

## **Genetic and social control of male maturation in *Phallichthys quadripunctatus* (Pisces: Poeciliidae)**

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### **Abstract**

Age and size at maturity can have significant fitness consequences. Selection often favors early-maturing individuals because of their higher survival to maturity and greater relative contribution to population growth rate, but it may also favor delayed maturation if fitness increases with age or size at maturity. Males of several poeciliid fishes exhibit variation in age and size at maturity primarily controlled by a sex-linked gene called the P-locus. Wild-caught *Phallichthys quadripunctatus* males show a bimodal size distribution, which is often associated with a P-locus polymorphism in other poeciliids. We conducted two experiments to evaluate the inheritance of male age and size at maturity and the influence of social environment (presence of mature or juvenile males during development) on these traits. We specifically tested the hypothesis that male age and size at maturity in *P. quadripunctatus* are governed by a single Y-linked locus, and modified by the social environment. Although our results imply both a genetic and an environmental component to the dimorphism in maturation, both large and small males were able to sire both large and small sons, allowing us to reject the hypothesis that age and size at maturity in this species are controlled by a single, Y-linked locus. Our data do not conform adequately to any of the genetic mechanisms described to date for maturation polymorphism in poeciliids. We suggest alternative mechanisms that may operate in *P. quadripunctatus*.

## Introduction

Maturation is a complex process involving the correlated parameters age, size, and development rate. The age and size at which individuals sexually mature can have important fitness consequences (Peters, 1983). Early-maturing individuals have a greater probability of survival to maturity (Ekstig, 1994), and in growing populations, progeny born early will contribute more to the population growth rate (Cole, 1954; Lewontin, 1965; Caswell and Hastings, 1980). Lewontin (1965) demonstrated this advantage through simulations showing that decreasing age at maturity by as little as 10% has as much impact on the rate of increase in population size as a doubling of total fecundity. However, selection may also favor delayed maturation if reproductive success or offspring quality depend on size or age at maturity of the parent (Stearns, 1976; Stearns and Koella, 1986). Early-maturing individuals may then suffer a disadvantage in terms of size-dependent fitness traits such as fecundity, dominance status, and mating success (Sibly and Calow, 1986; Bernardo, 1993). Because of the frequent, close association between age and size at maturity and fitness, it is of special interest to investigate populations that are polymorphic for these traits.

Male poeciliid fishes greatly reduce growth upon reaching sexual maturity, so that adult size distributions reflect size at maturity (Hughes, 1986; Snelson, 1989). Several poeciliids display a wide size range and sometimes polymodal distribution of adult male sizes in natural and laboratory populations (Constantz, 1975; Snelson, 1985; Kallman, 1989; Reznick et al., 1993). In some of these species, maturation is known to be governed by a sex-linked gene called the P-locus (McKenzie et al., 1983; Borowsky, 1987b; Kallman, 1989; Ryan et al., 1992; Erbeling-Denk et al., 1994). This locus indirectly controls testis maturation, which in turn causes a decrease in growth rate and metamorphosis of the anal fin into a gonopodium (Turner, 1941; Kallman and Schreibman, 1973). The P-locus has up to nine alleles in some species, and the smallest and largest males may differ in age and size at maturity by more than 100% (Kallman, 1989). The maintenance of this polymorphism is of interest given the potential fitness effects of such variation in age and size at maturity.

In some poeciliids, maturation is also influenced by the social environment experienced during development, by a process called "leap fish" (Snelson, 1989). Juvenile males reared in the presence of larger conspecifics delay maturation until they reach a body size similar to or larger than that of their companions (Borowsky, 1973; Campton, 1992). This effect has been shown to be associated with agonistic interactions in *Xiphophorus maculatus* and *X. variatus* (Borowsky, 1987a). One hypothesis is that the larger, dominant males suppress maturation by directing aggressive behavior towards the juveniles and activating hormonal pathways that inhibit the maturation process (Campton and Gall, 1988; Snelson, 1989; Zulian et al., 1993). In this manner, the juvenile size and dominance hierarchies are reversed in the adults (Borowsky, 1973). Social inhibition of maturation has been demonstrated in several poeciliids (Sohn, 1977a, b; Farr, 1980; Campton and Gall, 1988; Campton, 1992) and in other fish (Davis and Fernald, 1990; Bushmann and Burns,

1994). Variation in age and size in natural populations of poeciliids thus can have both a genetic and an environmental component.

