A GENETIC ANALYSIS OF A HYBRID SWARM

BETWEEN TWO PUPFISHES, <u>CYPRINODON</u>

<u>PECOSENSIS</u> AND <u>C. VARIEGATUS</u>

(CYPRINODONTIDAE):

GEOGRAPHICAL

PATTERN

By

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ACKNOWLEDGMENTS

Any dead fish can float downstream.

It takes a live one to swim upstream.

W. C. Fields

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INTRODUCTION

The Pecos pupfish, _Cyprinodon pecosensis_ (Family
Cyprinodontidae), is known only from the saline mid-reaches
of the Pecos River and closely associated springs and
sinkholes in southeastern New Mexico and West Texas
(Echelle and Echelle, 1978). This paper presents an
allozymic analysis of the evolutionary change that occurred
after a widespread coastal species, the sheepshead minnow,
C. variegatus, was introduced into the Texas portion of the
Pecos River. The apparent rapidity and extent of the
ensuing genetic changes seem unprecedented in the
literature on hybridization.

Prior to 1984, _C. pecosensis_ was the only pupfish known
to occur in the Pecos River proper. Two other pupfishes,
C. bovinus and _C. elegans_, occur in the Pecos River basin
but they are isolated in separate spring-systems and have
never been reported from the Pecos River (Echelle and
Echelle, 1978). In August of 1984, specimens which
allozymically appeared to be products of hybridization and
backcrossing between _C. pecosensis_ and _C. variegatus_ were
taken from the Pecos River at two localities in Texas
(Echelle et al., 1987). As late as 1980, collections of
pupfish from the Pecos River in Texas revealed no
morphological evidence of _C. variegatus_. Thus,
hybridization probably ensued sometime between 1980 and
1984 when _C. variegatus_ was introduced into the area.

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With few exceptions (Humphries and Miller, 1981; Humphries, 1984 a,b; Minckley and Minckley, 1986), the 30 or so species of Cyprinodon are allopatrically distributed. Therefore, there are relatively few situations in which reproductive isolation between naturally-occurring sympatric forms can be observed. In two such instances, morphological data indicate some degree of hybridization, but not at a particularly high level (Humphries and Miller, 1981; Minckley and Minckley, 1986). However, two accidental introductions of C. variegatus into the ranges of other pupfishes, both in the Pecos River basin, apparently were followed by high levels of genetic interaction:

1) Stevenson and Buchanan (1973) presented morphological evidence of hybridization in a narrow zone of contact between an introduced, reservoir population of C. variegatus and the spring-dwelling species, C. elegans. The spatial pattern of morphological variation indicated the possibility that the reservoir population had been genetically introgressed by the endemic species. Any introgression of the latter species was prevented by a physical barrier to upstream migration.

2) C. variegatus also was introduced into Leon Creek, Pecos Co., Texas, where it apparently hybridized extensively with the endemic species, C. bovinus (Kennedy, 1977). However, no analysis of the extent of hybridization has been reported. Because of concern for the C. bovinus genome, a massive, apparently successful

effort was made to eliminate C. variegatus and putative hybrids from C. bovinus habitat (Hubbs et al., 1978; Hubbs, 1980; Echelle et al., 1987).

Most pupfishes appear to be rather recent evolutionary products that may not have fully crossed the threshold of speciation: (1) Laboratory breeding experiments reveal little or no pre- or postmating reproductive isolation (Turner and Liu, 1977; Drewry, 1967; Cokendolpher, 1980; Loiselle, 1982; Garrett, 1979); (2) allozyme studies on morphologically divergent pupfishes in two separate regions, the Death Valley area, California and Nevada (Turner, 1974; 1983) and Laguna Chichankanab, Yucatan (Humphries, 1984 a; b), revealed levels of differentiation typically associated with intraspecific variation in other vertebrates, and (3) divergent species of Cyprinodon have the diploid number of 48 with no evidence of karyotypic differentiation (Stevenson, 1975, 1981; Miller and Echelle, 1975), although no detailed banding studies have been reported.

Thus, Turner and Liu (1977) and Stevenson (1981) suggested that Cyprinodon consists of several superspecies each containing a number of morphologically distinct semispecies. The superspecies have not been diagnosed. However, C. pecosensis and C. variegatus have generally been recognized as belonging to the same species complex within the genus (Echelle and Echelle, 1978; Miller, 1981; Echelle et al., 1987; Smith and Miller, 1986).

MATERIALS AND METHODS

Collections of pupfish totalling 4150 specimens were
made in March and August of 1985 from 20 localities in the
Pecos River drainage of Texas (Fig. 1). A sub-sample of 1-
681 of the specimens was preserved at each collection site
and catalogued in the Oklahoma State University Museum of
Natural and Cultural History (OSUS 12189, 12192, 12196,
12201, 12204, 12208, 12216, 12221, 12234, 12239, 12245,
12251, 12257, 12262, 12266, 12270, 12275, 12279, 12282). A
second sub-sample of 8-90 specimens was placed on dry ice
in the field, transported to the laboratory, and stored at
-70°C. Reference specimens of C. pecosensis and C.
variegatus from the Bitter Lake National Wildlife Refuge,
Chaves Co., New Mexico and the Galveston Bay area,
Galveston Co., Texas, respectively, were treated similarly.

Standard methods of horizontal starch-gel
electrophoresis (Selander et al., 1971; Siciliano and Shaw,
1976) were used to examine the products of four presumptive
gene loci: Alcohol dehydrogenase (Adh-A, EC 1.1.1.1),
Esterase (Est-1, EC 3.1.1.1), Glucose-6-phosphate
isomerase (Gpi-A, EC 5.3.1.9), and Proline dipeptidase
(Pdp-A, EC 3.4.13.9). Est-1 is the esterase locus of
greatest anodal mobility in pupfish liver and is the
esterase predominant in eye tissue. A high degree of
variability occurred among electromorphs for Est-1 in C.
variegatus; thus this locus was difficult to score

