 generations) is substantially less than between lower Mayo and Río Yaqui populations ($Nm=1.21$), a number comparable to the average dispersal rate between rivers. A 40-year-old impoundment (Presa Mocuzari) separates the upper and lower Mayo populations, but it seems unlikely this degree of divergence of putatively neutral alleles could accumulate in so short a time. Perhaps cascades in the region of high relief have long separated these populations. The region

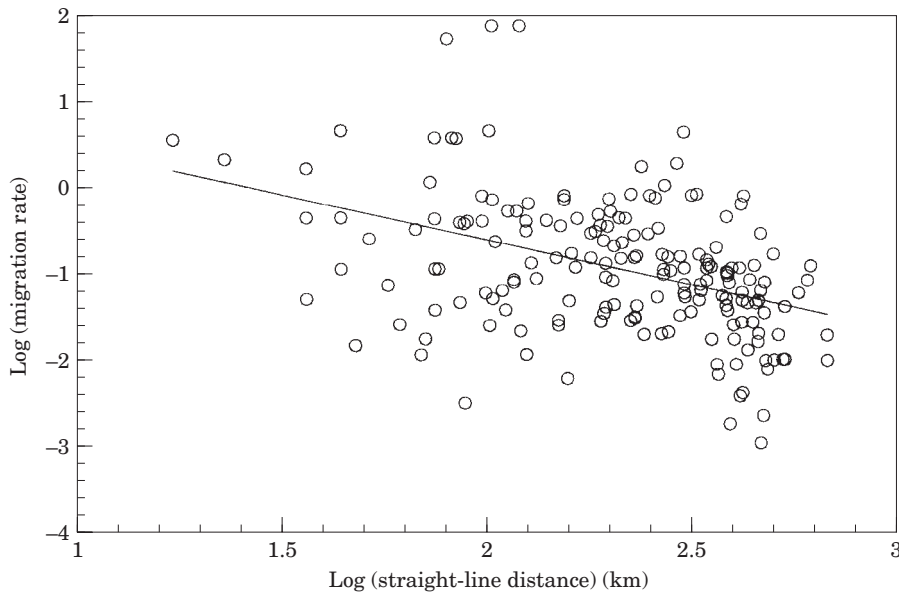


FIG. 1. A decline in gene flow with increasing geographical distance among 21 populations of *Poeciliopsis occidentalis*. The migration rate (Nm) and geographical distance (km) are shown on a log-log scale. Distance was determined as the straight-line distance between the sampled localities.

separating lower portions of the Río Mayo and Río Yaqui is coastal flood plain. Dispersal may occur across this region during severe floods.

The present result suggests that modelling dispersal on hydrography alone may not reflect the history of fish movements accurately. As seen, an approach using indirect genetic estimators of dispersal may be more informative than hydrographical information alone. Distinct fish species may share the same hydrographical system, but their unique dispersal behaviours and life histories may produce different patterns and rates of gene flow. Comparative studies of species with parallel distributions and distinct life histories can be used to test this hypothesis. Waples (1987) provides an excellent example with 10 syntopic species of marine fish from the region of southern California and Baja California. The different life histories of these marine species had a significant effect on rates of gene flow, with the lowest dispersal found in a viviparous fish and the highest found in species with pelagic larvae. Similarly, comparative studies of 14 invertebrate species endemic to deep-sea hydrothermal vents in the eastern Pacific revealed a range of gene flow rates (Vrijenhoek, 1997). Species with a free-swimming larval stage could disperse across gaps separating distinct oceanic ridge axes and apparently a species that brooded its young could not. Similar comparative studies with freshwater fish may provide a clearer picture of the roles of hydrography, life history and behaviour in realized dispersal rates.

CASE STUDIES WITH DESERT TOPMINNONS

Desert stream-dwelling fishes of the genus *Poeciliopsis* (Poeciliidae) provide a model system for assessing the merits of various genetic approaches to biological conservation. Natural subdivision of these topminnow populations into small,

partially isolated subpopulations have allowed us to examine the effects of fragmentation on formerly widespread and continuous fish populations. Field and experimental studies conducted with these topminnows during the past 30 years provide comprehensive analyses of temporal and spatial factors affecting genetic diversity and fitness.