Despite the overall agreement among these studies, neither genetic nor social effects on male maturation should be generalized to all poeciliids. Almost all that is known is confined to about 10 species of *Xiphophorus* (reviewed by Kallman, 1989), and there is variation even within this genus. For example, the P-locus may control either age or size at maturity (Sohn and Crews, 1977; Kallman, 1989), and may be linked to either or both sex chromosomes (Kallman, 1989). Busack and Gall (1983) determined different heritability estimates for age and size at maturity in *Gambusia affinis*, suggesting a genetic mechanism different from the P-locus. Also, in one species the P-locus may control susceptibility to social inhibition, not age or size directly (Kallman, 1989), and in others the social environment does not appear to affect maturation at all (Yan, 1987; Farr and Travis, 1989).

The P-locus is sometimes manifested in natural populations as a polymodal size distribution of mature males with each mode representing a different genotype (Kallman, 1989). In some poeciliids, males have a polymodal or wide ranging size distribution whose cause is unknown, but has been attributed to the P-locus (Kallman, 1989; Reznick et al., 1993). It has also been suggested that all poeciliids possess allelic variation at the P-locus (Kallman, 1989). However, given the differences among those species that have been studied, more information on less well-known genera is needed before we have a complete understanding of the determinants of maturation in this family.

*Phallichthys quadripunctatus* is a Central American species known only from two small tributaries of the Rio Sixaola in Costa Rica (Bussing, 1979). Aside from the original species description (Bussing, 1979), there is no published scientific information on this species. Like most poeciliids, sexually mature males possess a gonopodium and are smaller than females. Preliminary observations suggest that adult male *P. quadripunctatus* in natural populations exhibit substantial variation in body size; however, a formal investigation of this variation has not yet been undertaken.

In this paper we report variation in male age and size at maturity in *P. quadripunctatus*. By conducting two generations of controlled crosses, we tested the hypothesis that this variation is paternally inherited, which would suggest a Y-linked, P-locus type of genetic control. We also reared males in different social environments to determine if social inhibition of maturation operates in this species.

## Methods and materials

### *Study population*

In July, 1992, approximately 300 *P. quadripunctatus* individuals of mixed ages and both sexes were collected with dip nets from a small tributary of the Rio Sixaola in eastern Costa Rica. The stream was turbid and slow-moving, with an approximate width of 1 m and depth of 0.3 m. A group of mature males represent-

ing the extremes in body size was measured to the nearest mm (standard length, SL) and transported to the University of California at Riverside along with about 20 gravid females. The remaining fish were preserved in the field and measured to the nearest 0.01 mm to determine the size distribution of individuals in the population.

The fish were maintained at densities of approximately 3 fish/gallon H<sub>2</sub>O for several weeks prior to being individually isolated. All fish were fed *ad lib.* daily with freshly hatched brine shrimp (*Artemia*) in the morning and liver paste in the evening, supplemented with commercial flake food. All fish were maintained at  $26 \pm 2$  °C on a 12 L/12 D photoperiod.

Museum collections ANSP 169072 and ANSP 169073 ( $n = 14$  immature males, 23 mature males), made by D. P. Fromm in 1988 were obtained from the Academy of Natural Sciences of Philadelphia.

#### *Morphological measurements*

Sexual maturation in poeciliid males is characterized by metamorphosis of the anal fin into an elongated intromittent organ called the gonopodium (Turner, 1941; Rosen and Bailey, 1963). The physical appearance of the gonopodium has been shown to be an accurate external indicator of internal development (Kallman and Schreibman, 1973). Metamorphosis can be divided into discrete stages based on the physical appearance of the gonopodium and the number of segments present in the third fin ray (Turner, 1941). Mature male *P. quadripunctatus* have an easily-distinguishable hook at the tip of the gonopodium (Bussing, 1979). When a male is sexually mature, the cuticle surrounding this hook disappears, and the tip structures are fully formed.

Prior to being measured and staged, fish were anesthetized using tricaine methane sulfonate (MS222) (230 mg/L). Each fish was measured to the nearest 0.01 mm using digital Vernier calipers, blotted dry, and weighed to the nearest mg using an electronic balance. Staging was done according to Turner (1941; description for *Gambusia affinis*), but modified to accommodate differences between *P. quadripunctatus* and *G. affinis*. Males were assigned to stages from 0 through 4, with 0 being distinguishable from females only by slight thickening of the third ray, 1 being initiation of metamorphosis (10 or 11 segments in the third ray), 2 and 3 being further thickening and lengthening of the gonopodium, and 4 being maturity (cuticle on apical hook completely disappears).

