THE GENETIC STRUCTURE OF ENDANGERED DESERT FISHES: NATURAL VERSUS CAPTIVE POPULATIONS

By

DAVID RAY EDDS

Bachelor of Science

University of Kansas

Lawrence, Kansas

1977

Submitted to the Faculty of the
Graduate College of the
Oklahoma State University
in partial fulfillment of
the requirements for
the Degree of
MASTER OF SCIENCE
July, 1984

Thesis
1984
E21g
Cop.2

THE GENETIC STRUCTURE OF ENDANGERED
DESERT FISHES: NATURAL VERSUS
CAPTIVE POPULATIONS

Thesis Approved:

Anthony A. Echelle
Thesis Adviser

O. Eugene Maughan

Stanley Fox

Michael E. Douglas

Norman N. Durham
Dean of the Graduate College

1189260

ACKNOWLEDGMENTS

Thanks go to my major professor, Dr. Anthony A. Echelle, who gave me the opportunity to study and learn. Thanks to Alice F. Echelle for teaching me electrophoretic techniques. My committee members, Dr. Michael E. Douglas, Dr. Stanley F. Fox, and Dr. O. Eugene Maughan provided helpful discussions. Dr. Douglas was especially helpful with data analysis. Bruce Wagner, Allen Rutherford, and George Cline provided much support, as did other students, faculty, and staff of the Department of Zoology. Financial assistance was provided by the U. S. Fish and Wildlife Service, Contract No. 14-16-0002-82-213. Special thanks go to my parents, Mr. and Mrs. Neil Edds, for their love, support, and teaching throughout my continuing education.

TABLE OF CONTENTS

Chapter | Page

I. INTRODUCTION 1

Factors Causing Genetic Change in Captive Fishes. 4
Historical Perspective. 7
Present and Future Threats. 13

II. MATERIALS AND METHODS. 15

III. RESULTS AND DISCUSSION 20

Cyprinodon bovinus. 20
Cyprinodon elegans. 27
Gambusia nobilis. 35

IV. SUMMARY AND CONCLUSIONS. 47

LITERATURE CITED. 53

APPENDICES. 60

APPENDIX A - GENOTYPIC FREQUENCIES FOR ALL SAMPLES OF *CYPRINODON BOVINUS* 61

APPENDIX B - GENOTYPIC FREQUENCIES FOR ALL SAMPLES OF *CYPRINODON ELEGANS* 63

APPENDIX C - GENOTYPIC FREQUENCIES FOR ALL SAMPLES OF *GAMBUSIA NOBILIS* 66

LIST OF TABLES

Table Page

I. Presumptive Loci, Coded Proteins, Species Surveyed, Tissues Scored, and Analytical Systems Used in Horizontal Starch Gel Electrophoresis 16

II. Allelic Frequencies Among Samples of *Cyprinodon bovinus*. 21

III. Genetic Variability at 28 Loci in Samples of *Cyprinodon bovinus*. 22

IV. Chi-Square Analysis of Heterogeneity at Polymorphic Loci Among Samples of *Cyprinodon bovinus*. 24

V. Matrix of Rogers' (1972) Genetic Distance Coefficients Between Samples of *Cyprinodon bovinus*. 26

VI. Allelic Frequencies Among Samples of *Cyprinodon elegans*. 28

VII. Genetic Variability at 28 Loci in Samples of *Cyprinodon elegans*. 30

VIII. Chi-Square Analysis of Heterogeneity at Polymorphic Loci Among Samples of *Cyprinodon elegans*. 33

IX. Matrix of Rogers' (1972) Genetic Distance Coefficients Between Samples of *Cyprinodon elegans*. 34

X. Allelic Frequencies Among Samples of *Gambusia nobilis*. 36

XI. Genetic Variability at 24 Loci in Samples of *Gambusia nobilis*. 38

XII. Chi-Square Analysis of Heterogeneity at Polymorphic Loci Among Samples of *Gambusia nobilis*. 42

Table | Page

XIII. Matrix of Rogers' (1972) Genetic Distance Coefficients Between Samples of *Gambusia nobilis* 44

LIST OF FIGURES

Figure Page

1. Samples of *Cyprinodon bovinus* and *Gambusia nobilis* from Leon Creek. 9

2. Samples of *Cyprinodon elegans* from the Balmorhea Area 11

CHAPTER I

INTRODUCTION

The U.S. Fish and Wildlife Service is maintaining populations of threatened and endangered fishes at the Dexter, New Mexico, National Fish Hatchery (DNFH). The hatchery provides fish for reintroduction efforts in the event of a catastrophic loss of natural populations and functions as a gene bank for preserving the genetic variability of natural populations. There is some risk, however, that present management programs could exhaust the genetic resources they are intended to preserve. Present management practices at DNFH include obtaining one sample of live fish, usually fewer than 50, from a single collection site and transplanting them into 0.1- to 0.2-hectare hatchery ponds. A number of DNFH populations date to the original progenitor stocks introduced more than a decade ago. No data exist on how well the captive populations at DNFH typify the genetic variability of natural populations.

Recent discovery of reduced genetic variability in hatchery stocks of sport fishes evokes concern for gene bank programs. Allendorf and Phelps (1980) detected significant reduction of genetic variation in a hatchery stock of cutthroat trout when compared to the wild stock from which

it was derived. Ryman and Stahl (1980) detected rapid allelic frequency changes within a hatchery stock of brown trout, as well as differences between the hatchery stock and the natural population from which it was derived. Allendorf and Utter (1979) reported drastically reduced genetic variability in hatchery stocks of rainbow trout.

The genetic variability of a population affects that population's ability to adapt to a changing environment (Fisher 1930; Ayala 1968). It is important, however, to realize that genetic variability is a degradable resource. The major goal for gene bank programs like Dexter National Fish Hatchery should be to maintain high levels of genetic variability (Denniston 1978; Flesness 1977; Foose 1977), for if populations lose genetic variability, the species as a whole becomes a prime candidate for extinction in future variable environments (Wright 1951; Smith and Chesser 1981). In addition, if the founders of new populations are from captive populations which have undergone reduced variability, adaptive capability and hence potential for survival may be reduced.

Genetic variability affects the potential for a population to achieve adaptive characteristics by alterations in frequencies of specific alleles and by controlling the potential for overall heterozygosity per individual. Moon (1975) concluded that maintenance of enzyme heterogeneity in organisms such as salmonids increases flexibility and adaptability to fluctuating thermal regimes. Koehn (1969)

showed that variant allelic products differed in adaptive properties with respect to temperature in the Gila mountain sucker, Catostomus clarkii. Heterozygosity has been correlated with higher reproductive rates in mammals (Smith et al. 1975; Johns et al. 1977), with higher growth rates in oysters (Singh and Zouros 1978), with behavioral characteristics such as levels of aggression in mice (Garten 1976) and juncos (Baker and Fox 1978), with degree of morphological variation (Mitton 1978), and with functional sex ratios in fish (Simanek 1978). Female pigeons heterozygous at the transferrin locus hatch a larger percentage of their eggs than do homozygous females (Frelinger 1972). Smith et al. (1975) concluded that, in general, survivorship is probably higher for the more heterozygous individuals of a population.

Geographic variation in allelic frequencies is known for a wide variety of animals including mice (Selander 1970), sparrows (Baker 1975), lizards (Webster and Burns 1973), newts (Hedgecock 1978), and fish (Turner 1974; Yardley et al. 1974; Avise and Smith 1974; Nyman 1975; Allendorf et al. 1976; Darling 1976; Powers and Place 1978; Winans 1980; Stohl 1981). Genetic heterogeneity in fishes can occur over very short distances (Echelle et al. 1975, 1976; Yardley and Hubbs 1976; Avise and Felley 1979; Ryman et al. 1979; Christiansen et al. 1981; Ryman 1981). Microgeographic subdivision may, in fact, be a general rule for natural populations (Smith et al. 1978).

Knowledge of genetic variability in natural populations is a prerequisite for educated conservation efforts. Hatchery stocks from one segment of natural populations with marked genetic differentiation may not represent the diversity of the natural populations they are intended to preserve. Additionally, potential exists for significant genetic change during captivity (e.g., Allendorf and Phelps 1980; Ryman and Stahl 1980). The present study compares the genetic structure of captive populations at DNFH versus natural populations of three species of endangered fishes, the Leon Springs pupfish, Cyprinodon bovinus, the Comanche Springs pupfish, Cyprinodon elegans, and the Pecos gambusia, Gambusia nobilis. These fishes are restricted to isolated desert spring systems in the Pecos River drainage of western Texas and eastern New Mexico. My purpose was to assess the extent to which captive populations from DNFH typify the genetic resources of progenitor and other natural populations.

Factors Causing Genetic Change in Captive Fishes

Founder effect, inbreeding, genetic drift, and altered selection pressures may all contribute to unwanted genetic change in captive populations at DNFH.

The founder principle states that genotypes of the original stock determine, to a great extent, the gene pool for future generations (Mayr 1942). Founders of a new colony may contain only a small fraction of the total

genetic variation of the parental population. All subsequent evolution in the descendant population will proceed from the original genetic endowment of the founders. Founder effect at DNFH will be heightened by two factors: (1) Samples of natural populations typically are made at a single site, often with just two or three seine hauls. These samples may poorly represent the microgeographic variation of natural gene pools. (2) Populations at DNFH typically are initiated from a small number of individuals (less than 50), and hence may undergo "genetic bottlenecks" causing reduced heterozygosity and loss of rare alleles (Nei et al. 1975).

Founders carry such a small reservoir of genetic diversity that the population founded by them is highly susceptible to inbreeding and genetic drift (Mayr 1963; Dobzhansky 1970). The smaller the population size, the greater are random flunctuations in gene frequencies (genetic drift). At sufficiently small population sizes, drift can reduce genetic variability and, in turn, population fitness (Frankel and Soulé 1981). Production of large numbers of offspring from few parents raises the possibility of genetic deterioration as a result of inbreeding (Wright 1932, 1969). Some degree of reduced viability (inbreeding depression) usually occurs when a normally outbred population is suddenly subjected to intense inbreeding (Lerner 1954). Loss of fertility, increased susceptibility to disease, growth anomalies, and metabolic disturbances are some factors that have been attributed to inbreeding in

captive stocks of fishes (e.g., Ryman 1970; Aulstad and Kittelsen 1971; Kincaid 1976).

Populations at DNFH are initiated by introducing a small number of individuals but the number that survive to reproduce may be even smaller. The effects of genetic drift and inbreeding on the first generation of offspring at DNFH will be accentuated because the effective population size of the founding population is almost certain to be smaller than the actual number stocked (Kimura and Crow 1963). Several factors such as unequal sex ratios, territoriality, overlapping generations, and differential fecundity may act to reduce the effective population size (Falconer 1960; Crow and Kimura 1970).

The potential for genetic change is further enhanced by the ecological uniformity and unnatural character of the environment at DNFH. Selection pressures are drastically changed when fish hatch, breed, and spend their lives in the highly artificial hatchery ponds. In their native habitats, *Cyprinodon bovinus*, *C. elegans*, and *Gambusia nobilis* occupy springfed pools, spring runs, and shallow marshes with qualitatively and quantitiatively different predation/ competition regimes compared to those in hatchery ponds. Turner (1984) suggested that natural selection has played a major role affecting genetic variation and divergence of four refugium populations of the desert pupfish, *Cyprinodon macularius*. Selection for survival in a homogeneous environment could lead to reduced genetic variability in captive stocks at DNFH.

Exposure to new genetic factors may also reduce the diversity of the gene pool of a new colony. Sudden conversion from an open and relatively panmictic population to a small closed population may result in a "genetic revolution" (Mayr 1954), in which genes in homozygous condition are selected over those in heterozygous condition. This, in turn, can have pleiotropic effects at many loci.

Historical Perspective

The Leon Springs pupfish, Cyprinodon bovinus, the Comanche Springs pupfish, Cyprinodon elegans, and the Pecos gambusia, Gambusia nobilis, are endangered species as defined by the Endangered Species Act (U.S. Dept. Interior 1983).

Cyprinodon bovinus Baird and Girard, was originally discovered in 1851 by personnel of the U.S.-Mexico Boundary Survey seeking water (Gehlbach 1981). The type locality is Leon Springs, ca. 7 km north of Fort Stockton, Pecos County, Texas (Baird and Girard 1853). However, flow at Leon Springs declined to zero in the 1940's due to habitat alteration (Kennedy 1977), and the population at the type locality was extirpated. The species was believed extinct until recently, when it was rediscovered in Leon Creek, a few miles downstream from the type locality (Echelle and Miller 1974). The Leon Springs pupfish now occurs only in Diamond-Y Spring and in the lower portion of Leon Creek, 16 km north of Fort Stockton, Pecos County, Texas. The short, springfed area of Leon Creek is divided into two

separated units: an upper and a lower watercourse, approximately 2 and 4 km long, respectively (Figure 1). Between the units, the flow sinks into the soil. The resulting dry region is seldom less than 1 km long and the two segments are likely connected only during storm events (Hubbs et al. 1978). Springs occur throughout the watercourse and typically have high levels of chlorides, silica, and sulphates (Rohde and Guillory 1980). The available habitat ranges from springheads and spring runs to downstream areas with no marked spring influence. The pupfish is abundant throughout the system. Details of the ecology of Leon Creek and of the life history of C. bovinus are reported by Kennedy (1977).

