at the Malaria Branch, Centers for Disease Control, Atlanta, GA. All were identified as *P. vivax*; blood-stage parasites producing reactor and nonreactor sporozoites could not be distinguished morphologically.

12. The IFATs (*10*) were done with nonreactive sporozoites and anti-*P. vivax* MAbs: Navy Medical Research Institute (NMRI) NSV3 and NSV4; New York University (NYU) 2F2 (*1*, *7*); Centers for Disease Control (CDC) CDC1; and Walter Reed Army Institute of Research WR1, 3, 7, 9, 21, and 28. These MAbs recognize at least three CS protein epitopes (R. A. Wirtz *et al.*, unpublished data). MAbs to other sporozoite species were also used: *P. malariae* 109–179.4 and *P. ovale* 110–54.3 from CDC; *P. cynomolgi* Ceylon Cy6A4.E11, NIH-13E.11C9, Gombak GB1G11.G1, and *P. knowlesi* 2G3 from NYU; and *P. berghei* (3.28, 3.116, 3.135, 3.213), *P. yoelli*, and *P. inui* from NMRI. Not all MAbs were tested against nonreactive sporozoites from the same patient mosquito feed. Some MAbs were tested in pools. MAbs were protein A purified (*10*) and tested at 2.5 or 5 μg/ml and were IFAT positive with the homologous sporozoite.
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15. Species-specific regions of the small subunit rRNA were detected as described by A. P. Waters and T. F. McCutchan, *Lancet*, in press. The oligonucleotides have been described (*15*) with the exception of the diagnostic for *P. vivax*, which is designated 165R and has the sequence: 5′-AAATCA(A/T)CC(G/A)(G/A)ATTCAGTCCCACGT-3′. The probe used to diagnose *vivax* would distinguish between *P. cynomolgi* and *P. vivax*.
16. PAGE and Western blotting were performed as described (*7*) with the following modifications. The nitrocellulose paper was treated with 0.3% Tween 20, and strips were incubated overnight with the specific antibody diluted in 10 m*M* tris, *p*H 8, containing 0.05% Tween 20, they were washed, and then incubated with alkaline phosphatase–conjugated antibody to mouse antigen (Promega). The paper was washed and developed with nitro blue tetrazolium (Promega).
17. R. A. Wirtz *et al.*, unpublished data.
18. Oligonucleotide primers PvRI (5′-GTCGGAATT CAATAAGCTGAAACAACCA-3′ and PvRII (5′-CAGCGGATCCACAGGTTACACTGGTGG-3′) corresponding to regions I and II, respectively, of the published *P. vivax* sequence (*2*, *3*) were synthesized. In order to facilitate unidirectional subcloning, primers were designed to contain either an Eco RI (PvRI) or Bam HI (PvRII) site starting five bases proximal to their 5′ ends. DNA was phenol-chloroform extracted from 2.6×10^5 sporozoites, in the presence of 20 μg of carrier yeast transfer RNA, and amplified by PCR 5 min at 94°C, then amplified through 25 cycles [incubated (1.5 min at 94°C, 2 min at 45°C, and 4 min at 72°C), followed by 5 min at 72°C] (GeneAmp Kit, Perkin-Elmer). DNA was precipitated, resuspended, and digested with Eco RI and Bam HI. Amplified DNA was agarose-purified by electrophoresis, excised (GeneClean, Bio 101), and ligated into Bluescript plasmid SK^+. A single insert-containing clone was selected and sequenced (Sequenase, U.S. Biochemical). Sequencing primers were M13 and T3, which flanked the insert site in the SK^+ vector and an oligomer, Pv3 (5′-GGGGCATTCGCACCTTCATTA-3′), the reverse complement of bases 613 to 632 in the conserved post-repeat region of isolate VK247. To check for sequencing errors, PCR was repeated on VK247 genomic DNA. The sequences were identical. Sequences have been deposited in the GenBank database (IntelliGenetics) (accession numbers M25759 and M25760 for VK210 and VK247, respectively).
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Fitness Differences Among Remnant Populations of the Endangered Sonoran Topminnow

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Four correlates of fitness were measured in three stocks of the endangered Sonoran topminnow, *Poeciliopsis occidentalis*, from Arizona. Survival, growth, early fecundity, and developmental stability were greatest in laboratory-reared fish from the most heterozygous natural population studied. Conversely, all four traits were poorest in fish from a population with no electrophoretically detectable genetic variation. These results emphasize the need for genetic as well as demographic information for the development of comprehensive species recovery programs.