The Gila topminnow

Considerable controversy surrounds conservation studies and management plans for the Gila topminnow, *P. occidentalis*. The species is abundant in Sonora, Mexico, but the Gila River subspecies *P. o. occidentalis* is listed federally as endangered in the U.S.A. The goal of the 1984 Topminnow Recovery Plan was to introduce a stock of these fish from a national fish hatchery (Dexter, New Mexico) into 191 reclaimed sites in Arizona (U.S. Fish and Wildlife Service, 1983). Based on several criteria, it was recommended that the hatchery strain available at that time was a poor choice for restocking the Gila River (Vrijenhoek *et al.*, 1985). The Monkey Spring population from which the stock was taken, had low fecundity; may have limited physiological tolerance, coming from a thermally stable spring; and had no apparent genetic diversity, which might limit their adaptive potential. This study recommended restocking with genetically variable and more fecund fish from a thermally fluctuating environment. Soon after, the Dexter National Fish Hatchery replaced the Monkey Spring stock with new fish from Sharp Spring. Apparently, the new stock performed notably better in the hatchery (B. L. Jensen, pers. comm.).

Subsequently, several fitness-related traits in the Monkey Spring (MS) and Sharp Spring (SS) stocks were compared with those of a third stock that is considered a distinct sub-species, *P. o. sonoriensis*, from the Tule Spring (TS) (Quattro & Vrijenhoek, 1989). Average heterozygosity of the three stocks could be ranked as follows: SS>TS>MS. Laboratory-born progeny of wild-caught females from the three localities were raised in a common garden experiment. Their survival, growth rate, fecundity, and developmental stability (i.e. fluctuating asymmetry) were examined. For each of these four traits, the fish stocks could be ranked in the same order as heterozygosity: SS>TS>MS. Quattro & Vrijenhoek (1989) concluded cautiously that 'It is not our intention to imply that the enzyme polymorphisms . . . are the primary determinants of fitness in these fish . . . Nonetheless . . . severe population bottlenecks, founder events, inbreeding, and migration are expected to affect allelic diversity at all loci to a similar extent . . . (and thus estimates of heterozygosity may) reflect the recency and severity of these historical processes.' It was suspected that long-term isolation and inbreeding may have lowered the fitness of the homozygous Monkey Spring population. A current draft of the species recovery plan (U.S. Fish and Wildlife Service, 1993) does not recommend exclusive use of Sharp Spring fish for restocking the Gila River, however. Subsequent introductions will use stocks from the nearest natural population in the same hydrographical basin.

Sheffer *et al.* (1997, 1998) criticized the studies, because they could not reproduce some of the differences found between the Monkey Spring and Sharp Spring stocks. They reported no significant differences in survival between the two stocks, but 93.8% of fish in their study survived, and consequently no statistical power existed to test for survival differences. Our experiments were

designed to stress the fish by keeping the water hot (28° C) and food levels carefully rationed. They raised fish in large aquaria, and we used a controlled, flow-through incubator system developed to partition genetic and environmental sources of phenotypic variation in growth and survival carefully among other forms of *Poeciliopsis* (Wetherington *et al.*, 1989). Overall, about 50% of the fish studied by Quattro & Vrijenhoek (1989) died during the 12-week grow-out period, and this mortality provided the statistical power to test for survival differences.

Additionally, the rearing conditions used by Sheffer *et al.* (1997, 1998) did not reveal similar differences in growth rates or developmental stability, but fecundity differences between the Monkey Spring and Sharp Spring stocks were similar to ours. To explain the discrepancies between the two studies, they speculated that unnatural stresses and perhaps the soft water in New Jersey produced our results, and therefore the results were not relevant to conservation of this species in Arizona. We suggest that the high survival (93.8%) obtained in their study also is unnatural for an endangered species. The abilities to grow, reproduce and survive under stress are relevant to species restoration projects. Unfortunately, while we have engaged in these academic debates, only four natural populations of *P. o. occidentalis* remain in Arizona.