In November of 1974, a sample of 30-35 adults was taken from the lower watercourse of Leon Creek at the Texas State Highway 18 bridge and transplanted to DNFH (A. A. Echelle, pers. comm.). In February of 1976, a sample of C. bovinus from the upper watercourse was taken to DNFH (Hubbs 1980), and DNFH records show that the sample was released into the previously established captive population (B. Jenson, pers. comm.). The present DNFH population consists of more than 1000 adults, primarily derived from the lower watercourse of Leon Creek.

Cyprinodon elegans Baird and Girard, the Comanche Springs pupfish, was also discovered by personnel of the U.S.-Mexico Boundary Survey in 1851 (Gehlbach 1981). The type locality, Comanche Springs, within the present city

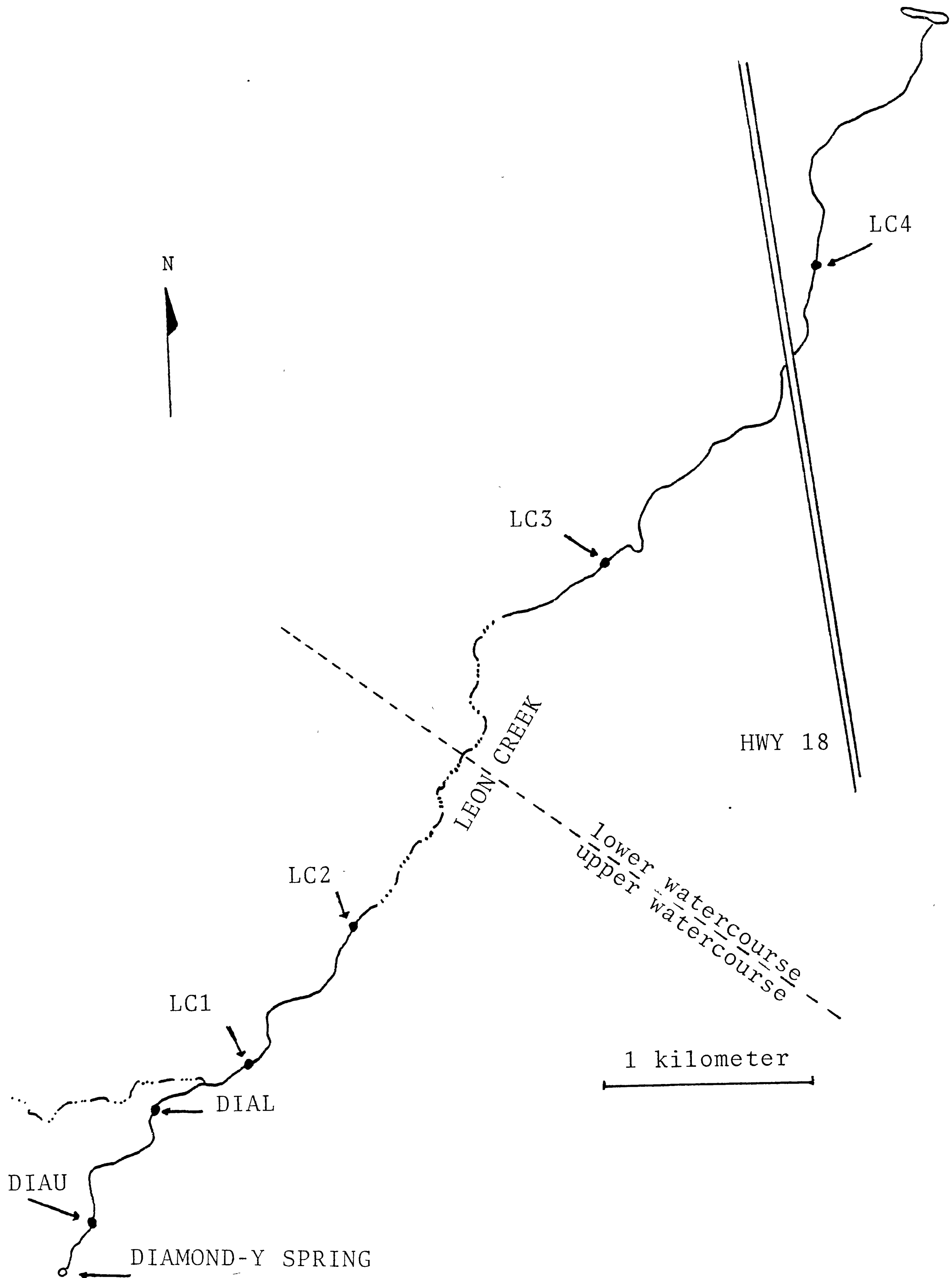

Figure 1. Samples of *Cyprinodon bovinus* and *Gambusia nobilis* from Leon Creek. (Acronyms refer to collection sites described in text.)

limits of Fort Stockton, Pecos County, Texas, has gone dry, eliminating the population (Miller 1961). The Comanche Springs pupfish now occurs only in a system of interconnected freshwater springs near Balmorhea, in Reeves and Jeff Davis counties, Texas. Earthen irrigation ditches and concrete flumes leading from Phantom Lake Spring, San Solomon Spring, and Giffin Spring represent most of the present habitat of the species (Figure 2). A small population also occurs in the nearby Toyah Creek. The pupfish population is sparse throughout most of the canal system, but occasionally is abundant in short segments. The flumes are characterized by swift current from constant spring discharge, but in downstream areas water flow varies with irrigation demands. A morphological analysis by Echelle (1975) revealed two extant morphs of the species: a streamlined form with reduced ventral scalation in the outflow of Phantom Lake Spring and a deep-bodied form with almost complete ventral scalation in Toyah Creek. The Phantom Lake Spring and Toyah Creek populations also differ in certain aspects of sexual dimorphism, while the Giffin Spring (includes Echelle's 1975 "San Solomon Spring") population is morphologically intermediate between these two forms.

Present contact between the two forms of _C. elegans_ apparently resulted from human activities. Prior to construction of the irrigation system, Phantom Lake Spring was isolated by more than 80 water-kilometers from the San

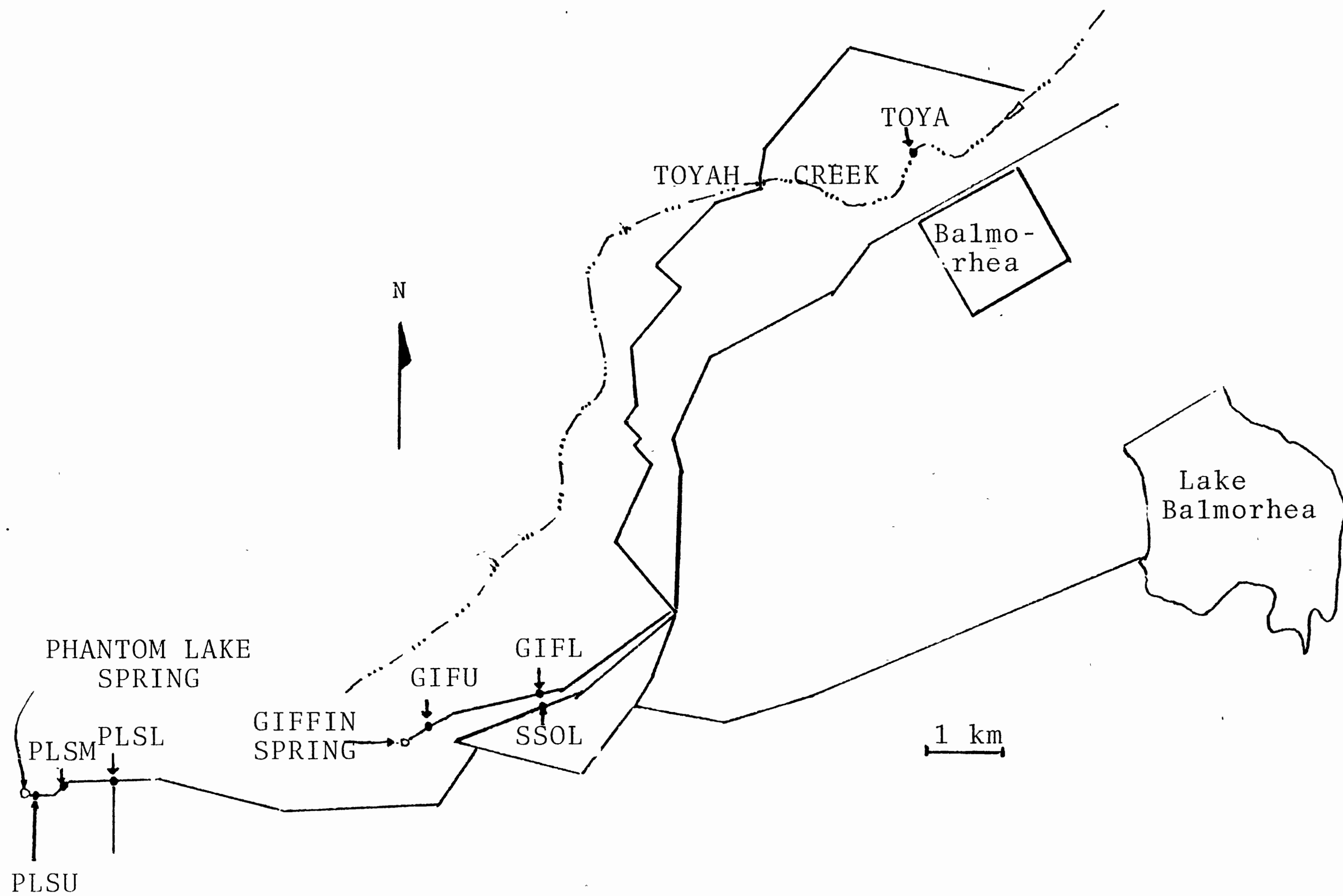

Figure 2. Samples of _Cyprinodon_ _elegans_ from the Balmorhea area. (Acronyms refer to collection sites described in text.)

Solomon Spring-Giffin Spring-Toyah Creek system (White et al. 1938). *Cyprinodon elegans* is a spring dweller poorly adapted for downstream environments (Stevenson and Buchanan 1973); thus, prior to human effects, Phantom Lake Spring populations would have been geographically isolated from other populations.

The Texas Parks and Wildlife Department constructed a small refugium at the Balmorhea State Recreation Area in 1974 to provide a stable, flowing-water habitat for a small population of *C. elegans*. The canal was stocked with adults from Giffin Spring and now supports a population of several thousand pupfish. In 1974, a sample of about 40 *C. elegans* was taken from an irrigation canal leading from Giffin Spring and transplanted to a hatchery pond at DNFH (A. A. Echelle, pers. comm.). The pond now supports more than 1000 adults of the species.

Gambusia nobilis Baird and Girard, the Pecos gambusia, occurs in four areas of the Pecos River drainage: (1) Leon Creek near Fort Stockton, Pecos County, Texas; (2) the springfed irrigation system near Balmorhea, Reeves and Jeff Davis counties, Texas; (3) Blue Spring near Black River Village, Eddy County, New Mexico; and (4) Bitter Lake National Wildlife Refuge near Roswell, Chaves County, New Mexico. Though its range is limited, the species is locally abundant in these areas. The type locality is Leon and Comanche Springs, near Fort Stockton, Pecos County, Texas (Hubbs and Springer 1957).

The Pecos gambusia occurs in a variety of habitats, with densest populations in sedge-covered marshes directly influenced by spring flow. The species also occurs in springheads, spring runs, downstream areas with no noticeable spring influence, and gypsum sinkholes with no surface flow. Absence and low abundance is associated with unstable habitats and lack of overhead cover. The strong preference for springfed habitats by the Phantom Lake Spring population, like that of the Comanche Springs pupfish, probably means that prior to human intervention the population was geographically isolated from other populations in the Balmorhea area. Echelle and Echelle (1980) reported that the Balmorhea population is declining in abundance and that it shows marked genetic and color divergence from populations in other areas.

In 1974, a sample of 30-40 _G. nobilis_ was taken from Leon Creek at the Texas State Highway 18 bridge and transplanted to a hatchery pond at DNFH (A. A. Echelle, pers. comm.). The introduced population reached large numbers and thrived. In the summer of 1981, the DNFH population was destroyed because the Leon Creek population seemed in little immediate danger. Presently, there is no population of _G. nobilis_ at DNFH.