#### *Experiment 1*

##### *Experimental design*

To test the hypothesis that male size and/or age at maturity are controlled by a single Y-linked locus, we designed a full-sib experiment with dams nested within sires. We used wild-caught sires representing the extremes of the male size distribution, and first generation lab-reared dams. To evaluate social inhibition of maturation by

adult males, we individually-reared (I-reared) some male offspring and paired others (P-reared) with mature males. Poeciliid females can store sperm for several months (Constantz, 1989), and a single litter can have multiple sires (Campton, 1992). Therefore, we reared sibships of virgin females to use as dams to ensure knowledge of the paternity of each offspring. Using a Latin Square design (Fisher and Yates, 1963), eight daughters (dams) from each of eight wild-caught females were randomly paired with eight sires (four large, four small). Each sire was mated to one female from each sibship, and each female was only mated to one sire, for a total of 64 crosses.

Unfortunately, only 14 females ( $\bar{X}_{SL} = 30.59$  mm), representing all four large sires and two of the four small sires (Appendix A), gave birth to a sufficient number of offspring ( $\geq 15$ ), so the planned Latin Square design was reduced to a one-way analysis of sire effects. We are unable to explain why so few of the small males were able to sire offspring, as all males had motile sperm and all fish appeared to be healthy and were not subjected to any stressful conditions. One possibility is that members of this species need to mate in groups to mate successfully, as suggested by the success of the second breeding experiment (see below).

#### *Experimental procedure*

The experiment to examine genetic and social effects on male age and size at maturity was conducted from February to October, 1993. Wild-caught small ( $n = 4$ ,  $\bar{X}_{SL} = 14.09$  mm) and large ( $n = 4$ ,  $\bar{X}_{SL} = 18.67$  mm) sires were isolated in 2-gallon tanks without visual contact with neighbors for the duration of the experiment, except when they were mated. Gravid wild-caught females were isolated into two-gallon tanks and allowed to give birth. Each litter was isolated into a divided ten-gallon tank and any fish showing signs of anal fin metamorphosis were removed from the tanks periodically and housed separately. Eight virgin females (= a sibship) from each litter were assigned to individual 8 L tanks arranged into random blocks of eight, such that each block contained a female from each sibship. Each sire was mated to a virgin female for three days, then moved to a new tank. This rotation was repeated until each sire was mated to his eight dams three times.

Each litter of offspring from these matings was isolated at birth into 21 L tanks at densities of  $12 \pm 2$  fish/tank. From 10 to 30 individuals were saved per litter. At 20–25 d of age, the fish were sexed, measured, and staged, and females were saved for later experiments. Also at 20–25 d of age, ten randomly selected I-reared males from each litter were isolated into identical 1.2 L plastic aquaria with gravel and one plant in each, in which they spent the duration of the experiment. These containers were arranged in groups according to litter. Up to ten P-reared males were randomly selected from those males remaining and returned to their group tanks. At 40–45 d of age, three I-reared males and three P-reared males were randomly chosen for the experiment. Each I-reared male's container (1.2 L) was relocated to a random slot in a randomized block array of containers, and each P-reared male was paired with a mature stock male in a 7.5 L tank. Each pair of males in the P-reared treatment were housed together in a randomly assigned 8 L tank without visual contact with neighbors for the duration of the experiment.

Males were measured and staged once weekly until stage 3, once every three days until just prior to maturity, and then once every day until maturity (stage 4). Mature males in the P-reared treatment were also measured periodically to minimize potential effects due to handling. All tanks and containers were cleaned once a week when the fish were removed to be measured.

## *Experiment 2*

### *Experimental design*

Because I-reared males in Experiment One were not isolated until 25 days old, it is possible that the social inhibition of maturation had already influenced growth of these individuals. Therefore, we conducted a second generation of crosses and isolated the males within the first few days after birth to evaluate the size variation without interference of social effects early in development. We used female offspring from the first generation as dams, and predominantly I-reared male offspring as sires. In spite of the potential effects of social inhibition acting within the first 25 d after birth, these males were reared under controlled conditions and their size is more likely to reflect genetic make-up than that of wild-caught sires used in the first experiment.

To increase the likelihood of insemination, we mated dams and sires in groups ( $n = 9$  matings). Although we could not retain individual identity of each sire, group mating did result in a higher insemination rate. The sires (2–3/group) in each group were either all small or all large full sibs and the dams (3–4/group) were full sibs, but sires and dams were descended from different wild-caught females. A total of 17 out of 22 isolated females gave birth to a sufficient number of offspring for the experiment, representing 10 large sires and 7 small sires (Appendix B). All male offspring in the subset of each litter that was saved were included in the experiment, resulting in variable numbers of males representing each litter. Offspring were either I-reared or reared as groups of male full-sibs (G-reared) to evaluate social inhibition of maturation by juveniles. Because we attempted to include a large number of males from each litter in the I-reared treatment, only 5 of the litters (representing four small sires and one large sire; Appendix B) had enough males to also be included in the G-reared treatment.