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Figure 1. Map of localities in study area. Stippled area represents a portion of the range of the sheepshead minnow, C. variegatus, and diagonal shading denotes the historic range of the Pecos pupfish, C. pecosensis. Continuous distribution is not implied by shading; only the known range.
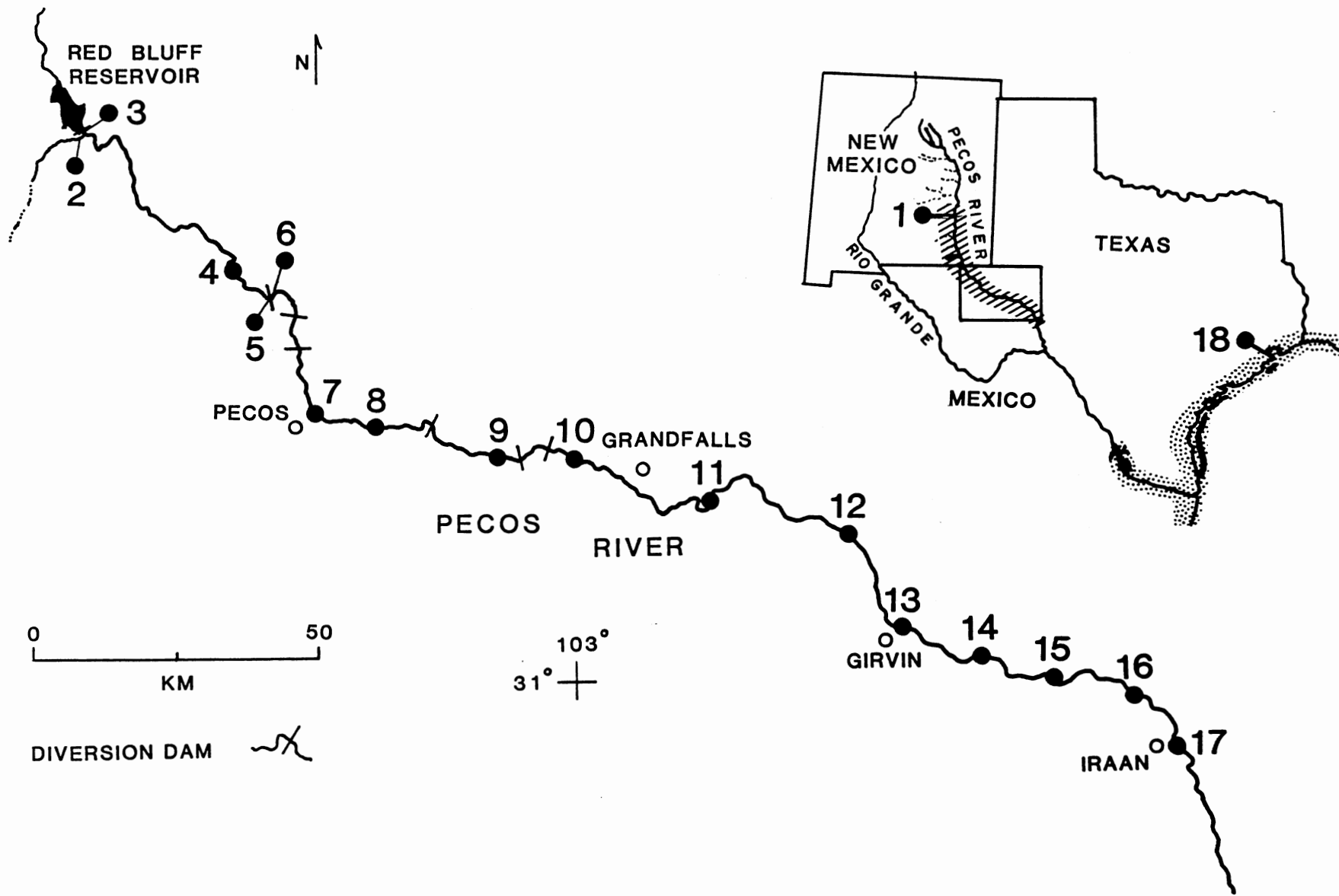
consistently. All of those electromorphs were slower than the one diagnostic of C. pecosensis and they are treated herein as representing a single allele. The four loci examined have fixed or nearly-fixed differences between C. pecosensis and C. variegatus (A. A. Echelle and A. F. Echelle, unpubl.). The liver was excised from each specimen, homogenized in distilled water, and centrifuged to obtain extracts of the water-soluble proteins. At the time of dissection, sex and standard length were recorded for each specimen, which was then tagged, preserved, and catalogued (OSUS 13647-13669, 13673, 13674, 13676, 13678). 12% starch gels, 341 cm² in area, were used. Electrophoresis employed the Tris-EDTA buffer system reported by Turner (1983).

Single-locus analyses were done using the BIOSYS-1 computer program (Swofford and Selander, 1981). To obtain a composite character-index for each specimen, a score of +1 and -1 was assigned for each allele typical of respectively, C. variegatus and C. pecosensis. The character-index is the sum of these scores.

For each sample, the composite measure of linkage disequilibrium (D, as described by Campton, 1986), and a standardized measure of linkage disequilibrium (D' = D/Dmax; see Hedrick, 1983) were computed from observed phenotypic frequencies in each pairwise combination of the four loci. Linkage disequilibrium addresses the question of whether alleles for the paired loci are assorting at random in gamete formation. In some cases, samples from

two to four different collections were pooled to increase
sample size and confidence in the estimation of **D**. The
heterogeneity G-test (Sokal and Rohlf, 1981) was used to
determine the likelihood that the gametic frequencies
inferred from the genotypes at these sites are
statistically homogeneous. Assuming gene frequencies are
estimated without error, I use the Nr^2 (X^2) statistic
to test the hypothesis that **D** is zero (Hill and
Robertson, 1968; Speiss, 1977).

RESULTS

Single Locus Analyses

The reference samples of C. pecosensis and C. variegatus were fixed or nearly fixed for different alleles at each of the four loci examined (Table 1, Fig. 2). At three of the four loci, Adh-A, Gpi-A, and Pdp-A, samples shared electromorphs at levels of 0.01 - 0.05, while no sharing was detected for Est-1. Thus, all four loci are effectively diagnostic of the two species.

The allele frequencies show a remarkable pattern of change downstream from Red Bluff Reservoir in the Pecos River drainage of Texas. The Salt Creek population showed no evidence of the influence of C. variegatus. However, at locality 3, approximately 1 km upstream from the mouth of Salt Creek, the frequency of the alleles typical of C. variegatus (= "variegatus alleles") averages 0.17 across the four loci. Continuing downstream, the frequency of variegatus alleles show a pattern of clinal increase which, in the vicinity of Pecos, Reeves Co., reaches a peak of 0.84. Then follows a reversed cline: the frequency of the variegatus alleles declines to a minimum of 0.40 at localities 12 and 13, approximately 175 km downstream from Pecos and effectively remains at that level in areas farther downstream. Abrupt changes were associated with the positions of irrigation-diversion dams (Fig. 1). The

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Table I. Allele frequencies at four loci in pupfish from the Pecos River. L denotes the locality.