Present and Future Threats

There are several major threats to _C. bovinus_, _C. elegans_, and _G. nobilis_: (1) habitat destruction;

(2) competition with introduced species; (3) degradation of genetic integrity caused by hybridization with introduced congeners; and (4) their rarity and restricted natural ranges.

CHAPTER II

MATERIALS AND METHODS

Samples were collected by seine 12-18 August 1982 throughout each species' natural range. Fish were immediately frozen on dry ice, and stored in the laboratory at -70 degrees C. One sample each of *C. bovinus* and *C. elegans* was collected 12 August from captive populations at DNFH. A sample of DNFH *G. nobilis* was frozen and shipped to Oklahoma State University when the captive population was terminated in 1981. Separate extracts from eye-brain, liver, and epaxial muscle tissue from each individual were subjected to standard methods of horizontal starch gel electrophoresis (e.g., Siciliano and Shaw 1976). I examined the protein products of 24-28 presumptive gene loci (Table I). Variance in sample sizes results from an occasional individual that could not be scored.

The nomenclatural system for electromorphs used herein is as follows. Enzyme abbreviations are shown in Table I. When designating a locus, the abbreviation is italicized. Individual loci in multiple-locus systems are assigned a hyphenated upper case letter which indicates the sequence of decreasing anodal mobility (e.g., *Phi-A* = most anodal Phi system, *Phi-B* = second-most anodal system, etc.).

TABLE I

PRESUMPTIVE LOCI, CODED PROTEINS, SPECIES SURVEYED, TISSUES SCORED, AND ANALYTICAL SYSTEMS USED IN HORIZONTAL STARCH GEL ELECTROPHORESIS

Protein	Abbreviation	Locus	Species Surveyed[1]	Tissues Scored[2]	Analytical System[3]
Adenylate kinase	AK	Ak-B	b,e,n	M*	1
Alcohol dehydrogenase	ADH	Adh	b,e,n	L	2
Aldolase	ALD	Ald	b,e,n	E*	2
Aspartate aminotransferase	AAT	Aat-A	b,e,n	L	1
Aspartate aminotransferase	AAT	Aat-B	b,e,n	M	1
Aspartate aminotransferase	AAT	Aat-C	b,e,n	L	1
Creatine kinase	CK	Ck-A	b,e,n	E	2
Creatine kinase	CK	Ck-B	b,e,n	E	2
Creatine kinase	CK	Ck-C	b,e	E*	2
Fumarase	FUM	Fum	b,e,n	M	2
General protein	GP	Gp	n	M	2
Glucose-6-phosphate dehydrogenase	G-6-PDH	G6pdh	b,e	L	2
Glyceraldehyde-3-phosphate dehydrogenase	GA-3-PDH	Ga3pdh-A	b,e,n	E	2
Glyceraldehyde-3-phosphate dehydrogenase	GA-3-PDH	Ga3pdh-B	b,e,n	E	2
Glycerol-3-phosphate dehydrogenase	G-3-PDH	G3pdh	b,e	M	1
Indophenol oxidase	IPO	Ipo	b,e,n	L	1
Isocitrate dehydrogenase	IDH	Idh-A	b,e,n	L	3
Isocitrate dehydrogenase	IDH	Idh-B	b,e,n	M	3
Lactate dehydrogenase	LDH	Ldh-A	b,e,n	E	2
Lactate dehydrogenase	LDH	Ldh-B	b,e,n	E	2
Lactate dehydrogenase	LDH	Ldh-C	b,e,n	E	2
Malate dehydrogenase	MDH	Mdh-A	b,e,n	L	3
Malate dehydrogenase	MDH	Mdh-B	b,e,n	L	3
Malate dehydrogenase	MDH	Mdh-C	b,e,n	M	3

TABLE I (Continued)

Protein	Abbre-viation	Locus	Species Surveyed[1]	Tissues Scored[2]	Analy-tical System[3]
6-Phosphogluconate dehydrogenase	6-PGD	*6Pgd*	b,e,n	L	2
Phosphoglucomutase	PGM	*Pgm*	b,e,n	L	1
Phosphohexose isomerase	PHI-A	*Phi-A*	b,e,n	L	2
Phosphohexose isomerase	PHI-B	*Phi-B*	b,e,n	L	2
Xanthine dehydrogenase	XDH	*Xdh*	b,e	L	2

[1]b = *C. bovinus*; e = *C. elegans*; n = *G. nobilis*.

[2]e = eye, brain; 1 = liver; m = muscle; tissue distributions generally are as given by Turner (1983) except that our Aat-B was scored from muscle, while according to Turner this locus is present only in the liver in cyprinodontoid fishes. Asterisks indicate loci not examined by Turner.

[3]Analytical systems as follows: 1. Electrode buffer: 0.1 M Trishydroxymethyl-aminomethane (= "Tris"), 0.1 M maleic acid, 0.01 M disodium EDTA, 0.01 M $MgCl_2$, pH 7.4; gel buffer: 1 in 10 dilution of electrode buffer. 2. Stock solution: 0.9 M Tris, 0.5 M boric acid, 0.1 M disodium EDTA, pH 8.6; electrode buffer: 1 vol. stock solution + 6.9 vols H_2O; gel buffer: 1 vol stock solution + 24 vols H_2O. 3. Electrode buffer: 0.7 M Tris, 0.17 M citric acid, diluted to 1 liter, pH 8.0; gel buffer: 0.02 M Tris, 0.006 M citric acid, diluted to 1 liter, pH 8.0.

Alleles are designated alphabetically in the order of decreasing anodal mobility by adding to the locus designation a hyphenated lower case letter; they are not italicized (e.g., Phi-A-a).

The sampled locations, by species, are as follows:

Cyprinodon bovinus (Figure 1). (1) Diamond-Y Spring (DIAY), combined collections from upper Diamond-Y Spring (DIAU) and lower Diamond-Y Spring (DIAL); (2) Leon Creek, beginning of upper watercourse (LC1); (3) Leon Creek, end of upper watercourse (LC2); (4) Leon Creek, beginning of lower watercourse (LC3); (5) Leon Creek, end of lower watercourse (LC4); (6) DNFH.

Cyprinodon elegans (Figure 2). (1) upper Phantom Lake Spring (PLSU); (2) middle Phantom Lake Spring (PLSM); (3) lower Phantom Lake Spring (PLSL); (4) upper Giffin Spring (GIFU); (5) lower Giffin Spring (GIFL); (6) lower San Solomon Spring (SSOL); (7) Toyah Creek (TOYA); (8) DNFH.

Gambusia nobilis (see Figure 1 for locations 11-16; maps of other locations are given by Echelle and Echelle 1980). (1) Bitter Lake National Wildlife Refuge, sinkhole 20 (BLNR); (2) Bitter Lake National Wildlife Refuge, Sago Spring (SAGO); (3) upper Blue Spring (BSU); (4) middle Blue Spring (BSM); (5) lower Blue Spring (BSL); (6) upper Phantom Lake Spring (PLSU); (7) middle Phantom Lake Spring (PLSM); (8) lower Phantom Lake Spring (PLSL); (9) Giffin Spring (GIFN); (10) East Sandia Spring near Balmorhea, Texas (ESAN); (11) upper Diamond-Y Spring (DIAU); (12) lower Diamond-Y

Spring (DIAL); (13) Leon Creek, beginning of upper watercourse (LC1); (14) Leon Creek, end of upper watercourse (LC2); (15) Leon Creek, beginning of lower watercourse (LC3); (16) Leon Creek, end of lower watercourse (LC4), (17) DNFH.

The following analyses were done with the BIOSYS-1 computer program (Swoffard and Selander 1981). The chi-square goodness of fit test allowed tests of agreement of observed genotypic frequencies with expectations from Hardy-Weinberg equilibrium at each variable locus in each population. Difficulties with using the chi-square distribution for small samples were avoided by computing exact significance probabilities (analogous to Fisher's exact test) and Levene's (1949) correction for small sample size. Tests for heterogeneity of gene frequencies among samples were performed with the chi-square statistic in an MxN contingency table with (M-1)(N-1) degrees of freedom, where M was the number of populations and N the number of alleles. Results were interpreted with caution when, occasionally, expected frequencies for some classes were low. Allelic frequencies were used to compute Rogers' (1972) genetic distance, a coefficient of dissimilarity, between all pairs of samples within each species.

CHAPTER III

RESULTS AND DISCUSSION

Cyprinodon bovinus

Twenty-eight systems of electromorphs (loci) representing 27 enzymes and one general protein were resolved for *C. bovinus*. Five loci were polymorphic at the .95 level (criterion for polymorphism = frequency of most common allele less than .95). Frequencies of alleles at the five polymorphic loci are given in Table II.

Mean number of alleles per locus, proportion of loci polymorphic, and mean heterozygosity for the captive population were similar to that of the natural population (Table III). The DNFH sample contained all alleles detected in natural populations of *C. bovinus* except Phi-A-a. The average frequency of Phi-A-a in the five samples of natural populations was .000-.078 ($\bar{x}$ = .018). Assuming a frequency of .018 at DNFH, the chance of missing Phi-A-a in my sample of 51 specimens is .16. Thus at the 95% level of confidence, I cannot reject the hypothesis that the allele is present at DNFH, but excluded from the sample by chance alone. No locus deviated significantly from Hardy-Weinberg equilibrium. There was no evidence of marked heterozygote deficiencies; thus inbreeding effects were not evident, either at DNFH

TABLE II

ALLELIC FREQUENCIES AMONG SAMPLES OF *CYPRINODON BOVINUS*[1]

Locus	Sample					
	DIAY	LC1	LC2	LC3	LC4	DNFH
Aat-C	(13)	(45)	(45)	(51)	(21)	(48)
a	0.500	0.556	0.567	0.461	0.571	0.615
b	0.500	0.444	0.433	0.539	0.429	0.385
Adh	(13)	(45)	(46)	(51)	(21)	(51)
a	0.000	0.000	0.000	0.059	0.000	0.098
b	1.000	1.000	1.000	0.941	1.000	0.902
Ald	(13)	(45)	(46)	(51)	(21)	(48)
a	0.769	0.822	0.837	0.912	0.929	0.823
b	0.231	0.178	0.163	0.088	0.071	0.177
6Pgd	(13)	(45)	(46)	(51)	(21)	(51)
a	0.115	0.011	0.033	0.069	0.048	0.010
b	0.885	0.989	0.967	0.931	0.952	0.990
Phi-A	(13)	(45)	(46)	(51)	(21)	(51)
a	0.000	0.011	0.000	0.078	0.000	0.000
b	0.231	0.233	0.261	0.186	0.167	0.167
c	0.769	0.756	0.739	0.735	0.833	0.833

[1]Sample size in parentheses.

TABLE III

GENETIC VARIABILITY AT 28 LOCI IN SAMPLES OF CYPRINODON BOVINUS[1]

Sample	Mean Sample Size Per Locus	Mean Number of Alleles Per Locus	Percentage of Loci Polymorphic[2]	Mean Heterozygosity	
				Direct-Count	HDYWBG Expected[3]
DIAY	13.0 (0.0)	1.1 (0.1)	14.3	0.055 (0.027)	0.053 (0.026)
LC1	45.0 (0.0)	1.2 (0.1)	10.7	0.044 (0.024)	0.043 (0.024)
LC2	46.0 (0.0)	1.1 (0.1)	10.7	0.043 (0.023)	0.044 (0.024)
LC3	51.0 (0.0)	1.2 (0.1)	17.9	0.044 (0.021)	0.047 (0.024)
LC4	21.0 (0.0)	1.1 (0.1)	10.7	0.041 (0.024)	0.036 (0.021)
DNFH	50.8 (0.1)	1.2 (0.1)	14.3	0.041 (0.020)	0.045 (0.022)

[1]Standard errors in parentheses.

[2]A locus is considered polymorphic if the frequency of the most common allele does not exceed 0.95.

[3]Unbiased estimate (see Nei 1978).

or in natural populations. Genotypic frequencies for all samples, as well as fixation indices, which measure the percent gain in homozygotes above that predicted from Hardy-Weinberg equilibrium, are given in Appendix A.

Heterogeneity chi-square analysis of allelic frequencies reveals statistically significant heterogeneity at two of five polymorphic loci in natural populations. Samples LC1 and LC2 from the upper watercourse of Leon Creek were statistically homogeneous across all five polymorphic loci. However, significant heterogeneity occurred at *6Pgd* when the Diamond-Y Spring sample was added to the set (Table IV). Adding samples LC3 and LC4 from the lower watercourse of Leon Creek produced highly significant heterogeneity at two additional loci (*Adh*, *Phi-A*). However heterogeneity at *6Pgd* disappeared with the addition of these samples because, on the average, LC3 and LC4 had intermediate frequencies between that at Diamond-Y Spring and those at LC1 and LC2.

