Genetic Differences in Male Development Time Among Populations of the Endangered Gila Topminnow

T. N. Cardwell, R. J. Sheffer, and P. W. Hedrick

Timing of male sexual maturity was compared in a common laboratory environment for populations from the four watersheds in which the Gila topminnow (Poeciliopsis occidentalis) still remains in Arizona. One population, Monkey Spring, was found to have an approximately 50% later development of male sexual maturity. Monkey Spring is the only population of the four whose natural habitat has both a constant and warm, year-round temperature and year-round reproduction. Year-round reproduction is a common strategy in tropical fish species in warm constant environments. Our findings are consistent with the hypothesis that the other three populations—Bylas Springs, Cienega Creek, and Sharp Spring—have adapted to temporada variable environments and seasonally limited reproduction with earlier male maturation. This genetic difference in a fitness-related trait lends support to the recommendation that Gila topminnows from different watersheds be managed and conserved separately.

The Gila topminnow (Poeciliopsis occidentalis) is a small, live-bearing fish (Poeciliidae) once common throughout lower elevations of the Gila River drainage in Arizona and New Mexico, and in Sonora, Mexico (Hubbs and Miller 1941), but now endangered and limited to a small fraction of its former range (U.S. Fish and Wildlife Service 1993). The Gila topminnow has been the subject of an ongoing series of investigations to determine the pattern and amount of molecular genetic variation over the few existing, and now quite isolated, populations and the possible association of genetic variation and fitness over these populations.

Published molecular genetic surveys have reported little or no variation among or within few extant natural Gila topminnow populations. For example, of 25 allozyme loci surveyed, only two were variable and only one population, Sharp Spring, was polymorphic for these loci while three other populations, Bylas Spring, Cienega Creek, and Monkey Spring, were monomorphic (Vrijenhoek et al. 1985). Recently Quattro et al. (1996) found that these four populations all had the same mitochondrial DNA haplotype using low-resolution six-cutter restriction enzymes.

Quattro and Vrijenhoek (1989) found differences in fitness measures between Sharp Spring and Monkey Spring, with Monkey Spring appearing to have lower fitness for all traits examined and suggested that there was a positive correlation of allozyme variation and fitness. However, their overall survival values were quite low, indicating that their laboratory environment was highly stressful to the fish and probably influenced the other fitness measures as well. In contrast, when the same fitness measures were examined by Sheffer et al. (1997), also in laboratory-reared fish, they showed a very high overall survival and no significant difference in survival among populations. Sheffer et al. (1997) also found that Monkey Spring males were significantly larger at 12 weeks than fish from three other populations.

Observations during the research reported in Sheffer et al. (1997) suggested that there may be differences in timing of male sexual maturity among the populations, a trait with potentially important fitness consequences. The present experiment was set up to quantify any differences among populations in the development of male secondary sex characteristics in a common, controlled environment. If differences are observed in a common environment, this indicates that there are genetic differences for this fitness trait among the populations.

Fish stocks were initiated in 1994 (Sheffer et al. 1997) from 20 pregnant females from four locations in Arizona—Bylas Spring, Cienega Creek, Sharp Spring, and Monkey Spring (Figure 1)—representing the four major watersheds in which natural populations of Gila topminnows are still extant. The first three locations are subject to large summer-winter temperature fluctuations. In contrast, Monkey Spring at its outflow is a warm spring and is chemically and thermally (27.4 ± 0.6°C) invariant (Schoenherr 1974). Monkey Spring is separated from other surface drainages by a 10 m high, natural travertine dam and modern diversion into an irrigation system.

Since summer 1994, stocks of approximately 1000 adults from each of these populations have been maintained in large, circular raceways under constant conditions. Seven gravid females were removed at random from each stock in July 1995 (after approximately three generations in captivity) and placed, one fish each, in 10 gal aquaria with the intent of obtaining five or more tanks of fish from each population. Broods of four or more juveniles were used in the study, making it likely that there would be one or more males in the brood. All the aquaria were maintained at the same light cycle (14 h of daylight), temperature (26°C), and water quality. Pregnant females from different populations were randomly placed in aquaria.