L	DATE	$\bar{N}$	Adh-A		Est-1		Gpi-A				Pdp-A		
			a	c	a	b	b	c	d	e	b	c	d
1	8/84	22	1.00		1.00				1.00		0.05	0.95	
2	3/85	60	1.00		1.00		0.01		0.98	0.01	0.02	0.98	
2	8/86	18	1.00		1.00				0.98	0.02		1.00	
3	3/85	7	0.81	0.19	0.71	0.29		0.13	0.87		0.06	0.94	
4	3/85	30	0.72	0.28	0.75	0.25		0.15	0.85		0.28	0.72	
5	3/85	11	0.46	0.54	0.36	0.64		0.14	0.86		0.70	0.30	
6	3/85	69	0.24	0.76	0.21	0.79		0.46	0.54		0.79	0.21	
6	8/85	50	0.24	0.76	0.12	0.88		0.50	0.50		0.79	0.21	
7	3/85	89	0.09	0.91	0.04	0.96		0.62	0.38		0.92	0.08	
7	8/85	60	0.10	0.90	0.03	0.97		0.58	0.42		0.80	0.20	

Table I continued

L	DATE	$\bar{N}$	Adh-A		Est-1		Gpi-A				Pdp-A		
			a	c	a	b	b	c	d	e	b	c	d
8	3/85	41	0.11	0.89	0.07	0.93		0.70	0.30		0.89	0.11	
8	8/85	59	0.05	0.95	0.05	0.95		0.58	0.42		0.88	0.12	
9	3/85	34	0.21	0.79	0.13	0.87		0.59	0.41		0.86	0.14	
9	8/85	60	0.26	0.74	0.14	0.86		0.57	0.43		0.76	0.24	
10	3/85	57	0.37	0.63	0.39	0.61		0.45	0.55		0.51	0.49	
11	3/85	67	0.63	0.37	0.44	0.56		0.43	0.57		0.45	0.54	0.01
12	3/85	60	0.62	0.38	0.46	0.54		0.36	0.63	0.01	0.32	0.68	
13	3/85	63	0.64	0.36	0.53	0.47		0.34	0.66		0.43	0.57	
14	3/85	68	0.52	0.48	0.51	0.49		0.35	0.65		0.37	0.63	
15	3/85	19	0.50	0.50	0.47	0.53		0.32	0.68		0.50	0.50	

Table 1 continued

L	DATE	$\bar{N}$	Adh-A		Est-1		Gpi-A				Pdp-A		
			a	c	a	b	b	c	d	e	b	c	d
16	3/85	51	0.63	0.37	0.44	0.56		0.41	0.59		0.40	0.60	
17	3/85	53	0.49	0.51	0.51	0.49		0.35	0.65		0.48	0.52	
18	2/86	83	0.01	0.99*		1.00*		1.00*			1.00*		
18	3/86	81		1.00*		1.00*		0.98*	0.02		1.00		
Allele assignment [a]			P	V	P	V	P	V	P	P	V	P	O

* Other *variegatus* alleles included.

[a]
P = *C. pecosensis*, V = *C. variegatus*, O = no assignment

Figure 2. The frequencies of <u>C. variegatus</u> alleles for all localities. Vertical bars on horizontal axis represent diversion dams for various irrigation districts. Reference samples are localities 1 and 18 for <u>C. pecosensis</u> and <u>C. variegatus</u>, respectively.
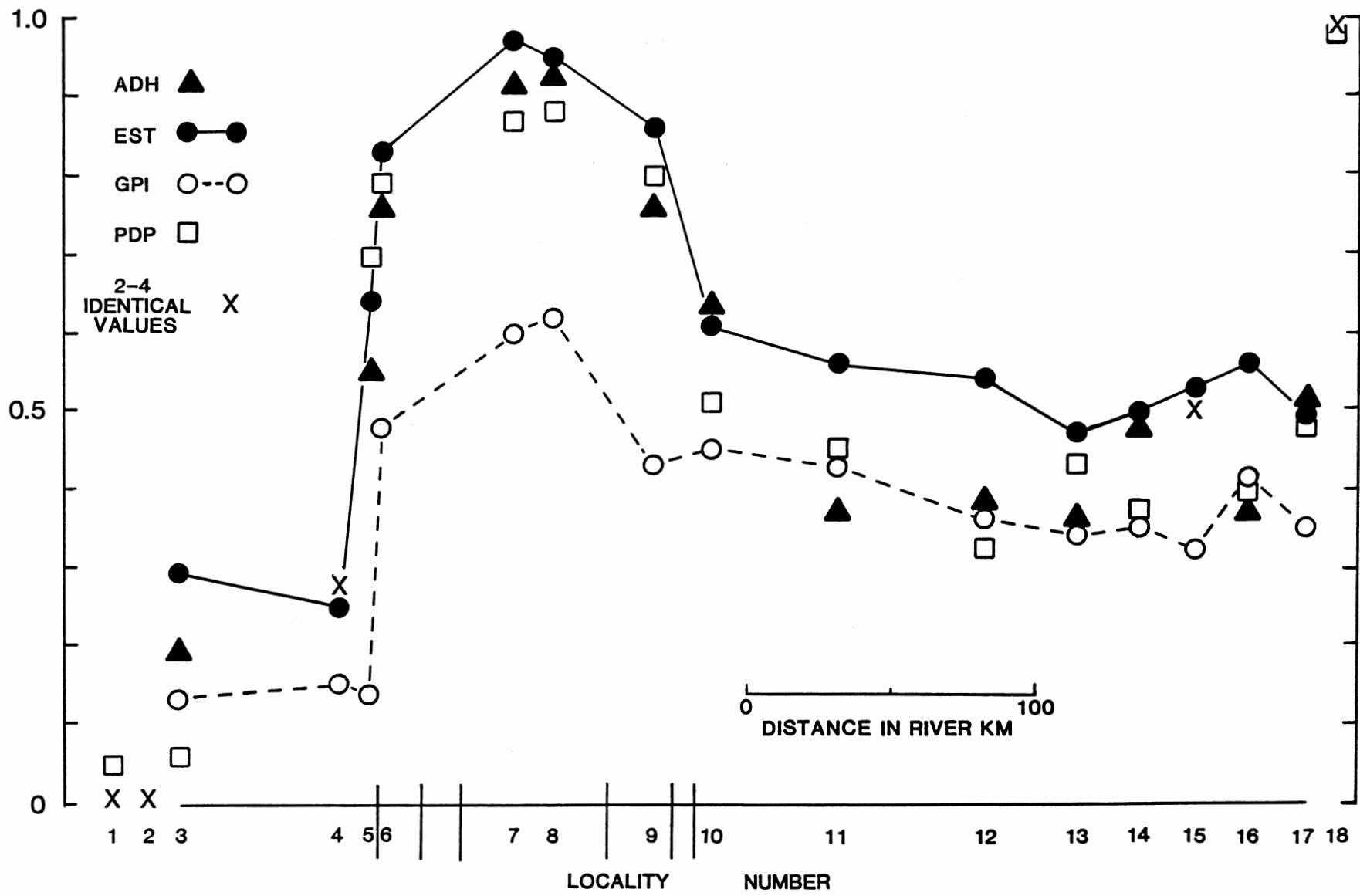
four diagnostic loci show similar clinal variation throughout the portion of the river studied. However, the frequency of the _variegatus_ allele for Est-1 is consistently higher than that for Gpi-A.