Adding the DNFH sample to the analysis allowed assessment of whether the captive population contributes added heterogeneity beyond that present in natural populations (Table IV). Adding DNFH to the set representing all natural populations reduced the probability of homogeneity at three of the five polymorphic loci, restored heterogeneity at *6Pgd*, and raised the number of loci showing significant heterogeneity from two to three. These results indicate that, overall, allelic frequencies among samples from natural populations are more similar than allelic frequencies between them and

TABLE IV

CHI-SQUARE ANALYSIS OF HETEROGENEITY AT POLYMORPHIC LOCI AMONG SAMPLES OF *CYPRINODON BOVINUS*

	Samples					
Locus	DIAY, LC1, LC2	DIAY, LC1, LC2, DNFH	LC3, LC4	LC3, LC4, DNFH	DIAY, LC1, LC2, LC3, LC4	DIAY, LC1, LC2, LC3, LC4, DNFH
Aat-C	ns*	ns	ns	ns	ns	ns
Adh	m**	$\chi^2 = 21.073$ 3 df $P = .0001$	ns	ns	$\chi^2 = 14.961$ 4 df $P < .005$	$\chi^2 = 22.632$ 5 df $P = .0004$
Ald	ns	ns	ns	ns	ns	ns
6Pgd	$\chi^2 = 6.750$ 2 df $P < .04$	$\chi^2 = 10.280$ 3 df $P < .02$	ns	ns	ns	$\chi^2 = 11.209$ 5 df $P < .05$
Phi-A	ns	ns	ns	$\chi^2 = 12.149$ 4 df $P < .02$	$\chi^2 = 18.270$ 8 df $P < .02$	$\chi^2 = 27.256$ 10 df $P < .003$

*Not significant at the .05 level.

**Monomorphic.

DNFH. This trend is further indicated by genetic distance (D) between samples (Table V). D-values computed from all 28 loci ranged from .003 to .013 ($\bar{x}$ = .010 ± .001 SE) among the samples of natural populations and from .008 to .016 ($\bar{x}$ = .012 ± .002) between them and DNFH. Although these distributions of D-values were not statistically significant (Mann-Whitney U = 33, n_1 = 10, n_2 = 5, P > .10 one-tailed), the direction of the difference is consistent with the locus-by-locus analysis of heterogeneity.

Comparison of the DNFH sample with its specific progenitor population in the lower watercourse of Leon Creek is confounded by uncertainties associated with the appearance in August, 1974, of the exotic congener, *C. variegatus*. An August, 1974, sample made by R. D. Suttkus contained specimens which appeared to be relatively "pure" *C. variegatus*, and there was little evidence of hybridization in the collection, while samples taken in 1975 contained large numbers of apparent hybrids (A. A. Echelle, pers. comm.). The DNFH population is derived from a sample taken in November of 1974, after the introduction of *C. variegatus*, but presumably before extensive contamination of the *C. bovinus* genome; the sample contained no obvious phenotypic evidence of *C. variegatus* influence (A. A. Echelle, pers. comm.). In February, 1976, the entire lower watercourse was poisoned with rotenone (see Hubbs et al. 1978). Two weeks later, the lower watercourse was examined for fish. None were found, and at that time *C. bovinus* from Diamond-Y

TABLE V

MATRIX OF ROGERS' (1972) GENETIC DISTANCE COEFFICIENTS BETWEEN SAMPLES OF *CYPRINODON BOVINUS*

Sample	DIAY	LC1	LC2	LC3	LC4
LC1	0.008	-	-	-	-
LC2	0.009	0.003	-	-	-
LC3	0.013	0.013	0.013	-	-
LC4	0.013	0.008	0.007	0.011	-
DNFH	0 016	0.008	0.010	0.015	0.010

Spring were introduced into the lower watercourse. However, the subsequent appearance of hybrid-like individuals indicated that the poisoning effort was not completely successful. During a subsequent series of repeated visits to Leon Creek, the lower watercourse was seined intensively and all hybrid-like specimens were killed (Hubbs et al. 1980). By 1978, there were no overt signs of C. variegatus traits in the population, and none were present in my 1982 samples.

The history of the present population in the lower watercourse limits my ability to directly assess the amount of change that has occurred between the DNFH population and that at the site from which the original DNFH stock was taken. Nonetheless, the DNFH population is only slightly divergent from present populations in Leon Creek. My data show no loss of heterozygosity, only minor (albeit statistically significant) changes in allelic frequencies, and possible loss of one allele present in low frequency in natural populations.

Cyprinodon elegans

Six of 28 loci I examined were polymorphic in C. elegans. The DNFH sample contained all alleles detected in natural populations except Ck-C-a (Table VI). This allele occurred at a frequency of .042 in the sample of the progenitor population (GIFL). The chance of missing an allele at that frequency in my sample of 30 specimens is .08. Thus at the 95% level of confidence, I cannot

TABLE VI

ALLELIC FREQUENCIES AMONG SAMPLES OF *CYPRINODON ELEGANS*[1]

Locus	Sample							
	PLSU	PLSM	PLSL	GIFU	GIFL	SSOL	TOYA	DNFH
Adh	(12)	(36)	(33)	(25)	(46)	(54)	(50)	(46)
a	0.833	0.597	0.500	0.440	0.413	0.463	0.290	0.228
b	0.167	0.403	0.500	0.560	0.587	0.537	0.710	0.772
Ck-A	(12)	(39)	(33)	(25)	(49)	(54)	(50)	(46)
a	0.125	0.192	0.136	0.000	0.051	0.065	0.060	0.054
b	0.875	0.808	0.864	1.000	0.949	0.935	0.940	0.946
Ck-C	(12)	(39)	(33)	(25)	(48)	(54)	(51)	(30)
a	0.000	0.000	0.000	0.000	0.042	0.000	0.010	0.000
b	1.000	1.000	1.000	1.000	0.958	1.000	0.990	1.000
Phi-B	(12)	(38)	(33)	(25)	(49)	(54)	(51)	(46)
a	0.250	0.539	0.333	0.000	0.020	0.185	0.039	0.043
b	0.750	0.461	0.667	1.000	0.980	0.815	0.961	0.957
Idh-A	(11)	(35)	(32)	(21)	(45)	(53)	(49)	(41)
a	0.136	0.271	0.125	0.167	0.556	0.245	0.469	0.646
b	0.864	0.729	0.875	0.833	0.444	0.755	0.531	0.354
Pgm	(12)	(38)	(33)	(25)	(47)	(54)	(51)	(44)
a	0.083	0.000	0.091	0.000	0.191	0.093	0.265	0.159
b	0.917	1.000	0.909	1.000	0.809	0.907	0.735	0.841

[1]Sample sizes in parentheses.

reject the hypothesis that the allele is present at DNFH, but excluded from the sample by chance. Mean number of alleles per locus, proportion of loci polymorphic, and mean heterozygosity for the captive population were similar to that of natural populations (Table VII). Genotypic frequencies for all samples, as well as fixation indices are given in Appendix B. Statistically significant deviation from Hardy-Weinberg equilibrium was detected at Pgm for the PLSU sample ($P < .05$), at Adh for PLSM ($P < .04$), at Adh for TOYA ($P < .02$), and at Adh for DNFH ($P < .001$). All significant deviation was due to heterozygote deficiency. This deficiency may or may not result from inbreeding. In the absence of strong selection, inbreeding should cause heterozygote deficiency across all polymorphic loci. However, selection favoring heterozygotes could counteract increased homozygosity caused by inbreeding in some loci. Although the PLSM sample showed a positive fixation index at four of six polymorphic loci (Appendix B), indicating an increase in homozygosity, the fixation index was not statistically significant at two of the four. The consistent deficiency (six of eight samples) at Adh is intriguing, but it alone is not sufficient to indicate inbreeding. The DNFH sample showed a higher heterozygote deficiency for Adh than did any other samples; however, a slight excess occurred at the other loci.

Gene frequencies at each of the six polymorphic loci are significantly heterogeneous among the seven natural

TABLE VII

GENETIC VARIABILITY AT 28 LOCI IN SAMPLES OF *CYPRINODON ELEGANS*[1]

Sample	Mean Sample Size Per Locus	Mean Number of Alleles Per Locus	Percentage of Loci Polymorphic[2]	Mean Heterozygosity	
				Direct-Count	HDYWBG Expected[3]
PLSU	12.0 (0.0)	1.2 (0.1)	17.9	0.048 (0.024)	0.047 (0.020)
PLSM	38.7 (0.2)	1.1 (0.1)	14.3	0.046 (0.022)	0.061 (0.029)
PLSL	33.0 (0.0)	1.2 (0.1)	17.9	0.067 (0.031)	0.057 (0.026)
GIFU	24.9 (0.1)	1.1 (0.0)	7.1	0.020 (0.014)	0.028 (0.020)
GIFL	48.6 (0.2)	1.2 (0.1)	14.3	0.050 (0.024)	0.054 (0.026)
SSOL	54.0 (0.0)	1.2 (0.1)	17.9	0.049 (0.022)	0.053 (0.024)

TABLE VII (Continued)

Sample	Mean Sample Size Per Locus	Mean Number of Alleles Per Locus	Percentage of Loci Polymorphic[2]	Mean Heterozygosity	
				Direct-Count	HDYWBG Expected[3]
TOYA	50.9	1.2	14.3	0.044	0.054
	(0.1)	(0.1)		(0.020)	(0.026)
DNFH	45.2	1.2	14.3	0.040	0.046
	(0.6)	(0.1)		(0.020)	(0.022)

[1]Standard errors in parentheses.

[2]A locus is considered polymorphic if the frequency of the most common allele does not exceed 0.95.

[3]Unbiased estimate (see Nei 1978).

populations (Table VIII). Significant heterogeneity at Adh, Ck-A, and Ck-C is due to the Phantom Lake Spring (PLS) samples; removal of those samples gives non-significant heterogeneity at those loci. Adding DNFH to the reduced set (natural populations minus PLS) restores heterogeneity at Adh and at Ck-C (Table VIII).

The upper Giffin Spring and lower Giffin Spring populations are significantly heterogeneous at two of the six polymorphic loci, Idh-A and Pgm. Inclusion of the DNFH data adds Adh to the number of significantly heterogeneous loci. The DNFH sample and the sample from its progenitor population (GIFL) show significant heterogeneity at Adh ($X^2 = 7.21$, 1 df, $P < .008$). The DNFH sample lacked an allele (Ck-C-b) present in low frequency (.042) in the progenitor population.

Genetic distance coefficients (Rogers 1972) ranged from .014 to .050 ($\bar{x} = .030 \pm .003$ SE) among samples of natural populations, and from .013 to .055 ($\bar{x} = .035 \pm .006$) between the DNFH sample and those from natural populations (Table IX). These distributions were not significantly different (Mann-Whitney U test, $P > .40$, one-tailed). The DNFH sample was most similar to the Toyah Creek population and its progenitor population from lower Giffin Spring ($D = .013$); it was least similar to the Phantom Spring populations (.044-.055).

In summary, statistically significant heterogeneity existed among natural populations of *C. elegans*, even when

TABLE VIII

CHI-SQUARE ANALYSIS OF HETEROGENEITY AT POLYMORPHIC LOCI AMONG SAMPLES OF *CYPRINODON ELEGANS*

Locus	Samples					
	GIFU, GIFL	GIFU, GIFL, DNFH	GIFU, GIFL, SSOL, TOYA	GIFU, GIFL, SSOL, TOYA, DNFH	PLSU, PLSM, PLSL, GIFU, GIFL, SSOL, TOYA	PLSU, PLSM, PLSL, GIFU, GIFL, SSOL, TOYA, DNFH
Adh	ns*	$\chi^2 = 9.414$ 2 df $P < .01$	ns	$\chi^2 = 16.492$ 4 df $P < .003$	$\chi^2 = 31.894$ 6 df $P < .00002$	$\chi^2 = 49.432$ 7 df $P < .00001$
Ck-A	ns	ns	ns	ns	$\chi^2 = 21.544$ 6 df $P < .002$	$\chi^2 = 23.721$ 7 df $P < .002$
Ck-C	ns	ns	ns	$\chi^2 = 9.801$ 4 df $P < .05$	$\chi^2 = 13.625$ 6 df $P < .04$	$\chi^2 = 15.748$ 7 df $P < .03$
Idh-A	$\chi^2 = 17.651$ 1 df $P < .00005$	$\chi^2 = 26.655$ 2 df $P < .00001$	$\chi^2 = 31.320$ 3 df $P < .00001$	$\chi^2 = 48.513$ 4 df $P < .00001$	$\chi^2 = 55.300$ 6 df $P < .00001$	$\chi^2 = 83.445$ 7 df $P < .00001$
Pgm	$\chi^2 = 10.942$ 1 df $P < .001$	$\chi^2 = 10.599$ 2 df $P < .005$	$\chi^2 = 22.668$ 3 df $P < .0001$	$\chi^2 = 22.588$ 4 df $P < .0002$	$\chi^2 = 43.196$ 6 df $P < .00001$	$\chi^2 = 42.563$ 7 df $P < .00001$
Phi-B	ns	ns	$\chi^2 = 29.890$ 3 df $P < .00001$	$\chi^2 = 33.354$ 4 df $P < .00001$	$\chi^2 = 118.777$ 6 df $P < .00001$	$\chi^2 = 141.724$ 7 df $P < .00001$

*Not significant at the .05 level.