After birth of a brood, the female was removed and offspring were kept in sibling groups of four to nine individuals. They were observed daily until the start of gonopodial development (which is an external signal of the initiation of male sexual development), termed initiation age (defined by elongation of anterior anal fin rays 3–5 in males). Once development started, male fish were observed (by T.N.C.) in a petri dish held upright against the side of the tank, every 4 days until gonopodial development was completed. Completion age is indicated by a fully fused and clear gonopodial tip which signals male sexual maturity. Initiation and completion age were analyzed for significant differences among populations with ANOVA and Tukey multiple-comparisons tests.
Results for individual tanks from the different populations are given in Table 1. The first tank initiated development at 19.5 days (Cienega Creek tank D) and the first tanks completed development at 47 days (Cienega Creek tanks D and E). Monkey Spring tanks A–D were last to initiate development and were the last to complete development. Of the 22 tanks, the five Monkey Spring tanks had the four latest initiation ages and completion ages and the four longest durations of development. On average, mean initiation age, mean completion age, and duration of development were 53%, 48%, and 45% longer, respectively, in Monkey Spring than the average of the other three populations.

Overall differences among populations in age of initiation and completion were highly statistically significant ($P < .001$). There were no significant differences in either initiation or completion age among Bylas Spring, Cienega Creek, and Sharp Spring, but Monkey Spring fish differed significantly from the other three populations ($P < .001$ for each comparison). Variation within populations among tanks was not a significant source of variation in initiation of maturation, but it was significant among tanks within populations for completion ages ($P < .05$). Regression of duration of development on initiation age was significant ($P < .01$), explaining 29% of the variance in duration.

The highly significant difference between the Monkey Spring population and the other populations in time to male sexual maturity, when raised in a common environment, indicates that there are genetic differences for this fitness-related trait between Monkey Spring fish and those from the other populations. This difference corresponds with the interpopulation fitness study by Sheffer et al. (1997) which found that 12-week-old, laboratory-reared Monkey Spring males were significantly larger than male offspring from the other three populations (male growth ceases after sexual maturity, so late male development is consistent with larger size).

The most obvious explanation for the large genetic difference in male sexual development between Monkey Spring and other populations is that they have been under different selection pressures. Southern Arizona is the northernmost extent of the geographic range of *P. occidentalis* (Rosen and Bailey 1963). Tropical derivation (tendency to breed year-round) of the Gila topminnow, along with a natural barrier separating warm Monkey Spring from the other populations in contrast with selection against year-round breeding in seasonally variable temperature habitats, could account for the genetic differences in male sexual maturation between Monkey Spring and other natural populations.

Although spring and summer surges in reproduction exist in all natural populations, the constant-temperature habitat of Monkey Spring supports year-round reproduction while the seasonally variable temperature habitats, such as Bylas Spring, Cienega Creek, and Sharp Spring, do not (Schoenherr 1974). Because of the year-round reproduction at Monkey Spring, some females are available for mating all year, which reduces selection against later starting, slower developing, and consequently larger males. On the other hand, male fish at temperature-variable sites are under selection pressure against late development because reproduction ceases from late fall to spring. This also imposes a trade-off in these locations between earlier development with potentially lower mating success because of smaller size or completion of development next spring with more mating success as a larger male. However, overwintering in cold temperatures poses survival risks and may increase potential costs of delayed male development. In other words, the slower male development time in Monkey Spring fish is consistent with predictions from life-history and sexual competition considerations.

We should note that the influence of environment on sexual development is well documented in fishes. Day length, temperature (Conover and Kynard 1981), and so-
cial interactions (Borowsky 1973, 1978, 1987; Braddock 1945) are all possible modifiers of developmental timing in the secondary sex characteristics. Some studies have found significant effects of the presence of other males on timing of development (Borowsky 1973, 1978, 1987). In this experiment, temperature, day length, and water quality were controlled. Per aquarium density and the numbers of male siblings in the same aquarium were kept as constant as possible but are two other possible sources of variation in developmental timing. Although we do not have enough observations per population and a wide enough range of values to statistically examine this effect, it does not appear to be significant.