Subsequent molecular studies provide relatively consistent information about genetic diversity within and among *P. occidentalis* populations, and suggest the action of common historical factors affecting dispersal and genetic drift. A study of restriction fragment length polymorphisms of mtDNA confirmed the genetic uniqueness of the *P. o. sonoriensis* sub-species, which persists in an isolated headwater portion of the Río Yaqui on the Arizona side of the U.S. border (Quattro *et al.*, 1996). No mtDNA variation was found within or among Gila River populations of *P. o. occidentalis*. As with allozymes, *P. o. occidentalis* have very low levels of diversity compared to populations of this species from Sonora, Mexico. Molecular analyses revealed that some MHC variation exists within and among remnant populations of *P. o. occidentalis* in Arizona (Hedrick & Parker, 1998). As with allozymes, the highest MHC variation was found in the Sharp Spring population. Although the other populations were not invariant, they clearly had fewer MHC alleles. Hedrick & Parker (1998) suggest the MHC differences among populations may reflect historical separation and warrant management of the stocks as evolutionarily significant units. However, variation at hypervariable genes like MHC can evolve very quickly, and MHC may represent one of the best examples of a selectively adaptive polymorphism (Hedrick, 1994). It remains unclear from their study what MHC polymorphisms tell us about the evolutionary history of these populations. Have these differences been accrued subsequent to anthropogenic fragmentation, or are they products of historical isolation and adaptive divergence? This is the relevant challenge for conservationists, if the goal is to restore the connections destroyed by recent anthropogenic changes and preserve the differences that accrued naturally.

Disequilibrium and intergenic correlations in Poeciliopsis monacha

Long-term field and experimental studies of *Poeciliopsis* from southern Sonora (Mexico) provide insight into the correlations between heterozygosity and fitness

TABLE III. Intergenic correlations due to finite population size (N_e) and the recombination rate (c), estimated from the equations of (Hill & Robertson, 1968; Ohta & Kimura, 1969):

$$r = \sqrt{\frac{1}{1 + 4N_e c}}$$

N_e	Recombination rate c					
	0.5	0.4	0.3	0.2	0.1	0.01
10 000	0.0071	0.0079	0.0091	0.0112	0.0158	0.0499
1000	0.0224	0.0250	0.0289	0.0353	0.0499	0.1562
100	0.0705	0.0788	0.0909	0.1111	0.1562	0.4472
10	0.2182	0.2425	0.2774	0.3333	0.4472	0.8452

found among the *P. occidentalis* stocks. *Poeciliopsis monacha* is a sexually reproducing species that coexists in the Río Fuerte with gynogenetic clones of the triploid fish, *P. 2 monacha-lucida* (Vrijenhoek, 1978). We have used the clones as genotypically uniform controls for events that altered genetic diversity in the sexual species. Survival of *P. monacha* during hypoxia, heat, and cold stresses was associated with balancing selection marked by genotypes at four polymorphic allozyme loci (Vrijenhoek *et al.*, 1992). At each locus, one homozygote was associated with greater survival during heat and hypoxic stress (which occur together in nature), and the alternate homozygote was associated with greater survival during cold stress. The heterozygotes at these loci generally were intermediate, although one locus exhibited overdominance for heat and hypoxic stress.

Population genetic data suggested that these polymorphic loci may not be the direct focus of natural selection. Strong and persistent linkage disequilibrium among alleles at these loci suggested that the allozymes marked larger blocks of genes that included the effectors of survival under stress. The association of these allozymes with differential survival may be a fortuitous consequence of genetic hitchhiking (Kojima & Schaffer, 1967; Hill & Robertson, 1968). Nevertheless, these allozymes marked balanced polymorphisms for which the heterozygotes were never the worst genotype. Thus, heterozygotes would have the highest geometrical mean fitness (Haldane & Jayakar, 1963) in a fluctuating environment such as these desert streams.

In a large randomly mating population, correlations between neutral markers and effector loci should be weak (Milton & Pierce, 1980; Chakraborty, 1981; Turelli & Ginzburg, 1983). However, markers can become associated with a heterozygote advantage (i.e. associative overdominance) because new deleterious mutations will be in linkage disequilibrium with the genes around them (Ohta, 1981). Although these associations decay rapidly in large populations, they can persist in small populations or populations that inbreed (Hedrick, 1982). The correlation (r) between alleles at linked loci is inversely correlated with effective population size (N_e) and the recombination rate c between genes (Table III). Very small populations (or those that went through a recent bottleneck or