All samples conformed closely with Hardy-Weinberg expectations for genotypic frequencies. An exact probability test provided by BIOSYS-1 (Swofford and Selander, 1981) revealed only three significant ($p < 0.05$) departures from Hardy-Weinberg expectations in a total of 72 single-locus comparisons: Gpi-A at locality 14 and Pdp-A at localities 6 and 7.

Multilocus Analyses

Table II shows how alleles were assigned to create the character-index score for each specimen. Table III summarizes the character-index scores at all sites including the reference samples (loc. 1 and 18). Frequency histograms at most localities were unimodal and broader than those for reference samples (Fig. 3). The modal shifts from locality to locality (Fig. 3) conform with the clinal changes in allele frequencies (Fig. 2): at upstream sites (localities 3 and 4) the mode was shifted towards the C. pecosensis mode; at sites 5 to 9 the mode was shifted toward that of C. variegatus; and at sites 10 to 17 the mode was intermediate between those of C. variegatus and C. pecosensis. Excepting the Salt Creek sample (loc. 2) which shows no evidence of C. variegatus influence, the frequency distributions of scores were much broader among Texas populations in the Pecos River than in either of the reference samples.

Values of standardized linkage disequilibrium (D') are presented in Table IV and Fig. 4. Overall, D' showed a trend towards positive values (sign test $X^2_{1 d.f.} = 19.0$, $P < 0.005$; Steel and Torrie, 1980). In all samples, D' for the Est-1 X Gpi-A combination of loci was markedly larger than for all other combinations of loci (Fig. 4). The trend toward positive values remained even after removal of the Est-1 X Gpi-A values ($X^2_{1 d.f.} = 11.8$, $P < 0.005$).

Table II. Alleles used in constructing hybrid index.

Locus	C. pecosensis	C. variegatus
Adh-A	a	b, c, d, e, f
Est-1	a	b
Gpi-A	b, d, e	a, c
Pdp-A	c	a, b

Index: -1 for each C. pecosensis allele in genotype,

+1 for each C. variegatus allele in genotype.

Table III. Character-index scores from Pecos River, 1985. Scores are the sum of values assigned to each allele in the genotype. L is the locality number. Q denotes the number of quadruple heterozygotes included in the 'O' class.

		C. PECOSENSIS						C. VARIEGATUS				
L	N	-8	-6	-4	-2	O	(Q)	+2	+4	+6	+8	MEAN
1	22	20	2									-7.82
2	78	75	3									-7.92
3	7	2	2	1	2							-5.14
4	30	7	5	7	6	2	(2)	3				-4.00
5	10		1	1	3	1	(0)	1	3			-0.20
6	117	1	1	2	3	10	(1)	25	41	30	4	3.40
7	143					5	(0)	11	39	57	31	5.37
8	97					1	(0)	11	27	31	27	5.48
9	93			2	5	6	(1)	16	30	20	14	3.94
10	56	1	1	5	10	11	(4)	14	9	4	1	0.75
11	65	1	11	4	16	10	(4)	12	9	1	1	-0.74
12	58	6	7	6	12	13	(7)	7	5	2		-1.59
13	61	4	9	8	9	18	(10)	7	5	1	.	-1.57
14	68	4	11	9	15	8	(2)	9	5	5	2	-1.18
15	19		3		7	5	(2)	1	1	1	1	-0.63
16	51	2	5	6	12	11	(5)	10	4	1		-1.02
17	52	3	4	7	13	7	(1)	7	6	5		-0.65
18	81									2	79	7.95

Figure 3. Histograms of the character-index scores by locality. Open bars denote "pure" populations. Locality 1 is Bitter Lake National Wildlife Refuge, Chaves Co., New Mexico and locality 18 is the Galveston Bay area, Galveston Co., Texas.

10% FREQUENCY
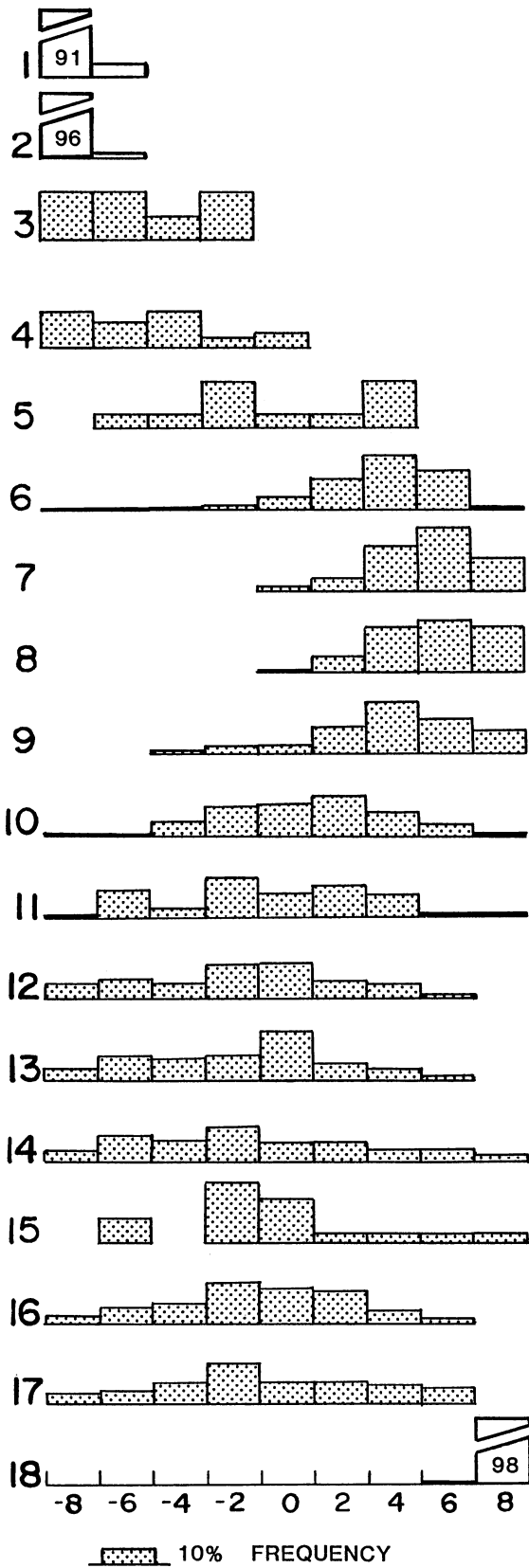
Correspondingly, all disequilibrium values significantly
(p < 0.05) different from zero were positive in sign.

Table IV. Values of D' (D/Dmax) in pupfish from the Pecos River of West Texas. Bottom and right margins are means of gene pair and locality values respectively. * $P < 0.05$, ** $P < 0.005$ using Nr^2 statistic: H_0: $D = 0$.