Our observation of a large genetic difference in a fitness-related trait between Monkey Spring fish and those from the other populations supports the suggestion of Simons et al. (1989) that “at least one representative lineage is preserved from each of the four geographic areas in Arizona.” It is also consistent with the recommendation (Sheffer et al. 1997; U.S. Fish and Wildlife Service 1993) that Gila topminnows from different watersheds be managed and conserved separately.

From the Department of Biology, Arizona State University, Tempe, AZ 85287–1501. We acknowledge the National Science Foundation and the Howard Hughes Medical Institute for support and Larry Nienaber for care of the topminnows. We appreciate the comments of W. L. Minckley. Address correspondence to Dr. Hedrick at the address above or e-mail: hedrick@hedricklab.la.asu.edu.

© 1998 The American Genetic Association

References

A Genetic Marker in the Growth Hormone Receptor Gene Associated With Body Weight in Chickens

X. P. Feng, U. Kuhnlein, R. W. Fairfull, S. E. Aggrey, J. Yao, and D. Zadworny

A genomic clone spanning 16 kb of the GH receptor gene was mapped and used as a probe for identifying restriction fragment length polymorphisms (RFLPs) in chickens. Several strains of meat-type and egg laying chickens were found to segregate for an HindIII RFLP located in the intron preceding exon 4. The polymorphic HindIII site overlapped with a poly(A) signal. Association of the HindIII RFLP with traits was analyzed in a random-bred White Leghorn strain in three generations using either selective or random genotyping. Both methods revealed significant association of the HindIII allele (presence of the poly(A) signal) with an increased juvenile body weight (130 days of age). In two meat-type strains divergently selected for size of the abdominal fat pad, the HindIII allele was coselected with leanness. The results indicate the presence of a genetic variant of the GH receptor gene which affects growth and abdominal fat deposition and which is relatively frequent in egg laying as well as in meat-type chickens.

The growth hormone (GH) axis affects many metabolic processes such as reproduction, growth, aging and immune responsiveness. In poultry it has been shown that GH levels are associated with the rate of growth, mature body weight, fatness and egg production (Anthony et al. 1990; Burke and Marks 1982; Picaper et al. 1986; Scanes et al. 1984). It has also been reported that the number of hepatic GH receptors (GHR) is affected by selection for growth or feed efficiency (Vanderpool et al. 1993).

Whether these differences in GH or GHR levels are due to genetic variations in either one of the two genes or in other regulatory genes of the GH axis is not known. However, in some sex-linked dwarf chickens, stunted growth and reduced immune responsiveness appear to be the consequences of mutations in the GHR gene (Agarwal et al. 1994; Burnside et al. 1991; Duriez et al. 1993; Huang et al. 1993; Pinard and Monvoisin 1994). This observation and the hypothesis by Robertson (1985) that genes which segregate for alleles with major effects are likely to also segregate for alleles with minor effects prompted us to search for genetic markers in the GHR gene which are associated with growth.

Materials and Methods

Isolation and Characterization of a Cosmid Clone of the GHR Gene

A cosmid library constructed from genomic DNA of a Cornish White Rock chicken was purchased from Stratagene (Stratagene, La Jolla, California). The genomic DNA had been partially digested with Sau3AI and inserted into the BamHI site of the cosmid vector pWEl5. Screening of 5 × 10⁶ colonies with the radiolabeled full-length chicken GHR cDNA as a probe (Burnside et al. 1991) yielded one positive clone.

A restriction map of the cosmid clone was constructed by analyzing the restriction fragment lengths and the hybridization signals obtained with probes for the T3 and T7 promoters which flank the vector and with individual subfragments (Figure 1A). The clone contained two vector segments interspersed with two chicken genomic DNA fragments. One of these (16.3 kb) hybridized to the GHR cDNA, while the other (10.5 kb) presumably represented an unrelated genomic DNA fragment.

The smallest contiguous segment of the cosmid clone which hybridized to the cDNA clone consisted of two adjacent HindIII fragments. The segment was 5.1 kb in length and located near one of the ends of the cosmid vectors (Figure 1B).