TABLE IV. Genetic disequilibrium in a *Poeciliopsis monacha* metapopulation

Loci:	<i>Idh-2</i>	<i>Ldh-C</i>	<i>Pgd</i>	<i>Ck-A</i>
<i>Idh-2</i>	—	0·106	0·109	— 0·053
<i>Ldh-C</i>	0·118	—	0·085	— 0·041
<i>Pgd</i>	0·053	0·072	—	— 0·041
<i>Ck-A</i>	— 0·042	— 0·041	— 0·021	—

Intergenic covariances (bold, upper-right matrix) were estimated from gene frequencies in three permanent springhead populations of the Arroyo de Jaguaro drainage. Gametic phase disequilibrium (lower-left matrix) was estimated in a temporary population that is recolonized periodically by migrants from the three source populations. From [Vrijenhoek et al. \(1992\)](#).

founder event; e.g. $N_e = 10$) can have high intergenic correlations ($r = 0.2182$) even for genes on different chromosomes (i.e. $c = 0.5$). For tightly linked loci ($c = 0.01$), correlations will be higher ($r = 0.8452$). Substantial correlations ($r = 0.0499$) can exist in large populations ($N_e = 10\,000$) for tightly linked genes ($c = 0.01$).

Random genetic drift among partially isolated sub-populations creates a hierarchical genetic covariance structure, composed of linkage disequilibrium within and between sub-populations ([Ohta, 1982](#)). When sub-populations mix due to dispersal, linkage disequilibrium increases as a function of the covariance in allelic frequencies among the source populations ([Nei & Li, 1973](#)). Population admixture explains the persistent linkage disequilibrium found in the temporary *P. monacha* populations that were sampled for our stress experiments ([Vrijenhoek et al., 1992](#)). During the dry season, these fish are fragmented into small sub-populations that persist in natural hot springs and cold seeps. The fish disperse to occupy temporary pools during the rainy season, which generates linkage disequilibrium in the mixed colonies. To predict disequilibrium in an admixed population, allelic frequency covariances were estimated ([Table IV](#), upper-right matrix) across three *P. monacha* sub-populations from permanent springs. Then, we estimated the observed linkage disequilibrium in a mixed population that is re-established periodically with migrants from these three springs ([Table IV](#), bottom-left matrix). The signs and magnitudes of the covariances were very similar to the estimated disequilibrium values. Periodic admixtures of topminnows from the springhead refugia provide a sufficient explanation for the linkage disequilibrium seen in the temporary populations. Nevertheless, we cannot rule out differential selection for co-adapted gene complexes involved with survival in this spatially and temporally heterogeneous environment ([Vrijenhoek et al., 1992](#)).

Other studies with these fish reveal that genetic diversity in *P. monacha* populations is highly correlated with fitness. Developmental stability was compromised in a founder population following a local extinction/recolonization event ([Vrijenhoek & Lerman, 1982](#)). The homozygous founder population also had a higher parasite load than local clones and genetically variable populations of *P. monacha* that occurred downstream ([Lively et al., 1990](#)). The founder population exhibited many manifestations of inbreeding depression, and their

ability to compete with the local clones was significantly compromised (Vrijenhoek, 1989). All these detrimental effects of inbreeding were quickly reversed when genetic diversity was re-introduced into the founder population. The ability of the sexual fish to live and compete with the clonal forms benefits from genetic diversity.

The relationships observed between allozyme heterozygosity and fitness-related traits in topminnow populations appear to be a consequence of inter-genic correlations. Even though the allozyme markers may be selectively neutral, they apparently mark larger blocks of genes that affect fitness in dynamic populations occupying a temporally and spatially heterogeneous natural environment. Clearly, such variation should not be discounted in species conservation programmes.

SUMMARY AND CONCLUSIONS

Considerable debate exists about the use of putatively neutral molecular markers in conservation genetic studies (Hedrick *et al.*, 1986; Lande, 1988; Caro & Laurenson, 1994). It may be more desirable to examine variability in traits that contribute more directly to fitness (e.g. survival, growth rate, fecundity, fertility, etc.), but the time needed to conduct such experimental studies with endangered species is often limited. Furthermore, as was illustrated by the Gila topminnows, experiments conducted in different laboratories can provide contradictory results and interpretations.