TABLE IX

MATRIX OF ROGERS' (1972) GENETIC DISTANCE COEFFICIENTS BETWEEN SAMPLES OF *CYPRINODON ELEGANS*

Sample	PLSU	PLSM	PLSL	GIFU	GIFL	SSOL	TOYA
PLSM	0.029	-	-	-	-	-	-
PLSL	0.016	0.021	-	-	-	-	-
GIFU	0.031	0.035	0.024	-	-	-	-
GIFL	0.046	0.049	0.038	0.026	-	-	-
SSOL	0.022	0.026	0.014	0.016	0.024	-	-
TOYA	0.048	0.050	0.040	0.030	0.012	0.026	-
DNFH	0.052	0.055	0.044	0.034	0.013	0.031	0.013

the divergent Phantom Lake Spring samples were excluded from the analysis. Adding DNFH to sets of samples from populations in the vicinity of the progenitor population (i.e., excluding Phantom Lake Spring) always increased the degree of heterogeneity. Comparison of the DNFH sample with its progenitor population revealed significant heterogeneity at one of six polymorphic loci and possible loss of one rare allele. However, despite measurable divergence, DNFH resembled natural populations in number of alleles per locus, proportion of polymorphic loci, and mean heterozygosity per individual. Thus, the overall change that has occurred in captivity appears slight.

Gambusia nobilis

Twenty-four loci were resolved clearly in all samples of *G. nobilis*. Six loci were polymorphic. Frequencies of alleles are shown for all samples in Table X.

Measures of genetic variability across all populations are quite diverse (Table XI). Percentages of loci polymorphic range from zero to 16.7; mean heterozygosities range from .004 ± .003 SE to .055 ± .029 based on direct counts of genotypes, and from .004 ± .003 to .067 ± .034 based on allelic frequencies and Hardy-Weinberg expectations (Nei 1978). Genotypic frequencies for all samples, as well as fixation indices are given in Appendix C.

Statistically significant deviation from Hardy-Weinberg equilibrium occurred at *Fum* for the LC4 sample ($P < .001$),

TABLE X

ALLELIC FREQUENCIES AMONG SAMPLES OF *GAMBUSIA NOBILIS*[1]

Locus	Sample									
	BLSH	SAGO	BSU	BSM	BSL	PLSC	PLS2	PLS3	GIFN	ESAN
Adh	(33)	(31)	(31)	(31)	(30)	(32)	(32)	(32)	(31)	(33)
a	1.000	1.000	1.000	1.000	1.000	0.063	0.172	0.219	0.790	0.636
b	0.000	0.000	0.000	0.000	0.000	0.938	0.828	0.656	0.210	0.364
c	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.125	0.000	0.000
Fum	(32)	(31)	(31)	(32)	(30)	(32)	(31)	(32)	(30)	(34)
a	0.000	0.000	0.000	0.000	0.000	0.000	0.016	0.000	0.000	0.029
b	1.000	0.952	0.984	1.000	1.000	0.984	0.984	1.000	1.000	0.971
c	0.000	0.048	0.016	0.000	0.000	0.016	0.000	0.000	0.000	0.000
Idh-A	(32)	(31)	(31)	(31)	(30)	(32)	(32)	(31)	(31)	(34)
a	0.031	0.000	0.000	0.000	0.000	0.047	0.016	0.129	0.032	0.000
b	0.969	0.984	1.000	0.968	1.000	0.531	0.609	0.677	0.952	0.941
c	0.000	0.000	0.000	0.000	0.000	0.422	0.344	0.194	0.016	0.059
d	0.000	0.000	0.000	0.016	0.000	0.000	0.031	0.000	0.000	0.000
e	0.000	0.016	0.000	0.016	0.000	0.000	0.000	0.000	0.000	0.000
Pgm-B	(31)	(31)	(24)	(31)	(30)	(32)	(32)	(31)	(31)	(33)
a	0.000	0.032	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
b	0.984	0.968	1.000	1.000	1.000	0.031	0.016	0.081	0.758	0.530
c	0.016	0.000	0.000	0.000	0.000	0.969	0.984	0.919	0.242	0.470
Phi-A	(32)	(32)	(31)	(31)	(30)	(32)	(31)	(31)	(30)	(33)
a	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
b	0.000	0.016	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
c	1.000	0.984	1.000	1.000	1.000	0.469	0.419	0.323	0.283	0.167
d	0.000	0.000	0.000	0.000	0.000	0.531	0.581	0.677	0.717	0.833
Phi-B	(32)	(32)	(31)	(31)	(28)	(32)	(32)	(32)	(30)	(33)
a	1.000	1.000	0.435	0.452	0.500	1.000	1.000	1.000	1.000	1.000
b	0.000	0.000	0.565	0.548	0.500	0.000	0.000	0.000	0.000	0.000

TABLE X (Continued)

Locus	Sample						
	DIAU	DIAL	LC1	LC2	LC3	LC4	DNFH
Adh	(32)	(31)	(30)	(12)	(30)	(32)	(31)
a	0.922	0.984	0.933	0.917	0.917	0.906	0.903
b	0.078	0.016	0.067	0.083	0.083	0.094	0.097
c	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Fum	(32)	(31)	(30)	(12)	(30)	(32)	(32)
a	0.031	0.000	0.000	0.000	0.000	0.203	0.000
b	0.969	1.000	1.000	1.000	1.000	0.797	1.000
c	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Idh-A	(32)	(31)	(30)	(12)	(30)	(32)	(31)
a	0.000	0.000	0.000	0.000	0.000	0.000	0.000
b	1.000	1.000	1.000	1.000	0.983	0.953	1.000
c	0.000	0.000	0.000	0.000	0.017	0.047	0.000
d	0.000	0.000	0.000	0.000	0.000	0.000	0.000
e	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Pgm-B	(32)	(31)	(30)	(12)	(30)	(32)	(31)
a	0.000	0.000	0.000	0.000	0.000	0.000	0.000
b	1.000	1.000	1.000	1.000	1.000	1.000	1.000
c	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Phi-A	(32)	(32)	(30)	(12)	(30)	(32)	(31)
a	0.016	0.000	0.050	0.000	0.000	0.000	0.032
b	0.172	0.203	0.250	0.167	0.150	0.219	0.161
c	0.813	0.797	0.700	0.833	0.850	0.781	0.806
d	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Phi-B	(32)	(32)	(30)	(12)	(30)	(32)	(31)
a	1.000	1.000	1.000	1.000	0.983	0.984	1.000
b	0.000	0.000	0.000	0.000	0.017	0.016	0.000

[1]Sample sizes in parentheses.

TABLE XI

GENETIC VARIABILITY AT 24 LOCI IN SAMPLES OF *GAMBUSIA NOBILIS*[1]

Sample	Mean Sample Size Per Locus	Mean Number of Alleles Per Locus	Percentage of Loci Polymorphic[2]	Mean Heterozygosity	
				Direct-Count	HDYWBG Expected[3]
BLSH	32.8 (0.1)	1.1 (0.1)	0.0	0.004 (0.003)	0.004 (0.003)
SAGO	31.8 (0.1)	1.2 (0.1)	0.0	0.009 (0.005)	0.009 (0.005)
BSU	30.7 (0.3)	1.1 (0.1)	4.2	0.019 (0.017)	0.022 (0.021)
BSM	31.8 (0.1)	1.1 (0.1)	4.2	0.022 (0.019)	0.024 (0.021)
BSL	29.9 (0.1)	1.0 (0.0)	4.2	0.021 (0.021)	0.021 (0.021)
PLSC	32.0 (0.0)	1.3 (0.1)	12.5	0.044 (0.027)	0.053 (0.030)
PLS2	31.9 (0.1)	1.3 (0.1)	12.5	0.055 (0.029)	0.057 (0.031)
PLS3	31.9 (0.1)	1.3 (0.1)	16.7	0.052 (0.030)	0.067 (0.034)
GIFN	30.9 (0.1)	1.2 (0.1)	12.5	0.054 (0.028)	0.051 (0.026)

TABLE XI (Continued)

Sample	Mean Sample Size Per Locus	Mean Number of Alleles Per Locus	Percentage of Loci Polymorphic[2]	Mean Heterozygosity	
				Direct-Count	HDYWBG Expected[3]
ESAN	33.8 (0.1)	1.2 (0.1)	16.7	0.053 (0.027)	0.060 (0.030)
DIAU	32.0 (0.0)	1.2 (0.1)	8.3	0.023 (0.016)	0.022 (0.014)
DIAL	31.8 (0.1)	1.1 (0.1)	4.2	0.016 (0.014)	0.015 (0.014)
LC1	30.0 (0.0)	1.1 (0.1)	8.3	0.026 (0.021)	0.024 (0.019)
LC2	12.0 (0.0)	1.1 (0.1)	8.3	0.021 (0.015)	0.019 (0.014)
LC3	30.0 (0.0)	1.2 (0.1)	8.3	0.019 (0.012)	0.020 (0.012)
LC4	32.0 (0.0)	1.2 (0.1)	12.5	0.033 (0.017)	0.040 (0.020)
DNFH	31.8 (0.1)	1.1 (0.1)	8.3	0.023 (0.017)	0.021 (0.015)

[1]Standard errors in parentheses.

[2]A locus is considered polymorphic if the frequency of the most common allele does not exceed 0.95.

[3]Unbiased e timate (see Nei 1978).

at *Phi-A* for PLSU ($P < .04$), and at *Adh* ($P < .001$), *Phi-A* ($P < .04$), and *Idh-A* ($P < .02$) for PLSL. All significant deviation was due to heterozygote deficiency, except at *Idh-A* for PLSL which showed a heterozygote excess. Only two loci were polymorphic at DNFH; both loci showed slight heterozygote excess (Appendix B).

Primarily because of geographic variation in presence-absence of alleles, the following alleles occured in one or more of the widely dispersed natural populations but were absent in the DNFH sample: Adh-c, Fum-a, Fum-c, Idh-A-a, Idh-A-c, Idh-A-d, Idh-A-e, Pgm-B-a, Pgm-B-c, Phi-A-d, and Phi-B-b.

Because of the diverse nature of the genetic structure of *G. nobilis* throughout its range, the remainder of my account considers only those natural populations from the Leon Creek area. This restricted analysis leaves a total of five polymorphic loci to be considered. The history of human interference in lower Leon Creek, as discussed in the narrative for *C. bovinus*, limits my ability to compare the captive population with its progenitor population from Leon Creek.

Alleles present in samples from the Leon Creek area but absent at DNFH include three alleles generally uncommon in natural populations: Fum-a, Idh-A-c, and Phi-B-b. If the DNFH sample is assumed to have allelic frequencies equal to the means of samples from Leon Creek (Table X), the probabilities of missing these three alleles range from

.08-.73. However, the product of three probabilities is .029. Thus the absence of all three alleles from DNFH by chance alone is not expected at the 95% level of confidence, suggesting that one or all of the three alleles are absent at DNFH or at least in lower frequencies than in natural populations. Another possibility is that the probability of missing the alleles at DNFH is an artifact of micro-graphic variation at the Fum locus. Fum-a was common (frequency = .203) in the LC4 sample but virtually absent in the remaining four samples from Leon Creek (.031, 1 sample). Omitting LC4 from the analysis indicates that the absence of the three alleles in the DNFH sample could be due to sampling error (P = .56).

The six samples from Leon Creek (FIAU, DIAL, LC1-4) showed relatively low heterogeneity compared to samples of C. bovinus from comparable sites in the Leon Creek area. This may have important implications for management practices. Heterogeneity occurred at only one of the five polymorphic loci compared to two of five loci in C. bovinus (Tables XII, IV). In C. bovinus, significant heterogeneity occurred between samples from the following sites: Diamond-Y Spring (DIAY) versus the upper watercourse of Leon Creek (LC1, LC2), DIAY versus the lower watercourse of Leon Creek (LC3, LC4), and the upper (LC1, LC2) versus the lower watercourse (LC3, LC4). In G. nobilis, the only heterogeneity was between LC4 and the remaining samples from Leon Creek, and again, this differentiation was due

TABLE XII

CHI-SQUARE ANALYSIS OF HETEROGENEITY AT POLYMORPHIC LOCI AMONG SAMPLES OF *GAMBUSIA NOBILIS*

	Samples					
Locus	DIAU, DIAL, LC1, LC2	DIAU, DIAL, LC1, LC2, DNFH	LC3, LC4	LC3, LC4, DNFH	DIAU, DIAL, LC1, LC2, LC3, LC4	DIAU, DIAL, LC1, LC2, LC3, LC4, DNFH
Adh	ns*	ns	ns	ns	ns	ns
Fum	ns	ns	$\chi^2 = 13.615$ 1 df $P < .0003$	$\chi^2 = 27.059$ 2 df $P < .00001$	$\chi^2 = 47.314$ 5 df $P < .00001$	$\chi^2 = 58.945$ 6 df $P < .00001$
Idh-A	m**	m	ns	ns	ns	ns
Phi-A	ns	ns	ns	ns	ns	ns
Phi-B	m	m	ns	ns	ns	ns

*Not significant at the .05 level.