					Locality Number (N)					
Paired Loci	6 (118)	7,8 (245)	9 (94)	10 (57)	11 (67)	12 (59)	13 (62)	14,15 (87)	16,17 (104)	MEAN
Adh X Est	.060	-.995	.055	-.060	.429*	.458*	.246	.262*	.110	.063
Adh X Gpi	-.097	-.199	.182	-.148	-.010	.389**	-.013	.443**	.095	.071
Adh X Pdp	.236	-.132	.090	-.062	.020	.079	.338*	.437**	.031	.115
Est X Gpi	.419	.809**	.861**	.829**	.668**	.925**	.469**	.731**	.617**	.703**
Est X Pdp	.086	-.533	.163	.303	.293	.170	.298*	.348*	.219	.150
Gpi X Pdp	-.081	.046	.199	.124	.166	.154	.178	.346**	.258*	.154
MEAN	.104	-.167	.259**	.165	.261	.362	.253	.428**	.222**	.209

Figure 4.   Block chart of values of D' (D/Dmax) for nine
localities and six gene pairs.   The stippled area
represents statistically significant (p < .05) linkage
disequilibrium.
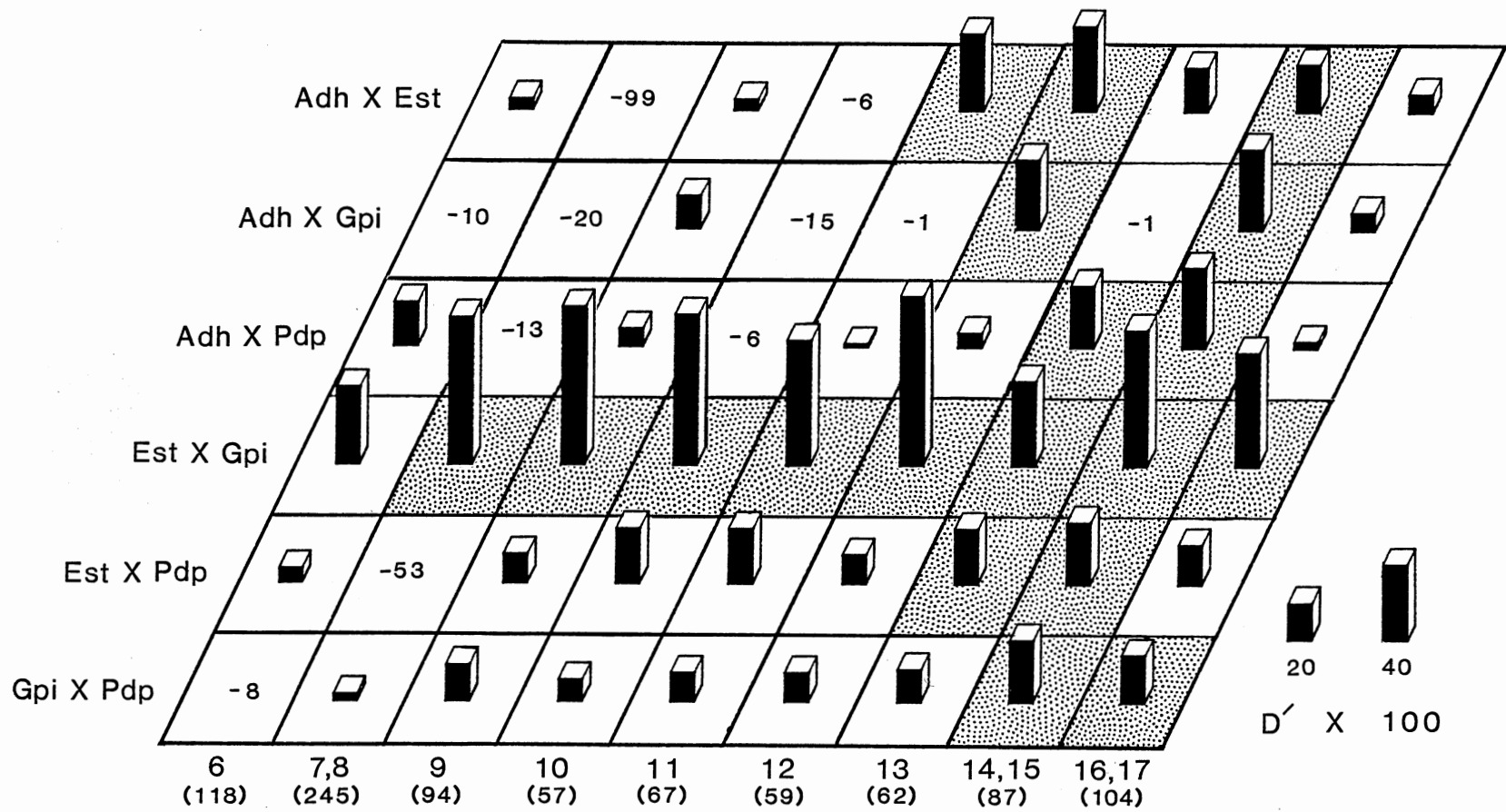
DISCUSSION

My data demonstrate that, throughout the Pecos River in the Texas portion of the range of C. pecosensis, pupfish are segregating for alleles diagnostic of the endemic species, C. pecosensis, and the introduced species, C. variegatus. This is the third known instance of an introduction of C. variegatus in the area, the others occurring in isolated springs (Stevenson and Buchanan, 1973; Hubbs, 1980). These introductions probably are accidental results of the stocking of sportfish. Stocking records of the Texas Parks and Wildlife Department show that, beginning in the 1960's, red drum, Sciaenops ocellatus, and striped bass, Morone saxatilis, have been introduced in several reservoirs in the Pecos River drainage and striped bass now occur in the Pecos River proper (pers. obs.). The original stocks were taken either directly from shallow coastal waters by seining (red drum), or they were obtained from coastal hatchery installations (red drum and striped bass). Because C. variegatus is abundant throughout the coastal waters of Texas, it may have been inadvertently included in the introduced stocks of fish.

Two lines of evidence sugggest that, despite the admixture of two recognized species of Cyprinodon, local populations in the Pecos River of Texas represent panmictic units: (1) the consistent lack of statistically

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significant single-locus deviations from Hardy-Weinberg
expectations and (2) the lack of bimodality in the
frequency histograms for the multilocus character index.
Thus, the original population of C. pecosensis has been
replaced by a hybrid population. It seems likely that no
individual in my samples from the Pecos River is free of
variegatus alleles. I examined only four loci, thus a
maximum of only four of the 24 pairs of chromosomes in C.
pecosensis (2N = 48). Nonetheless, only 31 (3.3%) of 927
specimens examined from the Pecos River had genotypes
expected of "pure" C. pecosensis. That percentage would
have been much lower if more chromosome markers (i.e.,
loci) had been examined. Similarly, it is unlikely that
any specimen sampled was a "pure" C. variegatus, even in
the vicinity of Pecos where the population has a high
proportion of variegatus alleles. Thus, it appears that,
over almost the entire Texas portion of its geographic
range, approximately 430 river km of the Pecos River, C.
pecosensis has been replaced by a hybrid population.