Molecular markers have proven their usefulness in solving many difficult taxonomic problems with endangered species, in designing and monitoring captive breeding programmes and understanding breeding systems, in detecting the geographical structure of genetic diversity, in managing gene flow, and in understanding factors contributing to fitness. The example with *P. monacha* suggests that it may be unwarranted to assume that molecular markers are invariably neutral. In small structured populations (e.g. most endangered taxa), these markers may become linked to effectors of fitness, and the persistence of molecular polymorphisms may be a consequence of genetic hitchhiking. Furthermore, Mitton (1997) recently reviewed a body of experimental evidence for selection acting on allozyme polymorphisms themselves. Similarly, DNA markers cannot be assumed to be neutral. Although most variation observed in mitochondrial DNA may not be expressed phenotypically (i.e. it occurs in non-coding regions or at synonymous sites), the entire mitochondrial haplotypes may be under selection and whole haplotypes may be in disequilibrium with nuclear genes (see Avise, 1994, for a review). Because selection can manifest itself in multifarious ways, it can obscure historical patterns of dispersal and vicariance that created present day population structure. Ultimately, we should strive to make our evolutionary inferences from as many molecular markers, nuclear and cytoplasmic, as is economically feasible. If a few markers stand out with a unique geographical distribution or population structure, they may provide clues about patterns of selection *v.* non-selective processes such as gene flow and genetic drift (Lewontin & Krakauer, 1973; Karl & Avise, 1992) that can be used in the design of species conservation programmes.

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References

- Allendorf, F. W. (1986). Genetic drift and the loss of alleles versus heterozygosity. *Zoo Biology* **5**, 181–190.
- Allendorf, F. W. & Leary, R. F. (1988). Conservation and distribution of genetic variation in a polytypic species, the cutthroat trout. *Conservation Biology* **2**, 170–184.
- Avise, J. C. (1994). *Molecular Markers, Natural History and Evolution*. New York: Chapman and Hall.
- Avise, J. C. & Hamrick, J. L. (1995). *Conservation Genetics: Case Histories from Nature*. New York: Chapman & Hall.
- Bartley, D., Bagley, M., Gall, G. & Bently, B. (1992). Use of linkage disequilibrium data to estimate effective size of hatchery and natural fish populations. *Conservation Biology* **6**, 365–375.
- Caro, T. M. & Laurenson, M. K. (1994). Ecological and genetic factors in conservation: a cautionary tale. *Science* **263**, 485–486.
- Chakraborty, R. (1981). The distribution of the number of heterozygous loci in an individual in natural populations. *Genetics* **98**, 461–466.
- Crow, J. F. & Denniston, C. (1988). Inbreeding and variance effective population numbers. *Evolution* **42**, 482–495.
- Crozier, R. H. & Kusmierski, R. M. C. (1994). Genetic distances and the setting of conservation priorities. In *Conservation Genetics* (Loeschcke, V. & Jain, S., eds), pp. 227–237. Basel: Birkhauser.
- Eccles, D. E. & Trewavas, E. (1989). *Malawian Cichlid Fishes: The Classification of Some Haplochromine Genera*. Herten, Germany: Lake Fish Movies.
- Echelle, A. A. (1991). Conservation genetics and genic diversity in freshwater fishes of North America. In *Battle Against Extinction: Native Fish Management in the American West* (Minckley, W. L. & Deacon, J. E., eds), pp. 141–153. Tucson, AZ: University Arizona Press.
- Echelle, A. A. & Conner, P. J. (1989). Rapid, geographically extensive genetic introgression after secondary contact between two pupfish species (*Cyprinodon*, Cyprinodontidae). *Evolution* **43**, 717–727.
- Echelle, A. A. & Dowling, T. E. (1992). Mitochondrial DNA variation of the Death Valley pupfishes (*Cyprinodon*, Cyprinodontidae). *Evolution* **46**, 193–206.
- Echelle, A. A. & Kornfield, J. (1984). *Evolution of Fish Species Flocks*. Orono, ME: University Maine Press.
- Echelle, A. A., Echelle, A. F. & Edds, D. R. (1989). Conservation genetics of a spring-dwelling desert fish, the Pecos Gambusia (*Gambusia nobilis*, Poeciliidae). *Conservation Biology* **3**, 159–169.
- Frankel, O. H. & Soule, M. E. (1981). *Conservation and Evolution*. Cambridge: Cambridge University Press.
- Fryer, G. (1972). Conservation of the Great Lakes of East Africa: a lesson and a warning. *Biological Conservation* **4**, 256–262.
- Gall, G. A. E. (1987). Inbreeding. In *Population Genetics & Fishery Management* (Ryman, N. & Utter, F., eds), pp. 47–87. Seattle, WA: University Washington Press.
- Gilpin, M. E. (1991). The genetic effective size of a metapopulation. *Biological Journal of the Linnaean Society* **42**, 165–175.
- Haldane, J. B. S. & Jayakar, S. D. (1963). Polymorphism due to selection of varying direction. *Journal of Genetics* **58**, 237–242.
- Hedrick, P. W. (1982). Genetic hitchhiking: a new factor in evolution? *BioScience* **32**, 845–853.