**Monomorphic.

to variation at a single locus. However, the difference in heterogeneity may be more apparent than real. For example, at three of the five polymorphic loci in G. nobilis, there was little opportunity for heterogeneity. Except for one case, Fum-a at LC4, all samples of those three loci were at or near fixation for a single allele. "Opportunity" for heterogeneity between most samples occurred at only two loci, while in C. bovinus it occurred at four. Nonetheless, heterogeneity did occur at two of the four in C. bovinus and in neither of the two in G. nobilis.

The difference in heterogeneity between G. nobilis and C. bovinus also extends to the comparison of the captive DNFH population with natural populations. Adding DNFH to the set of natural populations produced essentially no change of heterogeneity level in G. nobilis while in C. bovinus the probability of homogeneity was reduced an order of magnitude for three loci. Correspondingly, Rogers' coefficient of genetic distance (D) between DNFH and natural populations was significantly greater in C. bovinus than in G. nobilis (.008-.016 vs. .002-.013, respectively; Mann-Whitney $U = 27$, $n_1 = 6$, $n_2 = 5$, $P < .05$; Table V, XIII). Thus, the captive G. nobilis population seems to have diverged less from its progenitors in Leon Creek than has the captive population of C. bovinus.

The potentially reduced levels of heterogeneity among natural populations of G. nobilis and between them and the captive population may result from several aspects of

TABLE XIII

MATRIX OF ROGERS' (1972) GENETIC DISTANCE COEFFICIENTS BETWEEN SAMPLES OF *GAMBUSIA NOBILIS*

Sample	BLSH	SAGO	BSU	BSM	BSL	PLSC	PLS2	PLS3	GIFN	ESAN	DIAU	DIAL	LC1	LC2	LC3	LC4
SAGO	0.005	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
BSU	0.026	0.028	-	-	-	-	-	-	-	-	-	-	-	-	-	-
BSM	0.025	0.028	0.003	-	-	-	-	-	-	-	-	-	-	-	-	-
BSL	0.023	0.026	0.003	0.003	-	-	-	-	-	-	-	-	-	-	-	-
PLSC	0.119	0.120	0.144	0.143	0.142	-	-	-	-	-	-	-	-	-	-	-
PLS2	0.114	0.116	0.139	0.138	0.137	0.011	-	-	-	-	-	-	-	-	-	-
PLS3	0.107	0.109	0.133	0.131	0.129	0.027	0.020	-	-	-	-	-	-	-	-	-
GIFN	0.049	0.051	0.075	0.073	0.071	0.086	0.077	0.062	-	-	-	-	-	-	-	-
ESAN	0.072	0.072	0.096	0.096	0.094	0.075	0.065	0.051	0.024	-	-	-	-	-	-	-
DIAU	0.014	0.014	0.035	0.036	0.033	0.115	0.110	0.104	0.045	0.065	-	-	-	-	-	-
DIAL	0.011	0.013	0.033	0.033	0.030	0.117	0.112	0.105	0.047	0.069	0.005	-	-	-	-	-
LC1	0.016	0.018	0.039	0.038	0.035	0.115	0.109	0.102	0.043	0.066	0.006	0.006	-	-	-	-
LC2	0.012	0.014	0.035	0.034	0.031	0.115	0.110	0.102	0.044	0.067	0.002	0.004	0.006	-	-	-
LC3	0.012	0.014	0.034	0.033	0.031	0.115	0.110	0.103	0.045	0.067	0.004	0.006	0.008	0.002	-	-
LC4	0.025	0.024	0.046	0.045	0.044	0.120	0.115	0.109	0.052	0.070	0.012	0.015	0.015	0.014	0.013	-
DEX	0.013	0.015	0.036	0.036	0.032	0.114	0.109	0.101	0.043	0.066	0.003	0.005	0.005	0.002	0.004	0.013

Gambusia life history that tend to increase the effective population size of founding populations. *Gambusia* are known to exhibit sperm storage and delayed fertilization for a month or more (e.g., Hubbs 1971), and multiple insemination is known in several peociliid genera (e.g., Borowsky and Kallman 1976; Leslie and Vrijenhoek 1977), including Gambusia (Chesser et al. unpubl. manuscript). Chesser et al. found that in *Gambusia* *affinis* "a single female inseminated by one, two, or three males effectively contains 75, 83, or 88 percent, respectively, of the genetic variance of the parental population." Furthermore, the males of *C. bovinus* are highly territorial (Kennedy 1977), while *Gambusia* species generally do not defend large breeding territories.

At first glance, presence-absence of alleles in DNFH samples appears to argue against the hypothesis that effective population sizes might help explain the difference in heterogeneity observed in *G. nobilis* as compared with *C. bovinus*. The DNFH sample of *G. nobilis* lacked three alleles detected in natural populations in the Leon Creek area, while the DNFH sample of *C. bovinus* lacked only one. However, in both species, the alleles missing from the DNFH sample were also missing in the majority of the samples from Leon Creek. The DNFH sample of *G. nobilis* lacked three of the four such alleles in Leon Creek samples while *C. bovinus* lacked one of two.

The DNFH sample of *G. nobilis* is more similar to populations from Diamond-Y Spring (D = .003-.005), the

upper watercourse (D = .002-.005), and LC3 (.004) from the upper portion of the lower watercourse than it is to LC4 (.013) from the tailwater area of the lower watercourse (Table XIII). LC4 is no more similar to DNFH than are samples from the Bitter Lakes National Wildlife Refuge in New Mexico (D = .013-.015) which is 300 air-kilometers from Leon Creek. Uniqueness of the LC4 sample is due to its unusually high frequency of Fum-a. While this high frequency of Fum-a may be due to localized hybridization with *Gambusia affinis*, I found no evidence of such hybridization at other diagnostically useful loci (Rutherford 1980; Echelle and Echelle 1980). However, *G. affinis* was not examined for allelic composition at the Fum locus.

CHAPTER IV

SUMMARY AND CONCLUSIONS

1. Management of endangered species like those at DNFH must begin by consideration of which aspects of genetic structure should be managed. Hatcheries may: (1) maintain allelic frequencies at levels similar to those in natural populations; (2) maintain the variety of alleles present in wild stocks, perhaps those of the most endangered populations; or (3) maintain high genetic variability irrespective of allelic frequencies. Point 1 seems to be the least critical, while points 2 and 3 may be more important. Ryman and Stahl (1980) emphasize that "natural selection has a chance to adjust the frequency of an allele that is present, while this is impossible if the allele was lost during stocking." It is possible that a population with a variety of alleles might have alternative alleles at frequencies sufficiently low that favored allelic combinations would be virtually absent. To the extent that overall heterozygosity contributes to fitness, a highly variable population with high frequencies of many alternative alleles would be more desirable than a less variable one. Therefore my recommendations are designed to maximize both alleleic diversity and genetic variability (points 2 and 3).

For logistical reasons, these recommendations are made with the constraint that DNFH can maintain only a single stock of each endangered species/population. The validity of such recommendations could be confounded by one or more natural selection scenarios which may or may not be applicable in practice. As Allendorf (1983) emphasizes, such scenarios are poorly understood, both empirically and theoretically. Therefore, following Allendorf's (1983) recommendations, I accept as a working hypothesis the neutral allele model of population genetics.

2. DNFH stocks of *Cyprinodon bovinus*, *C. elegans*, and *Gambusia nobilis* show statistically significant differentiation from the genetic structure of their progenitor poulations. However, the differences are rather minor and primarily involve shifts in allelic frequencies. Some data suggest possible loss of rare alleles, but this loss is not well established because of the small size of my DNFH samples (30-51).

3. Natural populations of the three species show spatial heterogeneity in allelic frequencies, even within a single spring system. The highest heterogeneity occurs between populations of both *G. nobilis* and *C. elegans* in the complex system of springs and irrigation canals in the Balmorhea area of Texas. Lower, but statistically significant heterogeneity occurs between subpopulations in waters that have less effective barriers to gene flow. These include: (1) *C. bovinus* in Diamond-Y Spring versus

the upper watercourse of Leon Creek, (2) *C. elegans* from the head of Giffin Spring and a more downstream area, and (3) *G. nobilis* from two sample sites in lower Leon Creek. In all three cases the sample sites are partially isolated, either by irrigation diversion boxes and sluice gates (Balmorhea area), or by dense, intervening marshes with little connecting flow (Leon Creek--between Diamond-Y Spring and the upper watercourse, and between LC3 and LC4).

4. More striking heterogeneity occurs, as expected, between the completely isolated natural populations of *G. nobilis* in four widely separated spring systems of New Mexico and Texas. At three of the six polymorphic loci examined in this species, alleles common in one spring system are absent, or nearly so, in the other three systems. These alleles are: (1) Pgm-B-c, common (frequencies = .242-.984) in the Balmorhea area, nearly absent elsewhere (.016, one sample); (2) Idh-A-c, uncommon to common (.02-.42) in the Balmorhea area, rare elsewhere (.02-.05, 2 samples); (3) Phi-B-b, common (.50-.57) in Blue Spring, rare elsewhere (.02, 2 samples).

5. In general, the level of genetic differentiation among natural populations varies inversely with opportunity for gene flow. Whether genetic heterogeneity results from stochastic factors (genetic drift) or natural selection is beyond the scope of this study. However, geographic heterogeneity raises some questions regarding management of endangered fishes.

6. On the macrogeographic scale, a sample from a single location is a poor representation of genetic structure of the whole species. Alleles common in some areas will be absent from such a sample. If such alleles contribute to fitness in a given region, then the success of future attempts to reintroduce species into areas of past occurrence will be jeopardized. However, for species such as *G. nobilis* which occur in several different areas, the goal at DNFH is to protect individual populations under the most immediate threat of extinction. Thus, stocks at DNFH come, appropriately, from single populations. Reintroductions of fish from DNFH are done with hatchery stocks derived, as nearly as possible, from the areas of reintroduction.

7. The degree of microgeographic heterogeneity in natural populations of the three species suggests that the original DNFH stocks should be a mix from several different habitat situations. With small species like the three I examined, large numbers are easily obtained, and mixed stocks should be as large as logistical considerations allow. The stocks should include fish from each major spring system or watercourse in the area of concern. For populations in spring runs, such samples should include fish from the headspring area and an equal number from more variable environments.

8. Effective population sizes at DNFH seem large enough to minimize inbreeding effects. The *Adh* locus in

C. elegans shows strong heterozygote deficiency at DNFH, but this deficiency also occurs in natural populations of the species. Explanation of this effect awaits further analysis, and may or may not be related to inbreeding.

9. Bottlenecks that may have occurred in the history of the captive populations were sufficiently brief so that deleterious effects on levels of heterozygosity were minimized. These levels at DNFH are similar to those in the progenitor populations. Nei et al. (1975) emphasized that significant loss of heterozygosity is likely only if the bottleneck is prolonged. An extended period of low effective population size may expose successive generations to bottlenecks during which genetic drift may eliminate many low frequency alleles. Because of the high reproductive potentials of the species I examined, any severe bottlenecks at DNFH probably would have been associated with the initial founding events.

10. The present populations of all three species in the hatchery ponds at DNFH consistently contain several hundred adult individuals. Effective population sizes probably are above the "safe" level (>100) recommended by Allendorf and Phelps (1980) and may approximate the level (>500) recommended by Franklin (1980). However, Soulé (1980) stressed that long-term evolutionary potential may require substantially larger effective population sizes. Frankel and Soulé (1980) note that these are rough guidelines that depend on, among other factors, the demography of the

species concerned. More detailed studies of life histories and effective population sizes at DNFH are needed before this aspect of the management program can be thoroughly evaluated.

11. As measured by electrophoresis, present management practices at DNFH are maintaining captive populations at levels of genetic variability comparable to that in progenitor populations. However, electrophoresis almost certainly underestimates the change that has occurred in the captive populations. To ensure qualitative similarity between captive and progenitor populations, DNFH stocks should receive periodic infusions of individuals from wild populations. Because of microgeographic heterogeneity in natural populations, the infusion should comprise a sample similar to that described for the original DNFH stock. Kimura and Ohta (1971) showed that in the absence of selection, a single reproductively successful "immigrant" per generation would maintain qualitative genetic similarity between subpopulations independent of subpopulation size. Because not all alleles will conform to the neutral model, the infusions should be as frequent and as large as logistical considerations and the status of natural populations will allow.