Despite apparent panmixia, the linkage disequilibrium
analysis demonstrates that the pupfish population in the
Pecos River has not attained multilocus equilibrium. The
consistent pattern of positive D' values represents an
excess of coupling gametes. In other words, in my sampling
of genotypes, C. variegatus alleles at different loci
occurred together more often than random association would
predict and the same is true for C. pecosensis alleles.
Because the populations are panmictic, there are two

primary explanations for the observed disequilibrium
(Cavalli-Sforza and Bodmer, 1971): (1) selection for
particular pairs of chromosomes or for pairs of interlocus
allelic combinations, and (2) residual allelic associations
reflecting the original genetic differentiation of the two
species. Although it declines with each generation of
random mating, the residual effect occurs, even if the loci
involved assort independently in meiosis.

For one pair of loci, Est-1 and Gpi-A, it appears that
natural selection may be maintaining the observed
disequilibrium. The **D** values for this pair of loci were
exceptionally high ($D' = 0.41 - 0.92$, avg. 0.703) compared
to those for the other combinations of loci ($-0.99 - 0.46$,
average of loci combinations excluding Est-1 X Gpi-A 0.11)
At first clance, this suggests that Est-1 and Gpi-A are on
the same chromosome, thus retarding the decline in
disequilibrium relative to that in other pairs of loci.
However, such tight linkage should produce near equivalency
in the frequencies of variegatus alleles at the two loci.
Instead, those frequencies are strongly different (Fig. 2).
Thus, for at least the Est-1 X Gpi-A combination, natural
selection may be maintaining the observed disequilibrium.
The difference in allele frequencies between the two
loci may have occurred, when the introduced alleles were
rare and more susceptible to genetic drift.

Because disequilibrium values of the remaining pairs of
loci were small and approximately equivalent, it seems
reasonable to assume that the loci involved are assorting

independently in meiosis. Although I cannot completely
exclude the possibility, it seems unlikely that all of the
various combinations of loci are being subjected to natural
selection for coupling allele combinations. Therefore,
the consistently positive disequilibrium values support the
conclusion from past collections that the existing genetic
structure of pupfish in the Pecos River has developed
rather recently.

Five collections made by Duke et al. in Texas in March
and April, 1980, reveal no influence of C. variegatus in
Salt Creek (loc. 2), in the Pecos River at localities 11 and
13, nor 40 km downstream from locality 17 near Sheffield,
Pecos Co. (ENMU TX-RE-80-6.02, TX-LO-80-6.02, TX-PE-80-
012.02, TX-PE-80-7.04, TX-CR-80-9.09). However,
collections made in August, 1984 (reported by Echelle et
al., 1987) at Pecos (loc. 7) and Iraan (loc. 16) and my
collections thoughout the area in 1985 show abundant
evidence of morphological traits expected of C. variegatus.
It is likely, therefore, that C. variegatus was introduced
into the Pecos River of Texas sometime between 1980 and
1984.

The clinal patterns of variation in allele frequencies
suggest that the original introduction of C. variegatus
occurred in the vicinity of Pecos, Reeves Co., Texas. The
pattern also suggests that the introduction was followed by
the dispersal of C. variegatus alleles both upstream and
downstream from the Pecos area.

In both directions, the clines in allele frequencies

are steepest between sample sites separated by irrigation-
diversion dams. Thus, these dams apparently are retarding
gene dispersal. This would also explain the observation
(Fig. 2) that the upstream clinal changes are steeper than
the downstream changes. These dams range in height from
approximately 8-15 m and, although water often flows over
the smaller dams and sometimes is allowed to flow through
the larger ones, traversal by pupfish would be especially
difficult in the upstream direction. This "gene-flow"
hypothesis seems much more probable than the possibility
that environmental heterogeneity controls the clinal
patterns in allele frequencies. The Pecos River is
relatively uniform throughout the area and no obvious
environmental changes are associated with the clinal
patterns.

If the Pecos area was the initial point of
introduction for C. variegatus, the level of linkage
disequilibrium (D') in the pupfish of that area should be
lower than elsewhere in the Pecos River of Texas. This
assumes that with time, the level of disequilibrium is
eroding as a result of recombination and random mating. On
the other hand, if, as indicated for Est-1 and Gpi-A, the
observed D' values are being maintained by natural
selection, disequilibrium may persist. Because of the
skewed allele frequencies involved, my sample sizes
generally are not adequate to give high confidence in
estimates of D' in the Pecos area (Brown, 1975). If future
studies indicate little or no temporal change in D', then

natural selection for the coupling arrangement of chromosomes or alleles would be implicated.

Given the rapidity of change in pupfish populations in the Pecos River, it is clear that intense selection pressures have been operating. In allele frequencies, the population in the Pecos area is strongly shifted toward the genetic structure of C. variegatus. Furthermore, at least in superficial appearance, that population is morphologically indistinguishable from C. variegatus (pers. obs.). Apparently, C. variegatus-like individuals were favored over both C. pecosensis and intermediates between the two species. This suggests that once equilibrium is reached, the entire pupfish population will resemble C. variegatus in both morphology and allozyme structure.

Based on past collecting experience (A.A. Echelle, pers. comm.) the C. pecosensis population would have been very dense in the Pecos area at the time C. variegatus presumably was introduced. Thus, the original stock of C. varigatus must have been vastly outnumbered by C. pecosensis. Because of the relative densities, plus the generally low level of species recognition between allopatric pupfish (Turner and Liu, 1977; Cokendolpher, 1980; Loiselle, 1982), the progeny of the introduced stock of C. variegatus would have been dominated by C. variegatus X C. pecosensis hybrids. Thus, hybrid vigor may have provided the initial impetus for genetic change in the population.

An allozyme survey of several pupfish species in Texas and New Mexico (Echelle et al., 1987; Echelle and Echelle, 1987 in manuscript) found low averages of heterozygosity and polymorphism in C. pecosensis (H = 0.027; P = 0.07) relative to C. variegatus (H = 0.061; P = 0.18) and four of the remaining five species examined. As observed for a number of other organisms (Allendorf and Leary, 1986), heightened heterozygosity can confer enhanced fitness. This effect may help explain the high fitness of hybrids relative to C. pecosensis. However, it cannot be the whole answer as evolution in the Pecos area seems to be proceeding toward a C. variegatus-like form having lower heterozygosity than was presumably present in the earlier hybrid population in the area.