- Hedrick, P. W. (1994). Evolutionary genetics of the major histocompatibility complex. *American Naturalist* **143**, 945–964.
- Hedrick, P. W. & Miller, P. S. (1992). Conservation genetics: techniques and fundamentals. *Ecological Applications* **2**, 30–46.
- Hedrick, P. W. & Parker, K. M. (1998). MHC variation in the endangered Gila topminnow. *Evolution* **52**, 194–199.
- Hedrick, P. W., Brussard, P. R., Allendorf, F. W., Beardmore, J. A. & Orzack, S. (1986). Protein variation, fitness, and captive propagation. *Zoo Biology* **5**, 91–99.
- Hill, W. G. & Robertson, A. (1968). Linkage disequilibrium in finite populations. *Theoretical and Applied Genetics* **38**, 226–231.
- Hoogerhoud, R. J. C. (1984). A taxonomic reconsideration of the haplochromine genera *Gaurochromis* Greenwood 1980 and *Labrochromis* Regan 1920 (Pisces, Cichlidae). *Netherlands Journal of Zoology* **34**, 539–565.
- Hoogerhoud, R. J. C., Witte, F. & Barel, C. D. N. (1983). The ecological differentiation of two closely resembling *Haplochromis* species from L. Victoria (*H. iris* and *H. hiatus*): Cichlidae. *Netherlands Journal of Zoology* **33**, 283–305.
- Hughes, A. L. (1991). MHC polymorphism and the design of captive breeding programs. *Conservation Biology* **5**, 249–251.
- Jones, A. G. & Avise, J. C. (1997). Microsatellite analysis of maternity and the mating system in the Gulf pipefish *Syngnathus scovelli*, a species with male pregnancy and sex-role reversal. *Marine Biology* **6**, 203–213.
- Karl, S. A. & Avise, J. C. (1992). Balancing selection at allozyme loci in oysters: Implications from nuclear RFLPs. *Science* **256**, 100–102.
- Kimura, M. & Weiss, W. H. (1964). The stepping stone model of genetic structure and the decrease of genetic correlation with distance. *Genetics* **49**, 561–576.
- Kojima, K. & Schaffer, H. E. (1967). Survival processes of linked mutant genes. *Evolution* **21**, 518–531.
- Lacy, R. C. (1989). Analysis of founder representation in pedigrees: founder equivalents and founder genome equivalents. *Zoo Biology* **8**, 111–123.
- Lande, R. (1988). Genetics and demography in biological conservation. *Science* **241**, 1455–1460.
- Leberg, P. & Vrijenhoek, R. C. (1994). Genetic variation and the susceptibility of native populations to attack by parasites associated with exotic species. *Conservation Biology* **8**, 419–424.
- Leberg, P. L. (1991). Effects of genetic variation on the growth of fish populations: Conservation Implications. *Journal of Fish Biology* **37** (Suppl. A), 193–195.
- Leslie, J. F. & Vrijenhoek, R. C. (1977). Genetic analysis of natural populations of *Poeciliopsis monacha*. *Journal of Heredity* **68**, 301–306.
- Lewontin, R. C. & Krakauer, J. (1973). Distribution of gene-frequency as a test of the theory of the selective neutralism of polymorphisms. *Genetics* **74**, 175–195.
- Lively, C. M., Craddock, C. & Vrijenhoek, R. C. (1990). The Red Queen hypothesis supported by parasitism in sexual and clonal fish. *Nature* **344**, 864–866.
- Loudenslager, E. J., Rinne, J. N., Gall, G. A. E. & David, R. E. (1986). Biochemical genetic studies of native Arizona and New Mexico trout. *Southwestern Naturalist* **31**, 221–234.
- Manly, B. F. J. (1991). *Randomization and Monte Carlo Methods in Biology*. New York: Chapman & Hall.
- Maruyama, T. & Kimura, M. (1980). Genetic variability and effective population size when local extinction and recolonization of subpopulations are frequent. *Proceedings of the National Academy of Sciences of the USA* **77**, 6710–6714.
- Meffe, G. K. (1985). Predation and species replacement in American southwestern fishes: a case study. *Southwestern Naturalist* **30**, 173–187.
- Meffe, G. K. (1992). Techno-arrogance and halfway technologies: salmon hatcheries on the Pacific Coast of North America. *Conservation Biology* **3**, 350–354.
- Meffe, G. K. & Vrijenhoek, R. C. (1988). Conservation genetics in the management of desert fishes. *Conservation Biology* **2**, 157–169.