LITERATURE CITED

Allendorf, F. W. 1983. Isolation, gene flow, and genetic differentiation among populations. pp. 51-65 In Schonewald-Cox, C. M., S. M. Chambers, B. MacBryde, and W. L. Thomas (eds.). Genetics and Conservation. Benjamin/Cummings Pub. Co., Inc., Menlo Park, Calif.

Allendorf, F. W. and S. R. Phelps. 1980. Loss of genetic variation in a hatchery stock of cutthroat trout. Trans. Am. Fish. Soc. 109:537-543.

Allendorf, F. W. and F. M. Utter. 1979. Population genetics. pp. 407-454 In Hoar, W. S., D. J. Randall, and J. R. Brett (eds.). Fish Physiology Vol. VIII. Academic Press, New York.

Allendorf, F. W., N. Ryman, A. Stennek, and G. Stahl. 1976. Genetic variation in Scandinavian brown trout (*Salmo trutta* L.): evidence of distinct sympatric populations. Hereditas 83:73-82.

Aulstad, D. and A. Kittelsen. 1971. Abnormal body curvatures of rainbow trout (*Salmo gairdneri*) in inbred fry. J. Fish. Res. Bd. Can. 28:1918-1920.

Avise, J. C. and J. Felley. 1979. Population structure of freshwater fishes. I. Genetic variation of bluegill (*Lepomis macrochirus*) populations in man-made reservoirs. Evolution 33:15-26.

Avise, J. C. and M. H. Smith. 1974. Biochemical genetics of sunfish. II. Genetic similarity between hybridizing species. Am. Nat. 108:458-472.

Ayala, F. J. 1968. Genotype, environment, and population numbers. Science 162(3861):1453-1459.

Baird, S. F. and C. Girard. 1853. Descriptions of new species of fishes collected by Mr. John H. Clark on the U.S. and Mexican Boundary Survey under Lt.-Col. Jas. D. Graham. Proc. Acad. Nat. Sci. Phila. (1852-53) 6:387-390.

Baker, M. C. 1975. Song dialects and genetic differences in white-crowned sparrows (*Zonotrichia leucophrys*). Evolution 29:226-241.

Baker, M. C. and S. F. Fox. 1978. Dominance, survival, and enzyme polymorphism in dark-eyed juncos (Junco hyemalis). Evolution 32:697-711.

Borowsky, R. and K. D. Kallman. 1976. Patterns of mating in natural populations of Xiphophorus. I. X. maculatus from Belize and Mexico. Evolution 30:693-706.

Christiansen, F. B., B. V. Nielsen, and V. Somonsen. 1981. Genetical and morphological variation in the eelpout (Zoarces viviparous). Can. J. Genet. Cytol. 23:163-172.

Crow, J. F. and M. Kimura. 1970. An Introduction to Population Genetics Theory. Harper and Row, New York.

Darling, J. D. S. 1976. Electrophoretic variation in Cyprinodon variegatus and systematics of some fishes of the subfamily Cyprinodontinae. Ph.D. dissertation. Yale University.

Denniston, C. 1978. Small population size and genetic diversity. Implications for endangered species. In Temple, S. A. (ed.). Endangered Birds. Management techniques for preserving threatened species. Univ. of Wisconsin Press, Madison.

Dobzhansky, T. 1970. Genetics of the Evolutionary Process. Columbia Univ. Press, New York.

Echelle, A. A. 1975. A multivariate analysis of variation in an endangered fish, Cyprinodon elegans, with an assessment of populational status. Texas J. Sci. 26(3&4):529-538.

Echelle, A. A. and A. F. Echelle. 1980. Status of the Pecos gambusia. U. S. Fish and Wildlife Service Endangered Species Report No. 10. U. S. Fish and Wildlife Service, Albuquerque, New Mexico.

Echelle, A. A., A. F. Echelle, and B. A. Taber. 1976. Biochemical evidence for congeneric competition as a factor restricting gene flow between populations of a darter (Percidae: Etheostoma). Syst. Zool. 25: 228-235.

Echelle, A. A., A. F. Echelle, M. H. Smith, and L. G. Hill. 1975. Analysis of genic continuity in a headwater fish, Etheostoma radiosum (Percidae). Copeia 1975: 197-204.

Hubbs, C. 1980. The solution to the Cyprinodon bovinus problem: Eradication of a pupfish genome. Proc. Desert Fishes Council 12.

Hubbs, C. and V. G. Springer. 1957. A revision of the Gambusia nobilis species group, with descriptions of three new species, and notes on their variation, ecology, and evolution. Texas J. Sci. 9:279-327.

Hubbs, C., T. Lucier, E. Marsh, G. P. Garrett, R. J. Edwards, and E. Milstead. 1978. Results of an eradication program on the ecological relationships of fishes in Leon Creek, Texas. Southwest. Nat. 23:487-496.

Johns, P. E., R. Baccus, M. N. Manlove, J. E. Pinder III, and M. H. Smith. 1977. Reproductive patterns, productivity, and genetic variability in adjacent white-tailed deer populations. Proc. Conf. Southeast. Assn. Game Fish Commnrs. 31:167-172.

Kimura, M. and J. F. Crow. 1963. The measurement of effective population number. Evolution 17:279-288.

Kimura, M. and T. Ohta. 1971. Theoretical Aspects of Population Genetics. Princeton Univ. Press, Princeton, New Jersey.

Kennedy, S. E. 1977. Life history of the Leon Springs pupfish, Cyprinodon bovinus. Copeia 1977:93-103.

Kincaid, H. L. 1976. Inbreeding in rainbow trout (Salmo gairdneri). J. Fish. Res. Bd. Can. 33:2420-2426.

Koehn, R. K. 1969. Esterase heterogeneity: dynamics of a polymorphism. Science 163:943-944.

Lerner, I. M. 1954. Genetic Homeostasis. Wiley, New York.

Leslie, J. F. and R. C. Vrijenhoek. 1977. Genetic analysis of natural populations of Poeciliopsis monacha. Allozyme inheritance and pattern of mating. J. Hered. 68:301-306.

Levene, H. 1949. On a matching problem arising in genetics. Ann. Math. Stat. 20:91-94.

Mayr, E. 1942. Systematics and the Origin of Species. Columbia Univ. Press, New York.

Mayr, E. 1954. Change of genetic environment and evolution. In Huxley, J., A. C. Hardy, and E. B. Ford (eds.). Evolution as a Process. Allen and Unwin, London.

Mayr, E. 1963. Animal Species and Evolution. Belknap Press, Cambridge, Mass.

Miller, R. R. 1961. Man and the changing fish fauna of the American Southwest. Pap. Mich. Acad. Sci., Arts, Letters, 46:365-404.

Mitton, J. B. 1978. Relationship between heterozygosity for enzyme loci and variation of morphological characters in natural populations. Nature 273:661-662.

Moon, T. W. 1975. Temperature adaptation: isozymic function and the maintenance of heterogeneity. pp. 207-220 in Markert, C. L. (ed.). Isozymes II. Physiological function. Academic Press, New York.

Nei, M. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics 89:583-590.

Nei, M., T. Maruyama, and R. Chakraborty. 1975. The bottleneck effect and genetic variability in populations. Evolution 29:1-10.

Nyman, L. 1975. Allelic selection in a fish (Gymnocephalus cernua (L.)) subjected to hotwater effluents. Rep. Inst. Freshw. Drottingholm 54:75-82.

Powers, D. A. and A. R. Place. 1978. Biochemical genetics of Fundulus heteroclitus (L.). 1. Temporal and spatial variation in gene frequencies of Ldh-A, Mdh-A, Phi-B, and Pgm-A. Biochem. Genet. 16:593-607.

Rogers, J. S. 1972. Measures of genetic similarity and genetic distance. Studies in Genetics, Univ. Texas Publ. 7213:145-153.

Rohde, F. C. and V. Guillory. 1980. Cyprinodon bovinus Baird and Girard, Leon Springs pupfish. p. 493 in D. S. Lee et al. Atlas of North American Freshwater Fishes. N. C. State Mus. Nat. Hist. Raleigh.

Rutherford, D. A. 1980. Hybridization in Gambusia nobilis (Pisces: Poeciliidae); An endangered species in the Pecos River drainage of Texas and New Mexico. M. S. thesis. Baylor University, Waco, Texas.

Ryman, N. 1970. A genetic analysis of recapture frequencies of released young salmon (Salmo salar L.). Hereditas 65:159-160.

Ryman, N. 1981. Conservation of genetic resources: experiences from the brown trout (Salmo trutta) in Ryman, N. (ed.). Fish Gene Pools. Preservation of genetic resources in relation to wild fish stock. Ecol. Bull. (Stockholm) 34:61-74.

Ryman, N. and G. Stahl. 1980. Genetic changes in hatchery stocks of brown trout (Salmo trutta). Can. J. Fish. Aquat. Sci. 37:82-87.

Ryman, N., F. W. Allendorf, and G. Stahl. 1979. Reproductive isolation with little genetic divergence in sympatric populations of brown trout (Salmo trutta). Genetics 92:247-262.

Selander, R. K. 1970. Behavior and genetic variation in natural populations. Am. Zool. 19:53-66.

Siciliano, M. J. and C. R. Shaw. 1976. Separation and visualization of enzymes on gels. pp. 185-209 in Smith, I. (ed.). Chromatographic and Electrophoretic Techniques Vol. 2, Zone electrophoresis. Heinemann, London.

Simanek, D. E. 1978. Genetic variability and population structure of Poecilia latipinna. Nature 276:612-614.

Singh, S. M. and E. Zouros. 1978. Genetic variation associated with growth in the American oyster (Crassostrea virginica). Evolution 32:342-353.

Smith, M. H. and R. K. Chesser. 1981. Rationale for conserving genetic variation of fish gene pools in Ryman, N. (ed.). Fish Gene Pools. Preservation of genetic resources in relation to wild fish stock. Ecol. Bull. (Stockholm) 34:13-20.

Smith, M. H., C. T. Garten, and P. R. Ramsey. 1975. Genic heterozygosity and population dynamics in small mammals. pp. 85-102 in Markert, C. L. (ed.). Isozymes IV. Genetics and evolution. Academic Press, New York.

Smith, M. H., M. N. Manlove, and J. Joule. 1978. Spatial and temporal dynamics of the genetic organization of small mammal populations. pp. 99-113 in Snyder, D. P. (ed.). Populations of Small Mammals Under Natural Conditions. Pymatuning Symp. Ecol. Univ. of Pittsburgh Press, Pittsburgh, Penn.

Soulé, M. 1980. Thresholds for survival: maintaining fitness and evolutionary potential. pp. 151-169 in M. Soulé and B. Wilcox (eds.). Conservation Biology: An Evolutionary-Evological Perspective. Sinauer Associates, Sunderland, Mass.

Stahl, G. 1981. Genetic differentiation among natural populations of atlantic salmon (Salmo salar) in northern Sweden in Ryman, N. (ed.). Fish Gene Pools. Preservation of genetic resources in relation to wild fish stock. Ecol. Bull. (Stockholm) 34:95-105.

Stevenson, M. H. and T. M. Buchanan. 1973. An analysis of hybridization between the cyprinodont fishes *Cyprinodon variegatus* and *C. elegans*. Copeia 1973:682-692.

Swofford, D. L. and R. B. Selander. 1981. BIOSYS-1: a FORTRAN program for the comprehensive analysis of electrophoretic data in population genetics and systematics. J. Heredity 72:281-283.

Turner, B. J. 1974. Genetic divergence of Death Valley pupfish species: biochemical versus morphological evidence. Evolution 28:281-294.

Turner, B. J. 1983. Genic variation and differentiation of remnant natural populations of the desert pupfish, *Cyprinodon macularius*. Evolution 37:693-700.

Turner, B. J. 1984. Evolutionary genetics of artificial refugium populations of an endangered species, the desert pupfish. Copeia (in press).

U. S. Department of the Interior. 1983. Endangered and threatened wildlife and plants. U. S. Fish and Wildlife Service. 50 CFR 17.11 and 17.12.

Webster, P. and J. M. Burns. 1983. Dewlap color variation and electrophoretically detected sibling species in a Haitian lizard, *Anolis brevirostiris*. Evolution 27: 368-377.

White, W., H. Gale, and S. Nye. 1938. Groundwater resources of the Balmorhea area in western Texas. Texas Water Dev. Bd., Misc. Pub. 11.

Winans, G. A. 1980. Geographic variation in the milkfish, *Chanos chanos*. 1. Biochemical evidence. Evolution 34: 558-574.

Wright, S. 1932. The roles of mutation, inbreeding, crossbreeding, and selection in evolution. Proc. Int. Conf. Genetics 1:356-366.