### *Experimental procedure*

The experiment was conducted from September, 1993 until March, 1994. Sires (small:  $n = 8$ ,  $\bar{X}_{SL} = 15.12$  mm; large:  $n = 18$ ,  $\bar{X}_{SL} = 19.25$  mm) and dams ( $n = 17$ ,  $\bar{X}_{SL} = 28.06$  mm) were mated in groups in divided ten-gallon tanks. When females became gravid they were individually isolated in 7.5 L tanks to give birth. Each litter was temporarily housed in a 21 L tank for 0–5 d (with one exception of 7 d) after birth, at which time ten I-reared individuals were randomly selected and isolated into plastic containers arranged according to litter. G-reared fish remained in the 21 L tanks at densities of  $12 \pm 2$  fish/tank. All males were identifiable by age 40 d based on pilot studies and Experiment One. At 40–45 d of age each litter

was sexed, females were sacrificed, and males remained in the experiment. There were 1 to 9 I-reared males and 2 to 6 G-reared males per litter. We began measuring I-reared males at 50 d of age, when the first males were expected to mature. After this age the males were anesthetized and staged once per week, but were only measured and weighed upon attaining sexual maturity. The lengthening gonopodium in the early stages of development is opaque, and becomes translucent as the cuticle disappears and maturity nears. Because this difference is easily visible with the naked eye, G-reared fish were visually examined through the tank and were only staged, measured, and weighed when at least one of them appeared to be nearing maturity. Individual identity of G-reared fish could not be retained. I-reared males were staged (but not measured) once every three days upon reaching stage 3, and once every day when they were very close to maturity. All tanks and containers were cleaned once per week.

#### *Statistical analysis*

The Univariate Procedure (SAS Institute, 1985) was used to estimate kurtosis and skewness values and to conduct goodness-of-fit tests for normality of size distributions. The dip test (Hartigan and Hartigan, 1985) was conducted using a QuickBasic program (S. M. Scheiner in litt.) to evaluate departure from unimodality in the wild-caught, mature male size distribution. We divided the data into 10 equally-sized bins and performed the dip test on the resulting frequency distribution. The correlation matrix of male maturation traits was generated using the Correlation Procedure (SAS Institute, 1985).

We examined the associations between father size and son size at maturity for each rearing treatment and between rearing treatment and son size at maturity, using the *G*-test of independence (Sokal and Rohlf, 1995) with the sons classified as either small or large depending on into which of the non-overlapping modes they fell. For example, in Experiment One small males ranged from 14 to 16.5 mm and large males ranged from 17.5 to 21 mm (Fig. 2). We recognize that there may be an interaction between sire size and rearing treatment. We could not examine a potential interaction, however, because empty cells prevented us from applying multiway categorical analyses.

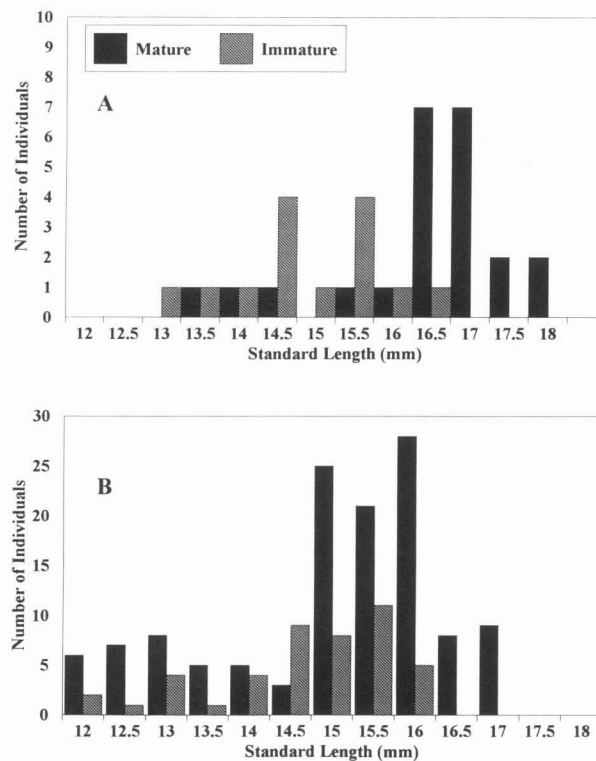
The *G*-test is appropriate for our data because we have a Model II design, with one fixed criterion (father size) and one criterion (son size) that is not fixed (Sokal and Rohlf, 1995: 724). Three *G*-tests were conducted for each experiment. One compares the effects of father size on son size. The second evaluates the effects of isolation versus rearing with conspecifics on age and size maturity. If there is a genetic basis for size at maturity then we predict a strong association between father size and son size. If there is an environmental effect on maturation, specifically if mature or immature males inhibit the development of their immature tank mates, then we predict that P- and G-reared males will be larger at maturity than I-reared males. The third test evaluates the effects of rearing treatment on the number of large sons produced. If there is an environmental effect on maturation via social inhibition, then we predict that there will be more large males in the P- and G-reared treatments than in the I-reared treatment.