- Meyer, A. (1993). Phylogenetic relationships and evolutionary processes in East African cichlid fishes. *Trends in Ecology and Evolution* **8**, 279–284.
- Miller, P. S. & Hedrick, P. W. (1992). MHC polymorphism and the design of captive breeding programs: simple solutions are not the answer. *Conservation Biology* **5**, 556–558.
- Mitton, J. B. (1997). *Selection in Natural Populations*. Oxford: Oxford University Press.
- Mitton, J. B. & Pierce, B. A. (1980). The distribution of individual heterozygosity in natural populations. *Genetics* **95**, 1043–1054.
- Moran, P., Kornfield, I. & Reinthal, P. N. (1994). Molecular systematics and radiation of the haplochromine cichlids (Teleostei: Perciformes) of Lake Malawi. *Copeia* **1994**, 274–288.
- Nei, M. (1975). *Molecular Population Genetics and Evolution*. Amsterdam: North Holland Publishing.
- Nei, M. & Li, W.-H. (1973). Linkage disequilibrium in subdivided populations. *Genetics* **75**, 213–219.
- Ohta, T. (1981). Associative overdominance caused by linked detrimental mutations. *Genetical Research* **18**, 277–286.
- Ohta, T. (1982). Linkage disequilibrium due to random genetic drift in finite subdivided populations. *Proceedings of the National Academy of Sciences, USA* **79**, 1940–1944.
- Ohta, T. & Kimura, M. (1969). Linkage disequilibrium due to random genetic drift. *Genetical Research* **13**, 47–55.
- Parker, A. & Kornfield, I. (1996). Polygynandry in *Pseudotropheus zebra*, a cichlid fish from Lake Malawi. *Environmental Biology of Fishes* **47**, 345–352.
- Quattro, J. M. & Vrijenhoek, R. C. (1989). Fitness differences among remnant populations of the Sonoran topminnow, *Poeciliopsis occidentalis*. *Science* **245**, 976–978.
- Quattro, J. M., Leberg, P. L., Douglas, M. E. & Vrijenhoek, R. C. (1996). Molecular evidence for a unique evolutionary lineage of endangered Sonoran Desert Fish (genus *Poeciliopsis*). *Conservation Biology* **10**, 128–135.
- Ralls, K. & Ballou, J. (1983). Extinction: lessons from zoos. In *Genetics and Conservation: a Reference for Managing Wild Animal and Plant Populations* (Schonewald-Cox, C. M., Chambers, S. M., McBryde, F. & Thomas, L., eds), pp. 164–184. Menlo Park, CA: Benjamin/Cummings.
- Richards, C. & Leberg, P. L. (1997). Temporal changes in allele frequencies and a population's history of severe bottlenecks. *Conservation Biology* **10**, 832–832.
- Ryman, N. (1983). Patterns of distribution of biochemical genetic variation in salmonids: differentiation between species. *Aquaculture* **33**, 1–21.
- Sheffer, R. J., Hedrick, P. W., Minckley, W. L. & Velasco, A. L. (1997). Fitness in the endangered Gila topminnow. *Conservation Biology* **11**, 162–171.
- Sheffer, R. J., Hedrick, P. W. & Shirley, C. (1998). No bilateral asymmetry in wild-caught endangered *Poeciliopsis o. occidentalis* (Gila topminnows). *Heredity* **80**, 214–217.
- Slatkin, M. (1993). Isolation by distance in equilibrium and non-equilibrium populations. *Evolution* **47**, 264–279.
- Slatkin, M. & Barton, N. H. (1989). A comparison of three indirect methods for estimating average levels of gene flow. *Evolution* **43**, 1349–1368.
- Slatkin, M. & Maddison, W. P. (1990). Detecting isolation by distance using phylogenies of genes. *Genetics* **126**, 249–260.
- Soltz, D. L. & Hirshfield, M. F. (1981). Genetic differentiation of pupfishes (genus *Cyprinodon*) in the American Southwest. In *Fishes of the North American Deserts* (Naiman, R. J. & Soltz, D. L., eds), pp. 291–333. New York: John Wiley & Sons.
- Turelli, M. & Ginzburg, L. R. (1983). Should individual fitness increase with heterozygosity? *Genetics* **104**, 191–209.
- Turner, B. J. (1974). Genetic divergence of Death Valley pupfish species: biochemical versus morphological evidence. *Evolution* **28**, 281–294.