A survey of genetic diversity in the endangered Sonoran topminnow, *Poeciliopsis occidentalis occidentalis* (Atheriniformes: Poeciliidae), revealed that remnant populations in Arizona contain low levels of genetic variation (*1*). A hatchery stock derived from one of these populations, Monkey Spring, was being used in a species recovery effort involving reintroductions within the Gila River drainage; however, these topminnows were not the best choice

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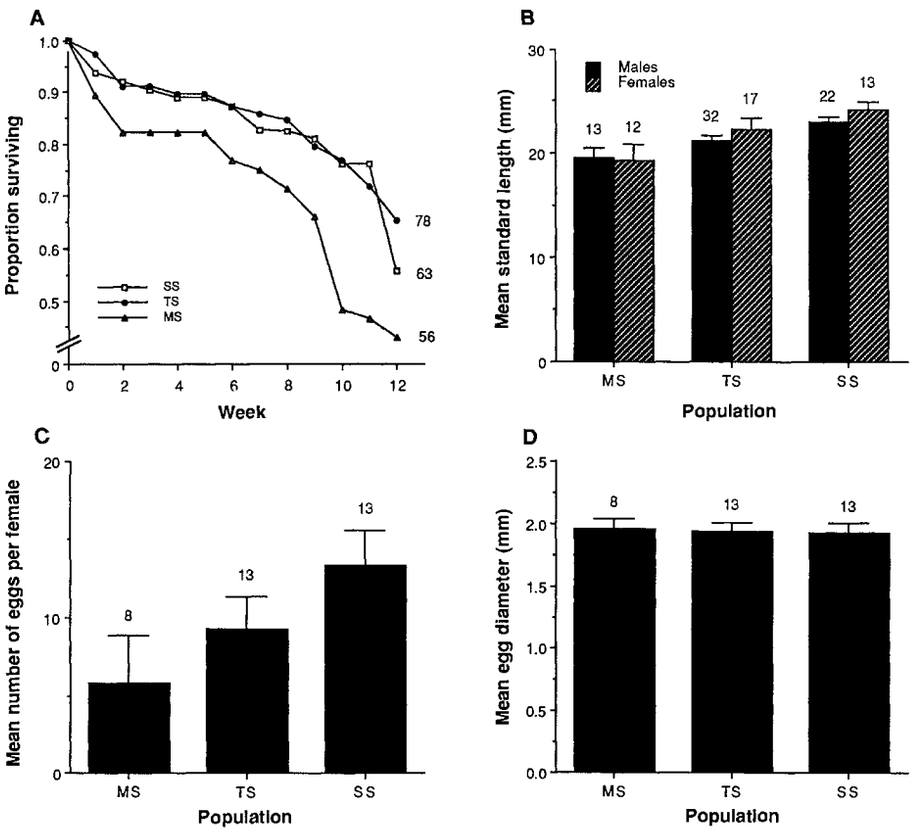

Fig. 1. Fitness differences among Sonoran topminnow populations. (**A**) Survival to 12 weeks. (**B**) Growth (mean standard length). (**C**) Fecundity at 12 weeks of age (least-squares means adjusted for standard length). (**D**) Mean egg diameter. Error bars represent 95% confidence intervals of the means. Sample sizes are shown.

for several reasons: (i) the Monkey Spring population is homozygous at all 25 loci surveyed in the electrophoretic study; (ii) fish from this thermally stable habitat might be incapable of adapting to thermally fluctuating environments; and (iii) females exhibit low fecundity in the wild (*2*). If genetic diversity is associated with immediate fitness and long-term evolutionary potential (*3*), the success of this recovery program might be enhanced by using fish from a more variable natural stock, such as Sharp Spring, a thermally fluctuating arroyo where topminnows are more fecund and coexist with the introduced predaceous mosquitofish *Gambusia affinis* (*4*). The U.S. Fish and Wildlife Service has adopted this plan, in part, for its current species recovery effort (*5*).

We have now tested the prediction that genetic diversity is associated with enhanced mean fitness of these fish populations. Four traits that should serve as components of overall fitness were measured: survival to 12 weeks of age, growth, fecundity at 12 weeks of age, and developmental stability. We examined three stocks with mean heterozygosities ($\overline{H}$) encompassing the range observed in Arizona: Monkey Spring ($\overline{H}$ = 0.0%), Tule Spring ($\overline{H}$ = 1.5%), and Sharp Spring ($\overline{H}$ = 3.7%) (*1*). Although Tule Spring topminnows represent a distinct subspecies (*P. o. sonoriensis*), they were selected for study solely on the basis of their intermediate level of mean heterozygosity (no Gila drainage *P. o. occidentalis* population studied, excepting Sharp Spring, has been shown to contain electrophoretically detectable genetic variation).