## Results

### *Male size variation*

The size distribution of wild-caught mature males reveals a large range in standard lengths and appears bimodal (Fig. 1B). The museum collections show similar size ranges but not bimodality, possibly due to a collection bias against small males (Fig. 1A). The distributions of immature males show a similar range, such that the values for mature and immature males overlap (Fig. 1). Therefore, the size variation in adult males is not due solely to post-maturational growth.

Skewness and kurtosis are usually used as indicators of the extent to which a distribution deviates from normality (Wyzomirski, 1992). A symmetrical, bimodal distribution is platykurtic and therefore has a high negative value for kurtosis; but if there is inequality in the abundance of large and small males, a high value for skewness may result (Reznick et al., 1993). Goodness-of-fit tests reveal significant



**Fig. 1.** Size distributions of mature and immature *P. quadripunctatus* males in nature (Rio Sixaola drainage, Costa Rica). Panel A shows specimens from museum collections ( $n = 14$  immature males, 23 mature males). Panel B shows our wild-caught sample ( $n = 45$  immature males, 125 mature males).



departures from normality of wild-caught *P. quadripunctatus* mature male size distributions from both the museum collections ( $n = 23$ ,  $P = 0.0024$ ) and from our collection ( $n = 125$ ,  $P = 0.0001$ ). The size distributions of our collections of immature males also differed significantly from a normal distribution, ( $n = 45$ ,  $P = 0.0009$ ); however, those of the museum collections did not ( $n = 14$ ,  $P = 0.9879$ ). Kurtosis values were: for museum collections, 1.660 for mature males and  $-0.312$  for immature males; for our collections,  $-0.361$  for mature males and 0.603 for immature males. Skewness values were: for museum collections,  $-1.307$  for mature males and 0.028 for immature males; for our collections,  $-0.637$  for mature males and  $-1.03$  for immature males.

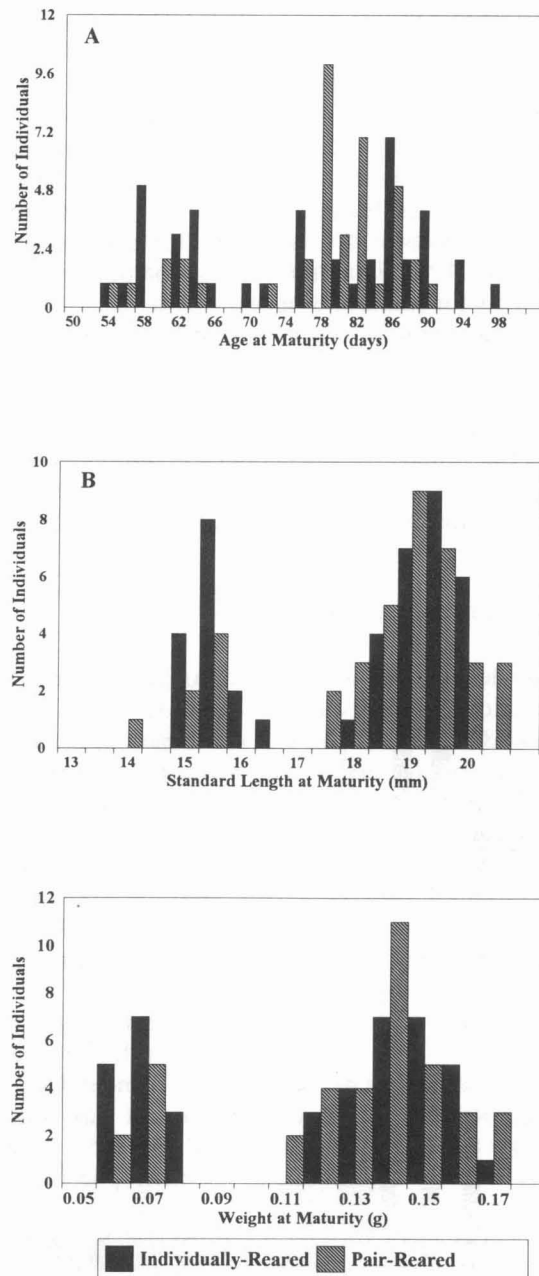
Although a much larger sample size is necessary to confirm bimodality in the presence of high skewness (Wright, 1968), our collections show a wide male size range and are consistent with bimodality such as that associated with the P-locus (Kallman, 1989). The dip test also implies that the data are not unimodally distributed, although the results are not significant (dip value = 0.0775,  $p < 0.10$ ). We therefore tested whether age and size at maturity in *P. quadripunctatus* are controlled by a single, Y-linked locus as in several other poeciliids (Kallman, 1989).