- U.S. Fish and Wildlife Service (1983). *Gila and Yaqui Topminnow Recovery Plan*. Albuquerque, NM: U.S. Fish and Wildlife Service.
- U.S. Fish and Wildlife Service (1993). Draft Gila topminnow recovery plan. Report Albuquerque, New Mexico: U.S. Fish and Wildlife Service.
- Vrijenhoek, R. C. (1978). Coexistence of clones in a heterogeneous environment. *Science* **199**, 549–552.
- Vrijenhoek, R. C. (1989). Genotypic diversity and coexistence among sexual and clonal forms of *Poeciliopsis*. In *Speciation and Its Consequences* (Otte, D. & Endler, J., eds), pp. 386–400. Sunderland, Massachusetts: Sinauer Associates.
- Vrijenhoek, R. C. (1997). Gene flow and genetic diversity in naturally fragmented metapopulations of deep-sea hydrothermal vent animals. *Journal of Heredity* **88**, 285–293.
- Vrijenhoek, R. C. & Leberg, P. L. (1991). Let's not throw the baby out with the bathwater: a comment on management for MHC diversity in captive populations. *Conservation Biology* **5**, 252–253.
- Vrijenhoek, R. C. & Lerman, S. (1982). Heterozygosity and developmental stability under sexual and asexual breeding systems. *Evolution* **36**, 768–776.
- Vrijenhoek, R. C., Douglas, M. E. & Meffe, G. K. (1985). Conservation genetics of endangered fish populations in Arizona. *Science* **229**, 400–402.
- Vrijenhoek, R. C., Pfeiler, E. & Wetherington, J. (1992). Balancing selection in a desert stream-dwelling fish, *Poeciliopsis monacha*. *Evolution* **46**, 1642–1657.
- Waples, R. S. (1987). A multispecies approach to the analysis of gene flow in marine shore fishes. *Evolution* **41**, 385–400.
- Waples, R. S. (1989). A generalized approach for estimating effective population size from temporal changes in allele frequency. *Genetics* **121**, 379–391.
- Waples, R. S. (1992). Pacific salmon, *Oncorhynchus* spp., and the definition of 'species' under the Endangered Species Act. *Marine Fisheries Reviews* **53**, 11–22.
- Weir, B. S. & Cockerham, C. C. (1984). Estimating F-statistics for the analysis of population structure. *Evolution* **38**, 1358–1370.
- Wetherington, J. D., Weeks, S. C., Kotora, K. E. & Vrijenhoek, R. C. (1989). Genotypic and environmental components of variation in growth and reproduction of fish hemiclones (*Poeciliopsis*: Poeciliidae). *Evolution* **43**, 635–645.
- Woodruff, D. S. (1989). The problems of conserving genes and species. In *Conservation for the Twenty-first Century* (Western, D. & Pearl, M., eds), pp. 76–88. Oxford: Oxford University Press.
- Wright, S. (1978). *Evolution and the Genetics of Populations, Vol. 4: Variability Within and Among Natural Populations*. Chicago, IL: The University of Chicago Press.