Ten gravid females from each locality were used to produce broods in the laboratory (*6*). Experimental progeny were isolated at birth and reared in individual containers for a 12-week test period (*7*). Survival was heterogeneous among populations [Wilcoxon rank test (*8*), $\chi^2(2) = 8.98$, $P < 0.02$; Fig. 1A]. Homozygous Monkey Spring topminnows exhibited the poorest survival ($\overline{S}$ = 9.0 weeks, SE = 0.5). Mean survival times were not different between the Tule Spring and Sharp Spring populations ($\overline{S}$ = 10.4 weeks, SE = 0.4).

Growth rates were estimated from the sizes achieved by the fish at the end of the experimental period (*9*). Following maturation, *Poeciliopsis* males grow more slowly than females; thus, growth was analyzed separately for each sex (Fig. 1B). Mean standard length differed among the three populations both for females [ANOVA, $F_{(2, 39)} = 26.92$, $P < 0.001$] and for males [ANOVA, $F_{(2, 64)} = 17.98$, $P < 0.001$]. Monkey Spring fish grew slower than Tule Spring fish, whereas Sharp Spring fish grew fastest.

Fecundity at 12 weeks differed among the three stocks (Fig. 1C). Sharp Spring females produced more eggs per unit of standard length than Tule Spring females, which in turn were more fecund than Monkey Spring females [ANCOVA, $F_{(2, 30)} = 6.68$, $P < 0.005$]. It became necessary, therefore, to test our assumption that fecundity was independent of the amount of energy invested in each egg. For example, Monkey Spring females might produce a few large eggs, whereas Sharp Spring females produce many small eggs. Because the diameter of a *Poeciliopsis* egg is predictive of its energy content (*10*), we measured a random sample of ova from all gravid females. Mean ovum diameter did not differ among the three stocks [ANOVA, $F_{(2, 31)} = 0.15$, $P = 0.86$] (Fig. 1D).

An important adaptive attribute of an organism is its ability to withstand random developmental accidents. Recent studies have shown that the more heterozygous individuals in a population often tend to exhibit reduced "fluctuating asymmetry," a sign of increased developmental stability (*11, 12*). This phenomenon has also been observed at the population level in a Mexican population of *Poeciliopsis monacha*, a related poeciliid fish that had suffered a severe loss of heterozygosity during a recent founder event (*13*).

To test whether differences in mean heterozygosity among *P. occidentalis* stocks are associated with reduced developmental stability, we examined fluctuating asymmetry in the progeny from the growth study. Since these fish were reared under controlled conditions, environmental factors that might disturb development were homogeneous across the stocks. Thus, differences among stocks in fluctuating asymmetry should be due to differences in their abilities to buffer random accidents of development.

Rank orders of individual character asymmetries were generally concordant across the three experimental populations (Table 1). Although only three characters showed significant differences among stocks, there is an overall tendency for fluctuating asymmetry to decrease with increasing mean heterozygosity. The heterozygous Sharp Spring stock exhibited the lowest fluctuating asymmetry for six of the seven characters examined, and differed significantly from the homozygous Monkey Spring stock.

Our study was performed under controlled environmental conditions that do

Table 1. Fluctuating asymmetry (FA) in bilateral meristic characters. Counts were made on 109 surviving offspring [Monkey Spring (MS), n = 25; Tule Spring (TS), n = 49; and Sharp Spring (SS), n = 35]. Raw asymmetry scores for each character (Right–Left) were distributed about a mean of zero and uncorrelated within populations. Scale corrections were unnecessary as individual (R–L) scores were independent of character sizes (*12*). For nonparametric statistical analyses, $(R–L)^2$ was used as a measure of asymmetry. No significant differences in FA existed between sexes within populations. Mean FAs among populations were compared with Kruskal-Wallis tests. Friedman's rank sums test was used to test for a difference in overall mean FA among populations. Results of post hoc multiple comparison tests are indicated; means with the same letter (a to c) are statistically homogeneous ($P > 0.05$). NS, not significant.