#### *Inheritance and social inhibition of maturation*

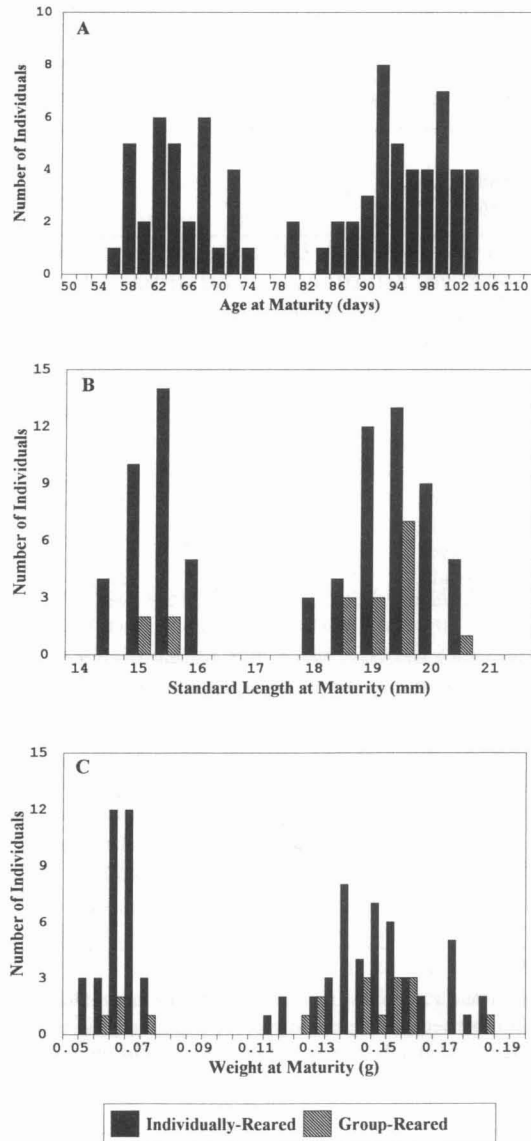
Distributions of age, length, and weight at maturity of male offspring from both experiments show distinct bimodality (Figs. 2 and 3). These three parameters are highly correlated ( $P = 0.0001$  for all pairwise combinations after sequential Bonferroni correction; Tab. 1); so “age and size at maturity” will be treated as one trait for the remainder of this paper. Contrary to the expectation under a single Y-linked locus hypothesis, sire size did not always predict son size (Tabs. 2 and 3, Appendices A and B), even when males were reared in isolation. However, there was a significant association between father and son size in both experiments ( $P = 0.0156$ , Experiment One and  $P = 0.0046$ , Experiment Two; Tabs. 2 and 3), suggesting a heritable component to male age and size at maturity, albeit by a more complex genetic mechanism than a single P-locus.

I-reared and P- and G-reared sons differed in length, weight, and age distributions (Figs. 2 and 3). The lack of association between father size and son size for the P- and G-reared treatments ( $P = 0.5031$ , Experiment One and  $P = 0.8003$ , Experiment Two) suggest that the social environment has obscured the genetic component of male age and size at maturity and that both juvenile siblings and mature males socially inhibit maturation, as in other poeciliid species (Snelson, 1989).

In the first experiment, P-reared males did not all delay maturation until they were larger than their mature tank mate, as predicted by the leapfish model (Appendix A; Borowsky, 1973; Sohn, 1977). However, for those litters that were represented in the P- or G-reared treatments, there were more large males in the P-reared treatment ( $P = 0.0697$ ) and significantly more large males in the G-reared treatment ( $P = 0.0028$ ) than in the respective I-reared treatments (Tab. 4), suggest-



**Fig. 2.** Distribution of ages, body lengths, and body weights at maturity of individually-reared and pair-reared *P. quadripunctatus* males from Experiment One ( $n = 44$  individually-reared males, 37 pair-reared males). Panel A shows the distribution of ages at maturity, Panel B shows the distribution of standard lengths at maturity, and Panel C shows the distribution of weights at maturity.



**Fig. 3.** Distribution of ages, body lengths, and body weights at maturity of individually-reared and group-reared *P. quadripunctatus* males from Experiment Two ( $n = 79$  individually-reared males, 18 group-reared males). Panel A shows the distribution of ages at maturity, Panel B shows the distribution of standard lengths at maturity, and Panel C shows the distribution of weights at maturity. The ages at maturity of group-reared males are not shown in Panel A because individual male identity could not be retained.