Wright, S. 1951. The genetical structure of populations. Ann. Eugen. 15:323-354.

Wright, S. 1969. Evolution and the Genetics of Populations, Vol. 2. The Theory of Gene Frequencies. Univ. of Chicago Press, Chicago.

Yardley, D. and C. Hubbs. 1976. An electrophoretic study of two species of mosquitofish with notes on genetic subdivision. Copeia 1976:117-120.

Yardley, D., J. C. Avise, J. W. Gibbons, and M. H. Smith. 1974. Biochemical genetics of sunfish III. Genetic subdivision of fish populations inhabiting heated waters. pp. 255-276 in Gibbons, J. W. and R. R. Sharitz (eds.). Thermal Ecology.

APPENDIXES

APPENDIX A

GENOTYPIC FREQUENCIES FOR ALL SAMPLES OF *CYPRINODON BOVINUS*

Genotypic Frequencies for All Samples of *Cyprinodon bovinus* (Fixation Indices in Parentheses)

Samples	Loci	Genotypic Frequencies	Fixation Indices
DIAY	ADH	BB:13	*
	ALD	AA:07 AB:06	(-.300)
	AAT-C	AB:07 BB:03 AA:03	(-.077)
	PHI-A	BC:04 CC:08 BB:01	(.133)
	6PGD	BB:10 AB:03	(-.133)
LC1	ADH	BB:45	*
	ALD	AA:29 AB:16	(-.216)
	AAT-C	AB:22 AA:14 BB:09	(.010)
	PHI-A	CC:26 BC:15 AC:01 BB:03	(.051)
	6PGD	BB:44 AB:01	(-.011)
LC2	ADH	BB:46	*
	ALD	AA:32 AB:13 BB:01	(-.035)
	AAT-C	AB:23 AA:14 BB:08	(-.041)
	PHI-A	CC:26 BC:16 BB:04	(.098)
	6PGD	BB:43 AB:03	(-.034)
LC3	ADH	BB:45 AB:06	(-.063)
	ALD	AA:42 AB:09	(-.097)
	AAT-C	AB:19 BB:18 AA:14	(.250)
	PHI-A	CC:27 BC:14 AC:07 AB:01 BB:02	(-.031)
	6PGD	BB:44 AB:07	(-.074)
LC4	ADH	BB:21	*
	ALD	AA:18 AB:03	(-.077)
	AAT-C	AB:12 BB:03 AA:06	(-.167)
	PHI-A	CC:14 BC:07	(-.200)
	6PGD	BB:19 AB:02	(-.050)
DNFH	ADH	BB:41 AB:10	(-.109)
	ALD	AA:33 AB:13 BB:02	(.071)
	AAT-C	AA:20 AB:19 BB:09	(.082)
	PHI-A	CC:36 BC:13 BB:02	(.082)
	6PGD	BB:50 AB:01	(-.010)

*Index not calculated due to absence of heterozygotes.

APPENDIX B

GENOTYPIC FREQUENCIES FOR ALL SAMPLES OF *CYPRINODON ELEGANS*

Genotypic Frequencies for All Samples of *Cyprinodon elegans* (Fixation Indices in Parentheses)

Samples	Loci	Genotypic Frequencies	Fixation Indices
PLSU	ADH	AA:08 AB:04	(-.200)
	CK-A	BB:09 AB:03	(-.143)
	CK-C	BB:12	*
	PHI-B	BB:06 AB:06	(-.333)
	IDH-A	BB:08 AB:03	(-.158)
	PGM	BB:11 AA:01	(1.000)
PLSM	ADH	AA:16 AB:11 BB:09	(.365)
	CK-A	BB:26 AB:11 AA:02	(.092)
	CK-C	BB:39	*
	PHI-B	AB:17 AA:12 BB:09	(.100)
	IDH-A	BB:21 AB:09 AA:05	(.350)
	PGM	BB:38	*
PLSL	ADH	AB:19 AA:07 BB:07	(-.152)
	CK-A	BB:24 AB:09	(-.158)
	CK-C	BB:33	*
	PHI-B	AB:20 BB:12 AA:01	(-.364)
	IDH-A	BB:24 AB:08	(-.143)
	PGM	BB:27 AB:06	(-.100)
GIFU	ADH	BB:10 AA:07 AB:08	(.351)
	CK-A	BB:25	*
	CK-C	BB:25	*
	PHI-B	BB:25	*
	IDH-A	AB:05 BB:15 AA:01	(-.143)
	PGM	BB:25	*
GIFL	ADH	AB:18 BB:18 AA:10	(.193)
	CK-A	BB:44 AB:05	(-.054)
	CK-C	BB:45 AB:02 AA:01	(.478)
	PHI-B	BB:47 AB:02	(-.021)
	IDH-A	AB:22 AA:14 BB:09	(.010)
	PGM	BB:30 AB:16 AA:01	(-.099)

Samples	Loci	Genotypic Frequencies	Fixation Indices
SSOL	ADH	AB:22 AA:14 BB:18	(.181)
	CK-A	BB:47 AB:07	(-.069)
	CK-C	BB:54	*
	PHI-B	BB:36 AB:16 AA:02	(.019)
	IDH-A	BB:31 AB:18 AA:04	(.083)
	PGM	BB:44 AB:10	(-.102)
TOYA	ADH	BB:29 AB:13 AA:08	(.369)
	CK-A	BB:44 AB:06	(-.064)
	CK-C	BB:50 AB:01	(-.010)
	PHI-B	BB:47 AB:04	(-.041)
	IDH-A	AB:20 BB:16 AA:13	(.181)
	PGM	BB:29 AB:17 AA:05	(.144)
DNFH	ADH	BB:32 AB:07 AA:07	(.588)
	CK-A	BB:41 AB:05	(-.057)
	CK-C	BB:30	*
	PHI-B	BB:42 AB:04	(-.045)
	IDH-A	BB:05 AB:19 AA:17	(-.014)
	PGM	BB:30 AB:14	(-.189)

*Index not calculated due to absence of heterozygotes.

APPENDIX C

GENOTYPIC FREQUENCIES FOR ALL SAMPLES OF *GAMBUSIA NOBILIS*

Genotypic Frequencies for All Samples of *Gambusia nobilis*
(Fixation Indices in Parentheses)

Samples	Loci	Genotypic Frequencies	Fixation Indices
BLSH	ADH	AA:33	*
	FUM	BB:32	*
	PHI-A	CC:32	*
	PHI-B	AA:32	*
	IDH-A	AB:02 BB:30	(-.032)
	PGM-B	BB:30 BC:01	(-.016)
SAGO	ADH	AA:31	*
	FUM	BB:28 BC:03	(-.051)
	PHI-A	BC:01 CC:31	(-.016)
	PHI-B	AA:32	*
	IDH-A	BB:30 BE:01	(-.016)
	PGM-B	AB:02 BB:29	(-.033)
BSU	ADH	AA:31	*
	FUM	BB:30 BC:01	(-.016)
	PHI-A	CC:31	*
	PHI-B	AA:07 AB:13 BB:11	(.147)
	IDH-A	BB:31	*
	PGM-B	BB:24	*
BSM	ADH	AA:31	*
	FUM	BB:32	*
	PHI-A	CC:31	*
	PHI-B	AA:07 AB:14 BB:10	(.088)
	IDH-A	BB:29 BD:01 BE:01	(-.025)
	PGM-B	BB:31	*
BSL	ADH	AA:30	*
	FUM	BB:30	*
	PHI-A	CC:30	*
	PHI-B	AA:07 AB:14 BB:07	(.000)
	IDH-A	BB:30	*
	PGM-B	BB:30	*
PLSU	ADH	AA:01 AB:02 BB:29	(.467)
	FUM	BB:31 BC:01	(-.016)
	PHI-A	CC:10 CD:10 DD:12	(.373)
	PHI-B	AA:32	*
	IDH-A	AC:03 BB:09 BC:16 CC:04	(-.104)
	PGM-B	BC:02 CC:30	(-.032)

Samples	Loci	Genotypic Frequencies	Fixation Indices
PLSM	ADH	AB:11 BB:21	(-.208)
	FUM	BB:32	*
	PHI-A	CC:06 CD:14 DD:11	(.073)
	PHI-B	AA:32	*
	IDH-A	AB:01 BB:12 BC:12 CC:05 BD:02	(.080)
	PGM-B	BC:01 CC:31	(-.016)
PLSL	ADH	AA:03 AD:08 BB:21	(.506)
	FUM	BB:32	*
	PHI-A	CC:06 CD:08 DD:17	(.410)
	PHI-B	AA:32	*
	IDH-A	AB:08 BB:11 BC:12	(-.325)
	PGM-B	BB:01 BC:03 CC:27	(.347)
GIFN	ADH	AA:18 AB:13	(-.067)
	FUM	BB:30	*
	PHI-A	CC:02 CD:13 DD:15	(-.067)
	PHI-B	AA:30	*
	IDH-A	AB:02 BB:28 BC:01	(-.039)
	PGM-B	BB:18 BC:11 CC:02	(.033)
ESAN	ADH	AA:15 AAB:12 BB:06	(.214)
	FUM	AB:02 BB:32	(-.030)
	PHI-A	CC:02 CD:07 DD:24	(.236)
	PHI-B	AA:33	*
	IDH-A	BB:30 BC:04	(-.063)
	PGM-B	BB:09 BC:17 CC:07	(-.034)
DIAU	ADH	AA:27 AB:05	(-.085)
	FUM	AB:02 BB:30	(-.032)
	PHI-A	AB:01 BC:10 CC:21	(-.109)
	PHI-B	AA:32	*
	IDH-A	BB:31	*
	PGM-B	BB:31	*
DIAL	ADH	AA:30 AB:01	(-.016)
	FUM	BB:31	*
	PHI-A	BB:01 BC:11 CC:20	(-.062)
	PHI-B	AA:32	*
	IDH-A	BB:31	*
	PGM-B	BB:31	*

Samples	Loci	Genotypic Frequencies	Fixation Indices
LC1	ADH	AA:26 AB:04	(-.071)
	FUM	BB:30	*
	PHI-A	AB:03 BC:12 CC:15	(-.124)
	PHI-B	AA:30	*
	IDH-A	BB:30	*
	PGM-B	BB:30	*
LC2	ADH	AA:10 AB:02	(-.091)
	FUM	BB:12	*
	PHI-A	BC:04 CC:08	(-.200)
	PHI-B	AA:12	*
	IDH-A	BB:12	*
	PGM-B	BB:12	*
LC3	ADH	AA:25 AB:05	(-.091)
	FUM	BB:30	*
	PHI-A	BB:01 BC:07 CC:22	(.085)
	PHI-B	AA:29 AB:01	(-.017)
	IDH-A	BB:29 BC:01	(-.017)
	PGM-B	BB:30	*
LC4	ADH	AA:26 AB:06	(-.103)
	FUM	AA:05 AB:03 BB:24	(.710)
	PHI-A	BB:01 BC:12 CC:19	(-.097)
	PHI-B	AA:31 AB:01	(-.016)
	IDH-A	BB:29 BC:03	(-.049)
	PGM-B	BB:32	*
DNFH	ADH	AA:25 AB:06	(-.107)
	FUM	BB:32	*
	PHI-A	AB:01 AC:01 BC:09 CC:20	(-.100)
	PHI-B	AA:31	*
	IDH-A	BB:31	*
	PGM-B	BB:31	*

*Index not calculated due to absence of heterozygotes.

VITA

David Ray Edds

Candidate for the Degree of

Master of Science

Thesis: THE GENETIC STRUCTURE OF ENDANGERED DESERT FISHES: NATURAL VERSUS CAPTIVE POPULATIONS

Major Field: Zoology

Biographical:

Personal Data: Born in Independence, Kansas, February 2, 1954, the son of Neil R. and Norma J. Edds.

Education: Graduated from Independence High School, Independence, Kansas, in May, 1972; received Associated of Arts degree in Liberal Arts from Independence Community College, Independence, Kansas, in May, 1974; received Bachelor of Science degree in Biology from the University of Kansas, in May, 1977; completed requirements for the Master of Science degree at Oklahoma State University in July, 1984.

Professional Experience: Teaching Assistant, Department of Zoology, Oklahoma State University, August, 1983 to December, 1983; Research Assistant, Department of Zoology, Oklahoma State University, August, 1982 to July, 1983; Fisheris Research Specialist, University of Oklahoma Biological Station, March, 1981 to July, 1982; U. S. Peace Corps/Nepal Conservation Program Director, October, 1979 to November, 1980; U. S. Peace Corps Volunteer/Volunteer Leader, Nepal, September, 1977 to September, 1979; Fisheris Aide, Kansas Forestry, Fish, and Game Commission, May, 1976 to August, 1976 and May, 1977 to August, 1977; Park Technician, Army Corps of Engineers, June, 1974 to October, 1974.