Character	Population rank	Mean FA	$\chi^2(2)$	P
Lateral line scales	MS	0.56 a		
	TS	0.39 b		
	SS	0.14 c	11.31	<0.01
Dorsolateral scale count	TS	0.96		
	MS	0.72		
	SS	0.43	3.88	NS
Pectoral fin rays	MS	0.28 a		
	TS	0.10 ab		
	SS	0.03 b	8.95	<0.02
Pelvic fin rays	TS	0.84 a		
	MS	0.68 ab		
	SS	0.26 b	10.81	<0.01
Scales above lateral line	MS	0.04		
	SS	0.03		
	TS	0.00	1.75	NS
Dentary outer teeth	TS	1.51		
	MS	1.24		
	SS	1.09	4.09	NS
Premaxillary outer teeth	MS	1.36		
	TS	0.96		
	SS	0.66	3.55	NS
Friedman's rank sums (across characters)	MS	18 a		
	TS	16 ab		
	SS	8 b	8.00	<0.02

not mimic the variable local environments in which these fish live. Nevertheless, our laboratory represents a novel environment, and thus tests the abilities of the three stocks to survive, grow, and reproduce in a new location. The near constant conditions of our flow-through incubator most closely match the thermally stable environment that the Monkey Spring stock has historically encountered. Yet with this apparent advantage, the homozygous Monkey Spring stock still performed most poorly overall. The most heterozygous stock (Sharp Spring) exhibited the highest survival, growth, fecundity, and developmental stability. Tule Spring topminnows, which were intermediate in heterozygosity, also exhibited intermediate growth and fecundity, but their survival equaled that of the Sharp Spring stock.

It is not our intention to imply that the enzyme polymorphisms detected in the electrophoretic study are the primary determinants of fitness in these fish. These genetic markers represent only a small fraction of the entire genome. Nonetheless, historical processes such as severe population bottlenecks, founder events, inbreeding, and migration are expected to affect allelic diversity at all loci to a similar extent, with the possible exception of those under strong selection. Thus, we suggest that population-level estimates of mean heterozygosity reflect the recency and severity of these historical processes (*14*).

Despite uncertainty regarding the predictive power of electrophoretic estimates of genomic heterozygosity, our results are consistent and unequivocal: the Sharp Spring stock currently offers the best choice for stocking in the Gila River system. These topminnows are less likely to suffer the negative effects associated with historical processes that lead to low heterozygosity. Additionally, the establishment of self-sustaining populations of these fish in reclaimed habitats should be rapid because of their higher fecundity and survival, thereby preventing further erosion of genetic variation. Continuous monitoring of the "genetic health" of stocked populations in reclaimed habitats is essential, and periodic restocking might be necessary to supplement genetic variation at isolated localities that are likely to experience wide fluctuations in population size.

The species recovery program that now includes Sharp Spring topminnows for stocking reclaimed habitats in the Gila River system (*15*) should spread the abundance of remnant genetic variation, thereby enhancing the likelihood that this diversity will be preserved. Although hybridization might provide a means of maximizing genetic diversity and fitness of a hatchery stock, indiscriminate mixing could lead to outbreeding depression, a decrease in fitness attributable to negative interactions between differentially adapted genotypes (*16*). Remnant natural populations, such as the Monkey Spring topminnows, should be maintained as isolated reservoirs of potential genetic variation.

Similar studies of fitness components are not feasible with many endangered species. For some species the time to perform such studies would forestall necessary immediate action. However, the small viviparous desert fish examined in this study are ideal for testing many hypotheses regarding the genetic and evolutionary consequences of various species recovery programs.

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Subplate Neurons Pioneer the First Axon Pathway from the Cerebral Cortex

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During the development of the nervous system, growing axons must traverse considerable distances to find their targets. In insects, this problem is solved in part by pioneer neurons, which lay down the first axonal pathways when distances are at a minimum. Here the existence of a similar kind of neuron in the developing mammalian telencephalon is described. These are the subplate cells, the first postmitotic neurons of the cerebral cortex. Axons from subplate neurons traverse the internal capsule and invade the thalamus early in fetal life, even before the neurons of cortical layers 5 and 6, which will form the adult subcortical projections, are generated. During postnatal life, after the adult pattern of axonal projections is firmly established, most subplate neurons disappear. These observations raise the possibility that the early axonal scaffold formed by subplate cells may prove essential for the establishment of permanent subcortical projections.

IN HIGHER MAMMALS, THE GENESIS OF neurons destined for the adult cerebral cortex is preceded by a period dedicated to the production of two transient populations of neurons, the subplate cells and the marginal zone cells. In the cat, [^{3}H]thymidine birth-dating studies have shown that these cells are generated between embryonic day (E) 24 and E30 (gestation, 65 days) (*1*), and the neurons of the six cortical layers are generated only thereafter (*2*). The later-born cortical cells then insert themselves between the early-generated neurons, splitting the

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