Pupfish are lek breeders (Loiselle and Barlow, 1978; Itzkowitz, 1977) and, as Kodric-Brown (1977, 1983) described for C. pecosensis, males show high variance in reproductive success. Thus, "runaway sexual selection" (Fisher, 1958; Lande, 1980; 1981) may explain the rapid evolutionary change toward a C. variegatus-like form in the Pecos area. Such selection might be due to female mating preferences and/or competition among males for breeding rights. Kodrick-Brown's (1983) study shows that, in C. pecosensis, territory space can be a limiting factor in male reproductive success, and Loiselle (1982) found that, on the average, females of some pupfish species actually prefer heterospecific mates. Loiselle (1982) explained heterospecific preference as a result of selection for

enhanced genetic variability in the female's offspring, a
process that might be especially important in
geographically restricted desert populations.

Mayr (1954) described the genetic changes which could
lead to speciation. He characterized polyploidy,
hybridization, and founder effects as those genetic changes
in a natural population which potentially can affect all
loci. He termed the rapid shift in genes in this
different genetic background a "genetic revolution". The
allozymic traits studied here indicate that hybridization
indeed has resulted in a genetic revolution. Data
presented are prima-facie evidence of intense natural
selection either for the loci surveyed or, more likely,
traits linked to those loci. That there has been a large
and rapid change is clear. The means by which this
happened is not as apparent. Studies of the relative
importance of mate choice versus intrasexual competition
and other aspects of natural selection should be
particularly rewarding with the pupfish population of the
Pecos River.

C. pecosensis is an endangered species. The only
known remaining "pure" populations exist in New Mexico,
Salt Creek, Reeves Co., Texas and in a gravel pit at
Phipps Sand and Gravel Co., Pecos Co., Texas. Given man's
proclivity to transport fish where they do not belong,
measures should be taken to educate the public on the Pecos
pupfish's precarious position and deter them from
eliminating a unique product of evolution.

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Appendix A. Genotypic scores of specimens surveyed across four loci by gene pairs for locality 6.

	Est-1			Gpi-A		
Adh-A	AA	AB	BB	DD	CD	CC
AA	2	3	3	5	4	0
AC	1	6	22	4	22	14
CC	3	32	48	22	36	11

	Est-1			Pdp-A		
Gpi-A	AA	AB	BB	CC	BC	BB
DD	18	19	4	19	13	8
CD	4	28	15	15	20	12
CC	2	4	10	2	10	5

	Est-1			Adh-A		
Pdp-A	AA	AB	BB	AA	AC	CC
CC	11	21	5	15	15	7
BC	7	20	15	10	20	11
BB	6	10	9	9	12	4

Appendix A continued. Genotypic scores of specimens surveyed across four loci for localities 7 and 8.

	Est-1				Gpi-A		
Adh-A	AA	AB	BB		DD	CD	CC
AA	0	0	3		0	2	1
AC	0	2	35		6	14	17
CC	0	20	187		32	97	75

	Est-1				Pdp-A		
Gpi-A	AA	AB	BB		CC	BC	BB
DD	0	9	30		2	5	32
CD	0	10	103		2	25	86
CC	0	3	90		3	14	75

	Est-1				Adh-A		
Pdp-A	AA	AB	BB		AA	AC	CC
CC	0	1	6		0	2	5
BC	0	2	45		0	6	41
BB	0	19	173		3	29	159

Appendix A continued. Genotypic scores of specimens surveyed across four loci for locality 9.

	Est-1			Gpi-A		
Adh-A	AA	AB	BB	DD	CD	CC
AA	0	1	3	2	0	2
AC	2	7	28	8	19	10
CC	0	13	40	7	27	19

	Est-1			Pdp-A		
Gpi-A	AA	AB	BB	CC	BC	BB
DD	1	9	7	3	4	10
CD	1	11	34	2	13	31
CC	0	2	29	2	7	21

	Est-1			Adh-A		
Pdp-A	AA	AB	BB	AA	AC	CC
CC	0	3	4	1	2	4
BC	1	6	17	1	11	12
BB	1	13	48	2	24	36

Appendix A continued. Genotypic scores of specimens
surveyed across four loci for locality 10.

	Est-1				Gpi-A		
Adh-A	AA	AB	BB		DD	CD	CC
AA	3	4	2		3	3	3
AC	1	10	13		8	9	7
CC	4	14	5		9	11	4

	Est-1				Pdp-A		
Gpi-A	AA	AB	BB		CC	BC	BB
DD	5	13	2		5	10	5
CD	3	13	6		3	19	1
CC	0	2	12		2	7	5

	Est-1				Adh-A		
Pdp-A	AA	AB	BB		AA	AC	CC
CC	3	6	1		3	1	6
BC	3	20	12		4	18	14
BB	2	2	7		2	5	4

Appendix A continued. Genotypic scores of specimens
surveyed across four loci for locality 11.

	Est-1				Gpi-A		
Adh-A	AA	AB	BB		DD	CD	CC
AA	7	14	6		10	11	6
AC	6	14	10		10	13	7
CC	0	4	6		4	4	2

	Est-1				Pdp-A		
Gpi-A	AA	AB	BB		CC	BC	BB
DD	11	11	2		9	12	3
CD	1	16	11		7	13	7
CC	1	6	8		3	9	3

	Est-1				Adh-A		
Pdp-A	AA	AB	BB		AA	AC	CC
CC	6	10	3		8	8	3
BC	5	17	12		13	16	5
BB	2	5	6		5	6	2

Appendix A continued. Genotypic scores of specimens
surveyed across four loci for locality 12.

		Est-1			Gpi-A		
Adh-A		AA	AB	BB	DD	CD	CC
AA		8	11	5	14	8	2
AC		5	11	8	9	12	3
CC		0	5	5	1	6	3

		Est-1			Pdp-A		
Gpi-A		AA	AB	BB	CC	BC	BB
DD		12	8	3	14	10	1
CD		1	19	7	8	16	2
CC		0	0	8	4	3	1

		Est-1			Adh-A		
Pdp-A		AA	AB	BB	AA	AC	CC
CC		10	5	11	11	13	2
BC		3	21	5	10	12	7
BB		0	2	2	3	0	1

Appendix A continued. Genotypic scores of specimens
surveyed across four loci for locality 13.

		Est-1				Gpi-A		
Adh-A		AA	AB	BB		DD	CD	CC
	AA	9	15	2		12	11	3
	AC	3	18	6		8	17	2
	CC	2	5	2		4	5	0

		Est-1				Pdp-A		
Gpi-A		AA	AB	BB		CC	BC	BB
	DD	13	10	2		11	10	3
	CD	0	27	6		8	17	8
	CC	1	2	2		2	2	1

		Est-1				Adh-A		
Pdp-A		AA	AB	BB		AA	AC	CC
	CC	8	11	2		13	6	2
	BC	5	20	4		10	15	3
	BB	1	7	4		3	6	3

Appendix A continued. Genotypic scores of specimens
surveyed across four loci for localities 14 and 15.