**Table 1.** Pairwise correlations between age, length and weight at maturity of *P. quadripunctatus* males, with results of Spearman Rank Correlation tests with sequential Bonferroni correction. Values below the diagonal are for Experiment One, values above the diagonal are for Experiment Two ( $n = 81$  for both).

| Spearman rank correlation coefficient |        |        |        |
|---------------------------------------|--------|--------|--------|
|                                       | Age    | Length | Weight |
| Age                                   |        | 0.812* | 0.840* |
| Length                                | 0.881* | –      | 0.976* |
| Weight                                | 0.861* | 0.965* | –      |

\* significant ( $P = 0.0001$ ).

**Table 2.** Numbers of sons in each size class within each rearing treatment, and outcome of *G*-test of independence for association between sire size and son size for Experiment One.

| Rearing treatment   | Sire size | Son size |       | <i>P</i> |
|---------------------|-----------|----------|-------|----------|
|                     |           | Large    | Small |          |
| Individually-reared | large     | 21       | 15    | 0.0156   |
|                     | small     | 6        | 0     |          |
| Pair-reared         | large     | 30       | 6     | 0.5031   |
|                     | small     | 2        | 1     |          |

**Table 3.** Numbers of sons in each size class within each rearing treatment, and outcome of *G*-test of independence for association between sire size and son size for Experiment Two.

| Rearing treatment   | Sire size | Son size |       | <i>P</i> |
|---------------------|-----------|----------|-------|----------|
|                     |           | Large    | Small |          |
| Individually-reared | large     | 34       | 14    | 0.0046   |
|                     | small     | 12       | 19    |          |
| Group-reared        | large     | 8        | 2     | 0.8003   |
|                     | small     | 6        | 2     |          |

**Table 4.** Numbers of sons in each size class within each rearing treatment, and outcome of *G*-test of independence for association between son size and rearing treatment, for both experiments. Only those litters represented in both the Individually-reared and the Pair- or Group-reared treatments are included.

| Experiment | Rearing treatment | Son size |       | <i>P</i> |
|------------|-------------------|----------|-------|----------|
|            |                   | Large    | Small |          |
| One        | individual        | 27       | 15    | 0.0697   |
|            | pair              | 32       | 7     |          |
| Two        | individual        | 9        | 18    | 0.0028   |
|            | group             | 14       | 4     |          |

ing that more sons delayed maturation when they were reared with conspecifics. There was a greater proportion of large sons in the G-reared treatment than in the I-reared treatment for four out of the five litters in Experiment Two (Appendix B). In Experiment One, 24 of the 32 P-reared males matured after exceeding the size of the mature male (Appendix A). Although we did not quantify intermale aggression in this study, we frequently observed agonistic encounters between males in the P-reared tanks.

#### *Summary*

Male age and size at maturity are highly correlated in *P. quadripunctatus*. Although sire size is significantly associated with son size, our data do not support the hypothesis that male maturation is controlled by a single Y-linked locus. Instead, it appears that a more complex genetic mechanism controls the maturation schedule in this species. Because we did not know the size at maturity of the females, we were unable to evaluate the maternal contribution to male maturation characteristics. Social inhibition by both adult males and juvenile siblings appears to affect male age and size at maturity.

#### **Discussion**

Luckner (1979) described discrete male size classes in *Poecilia latipinna* and proposed that male size variation in this species is due to a Y-linked gene with two alleles, “early” and “late”, similar to the P-locus found in some *Xiphophorus* species (Snelson, 1985; Kallman, 1989). Subsequent experiments with *P. latipinna* revealed a continuous distribution of male sizes and showed that, whereas male size is genetically polymorphic, the size classes are not discrete because of the presence of an autosomal modifier gene (Snelson, 1985; Travis, 1994). A genetic size polymorphism can therefore result in a continuous phenotypic distribution of sizes.

We tested the hypothesis that male size variation in *P. quadripunctatus* is controlled by a single Y-linked locus with alleles for early and late maturation. Although we observed a positive association between father size and son size, both large and small males produced both small, early-maturing and large, late-maturing sons. These results reject Y-linkage alone as the cause of variation in maturation and suggest a more complicated genetic control mechanism. For example, the P-locus mechanism may be complicated in several ways which lead to more complex inheritance patterns, often as a result of the sex-determining system of the species.