	Est-1				Gpi-A		
Adh-A	AA	AB	BB		DD	CD	CC
AA	9	13	3		15	6	3
AC	10	19	11		23	11	6
CC	4	9	9		6	8	8

	Est-1				Pdp-A		
Gpi-A	AA	AB	BB		CC	BC	BB
DD	17	22	5		17	25	2
CD	3	15	7		9	13	3
CC	2	4	11		3	8	6

	Est-1				Adh-A		
Pdp-A	AA	AB	BB		AA	AC	CC
CC	12	11	6		13	14	2
BC	11	25	11		11	23	14
BB	0	5	6		1	3	6

Appendix A continued. Genotypic scores of specimens
surveyed across four loci for localities 16 and 17.

	Est-1				Gpi-A		
Adh-A	AA	AB	BB		DD	CD	CC
AA	10	15	9		18	11	5
AC	8	27	12		14	24	9
CC	5	9	8		8	12	2

	Est-1				Pdp-A		
Gpi-A	AA	AB	BB		CC	BC	BB
DD	18	19	4		19	13	8
CD	4	28	15		15	20	12
CC	2	4	10		2	10	5

	Est-1				Adh-A		
Pdp-A	AA	AB	BB		AA	AC	CC
CC	11	21	5		15	15	7
BC	7	20	15		10	20	11
BB	6	10	9		9	12	4

Appendix B. Plot of the frequencies of <u>variegatus</u> alleles against the average cline. All open circles above the diagonal represent Est-1 and all open circles below the diagonal represent Gpi-A.
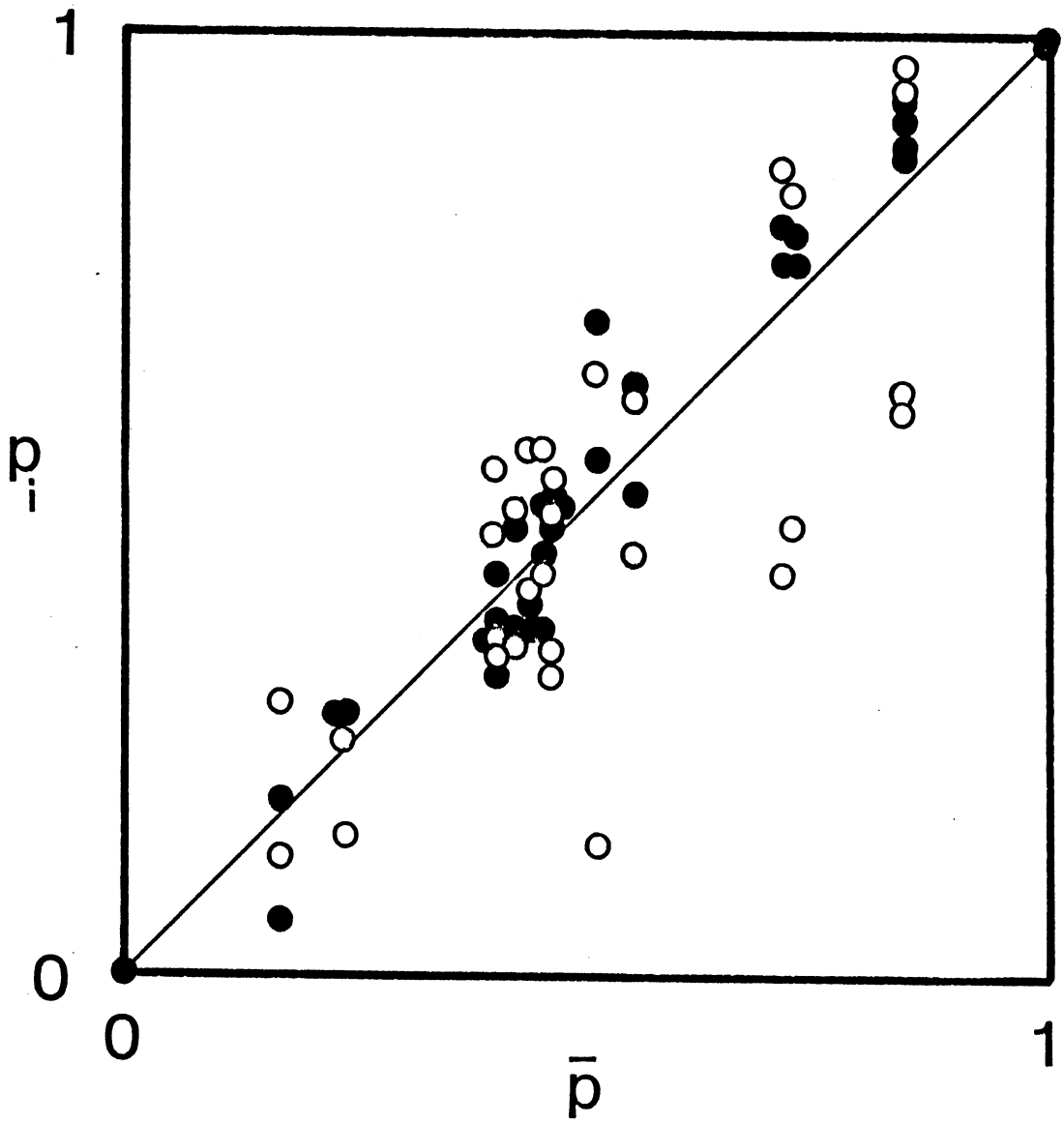
Appendix C. Values of $\hat{D}$, the unbiased estimate of the coefficient of linkage disequilibrium (Campton, 1986) for selected localities.

	Locality Number								
	(N)								
Paired	6	7,8	9	10	11	12	13	14,15	16,17
Loci	(118)	(245)	(94)	(57)	(67)	(59)	(62)	(87)	(104)
Adh X Est	.0089	-.0039	.0056	-.0090	.0703	.0807	.0483	.0640	.0230
Adh X Gpi	-.0126	-.0068	.0253	-.0306	-.0022	.0890	-.0017	.0787	.0206
Adh X Pdp	.0391	-.0014	.0141	-.0113	.0042	.0156	.0695	.0893	.0077
Est X Gpi	.0340	.0222	.0692	.1480	.1293	.1605	.0865	.1253	.1126
Est X Pdp	.0118	-.0030	.0184	.0618	.0595	.0248	.0689	.0698	.0464
Gpi X Pdp	-.0091	.0034	.0234	.0398	.0316	.0358	.0358	.0725	.0561

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Thesis: A GENETIC ANALYSIS OF A HYBRID SWARM BETWEEN TWO
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