#### *Genetic models of male maturation in Poeciliidae*

Sex-determining systems of poeciliids in the tribe Poeciliini show variation both within and among genera (Angus, 1989; Kallman, 1989; Nada et al., 1993; Erbeling-Denk et al., 1994). For example, *Xiphophorus maculatus* have X, Y, and

W sex chromosomes with male genotypes XY and YY and female genotypes WY, WX, and XX (Kallman, 1989). A consequence of this more complicated sex determining mechanism is that a male offspring could inherit the male determining factor from its mother rather than its father. In such circumstances, father size would not necessarily determine son size. It is difficult to propose a model of sex-linked control of age and size at maturity in *P. quadripunctatus* without knowing its sex-determining system, because a given sex may be determined by multiple genotypes and sex ratios may vary significantly from the 1:1 ratio typically seen with simple X-Y sex determination.

Although we only directly tested for Y-linked sex-determination, our results also indicate that control of maturation in *P. quadripunctatus* does not conform completely to any previously-described P-locus mechanisms (Angus, 1989; Kallman, 1989; Nanda et al., 1993; Erbelding-Denk et al., 1994). One impediment to future progress in understanding the genetics of male maturation in this species is that there are no color markers linked to size such as those used to unravel the genetic mechanisms operating in some *Xiphophorus* species (Kallman, 1983; Erbelding-Denk et al., 1994).

#### *Alternative explanations for age and size variation in P. quadripunctatus*

Despite the evidence suggesting that age and size at maturity in *P. quadripunctatus* are paternally inherited via a mechanism similar to the P-locus, we cannot exclude other explanations. For example, age and size at maturity were found to have different heritabilities in *Gambusia affinis*, indicating that both traits are not controlled by the same genetic mechanism (Busack and Gall, 1983). In *P. quadripunctatus*, genetic mechanisms other than the P-locus can result in bimodal size distributions. For example, size at maturity may be a threshold trait (e.g., Ebert, 1994), polygenically controlled but discontinuously manifested (Mittwoch, 1973; Falconer, 1989).

Bimodality can also result because of an environmental switch determining the timing of maturation. For example, Atlantic salmon exhibit bimodal size distributions because they either mature or delay maturation when they reach a threshold size, depending on their growth rates at the time (Skilbrei, 1991; Ebert, 1994). It can be argued that differences in growth rate between the size classes in *P. quadripunctatus* (Kolluru, 1994) are evidence for such a switch. However, male growth rate in *X. nigrensis* is directly controlled by the P-locus (Zimmerer and Kallman, 1989), and, as in *P. quadripunctatus*, large males grow faster than small males (Kallman, 1989). Therefore, growth rate differences do not necessarily indicate environmental control. Also, if maturation were purely controlled by the environment, we would not expect an association between the phenotypes of sires and sons.

#### *Social inhibition of maturation*

The social environment experienced during development is known to affect traits such as gender and maturation of individuals in several taxa (Borowsky, 1973;

Jones and Thompson, 1980; Kruczek et al., 1989; Ra'anani et al., 1991; Francis and Barlow, 1993). Direct evidence from lab experiments (Borowsky, 1973; Sohn, 1977; Campton, 1992) and indirect evidence from field studies (Borowsky, 1978; Hughes, 1985) support the existence of social inhibition of maturation in some poeciliids but not in others (Yan, 1987; Farr and Travis, 1989). In species that do exhibit it, the cause is believed to be aggressive behaviour directed by dominant males to subordinate males inhibiting maturation via the activation of hormonal pathways. A similar hormonal mechanism results in alternative male morphs in the plainfin midshipman (Grober et al., 1994), and is subject to social control in some cichlids (Davis and Fernald, 1990; Francis et al., 1993).

A hypothesis of social inhibition predicts that there will be more large relative to small males in the pair-reared and group-reared treatments than in the individually-reared treatments. Our results support this hypothesis and suggest that in *P. quadripunctatus* the presence of either adult males or juvenile siblings during development caused a delay in maturation. However, not all males delayed maturation until they were larger than their mature tank mates, as predicted by the leapfish hypothesis (Borowsky, 1973). There are two explanations for this inconsistency. Firstly, *P. quadripunctatus* may have a maturation threshold beyond which a male is committed to maturing at the age and size pre-determined by his genotype, regardless of the social environment, as reported for *X. variatus* (Borowsky, 1973). Small males especially were already halfway to maturity by the time they were introduced into the first experiment, and may therefore have reached this threshold by then.

The second possible explanation is that pair-reared juveniles were not always subordinate to the mature males, even though they were smaller. Borowsky (1987a), who also found that inhibited fish often matured while still smaller than the inhibiting fish, suggested that relative size was not as important as aggressive interactions in determining the extent of inhibition. He proposed that, for reasons unknown, aggression declines at some point, resulting in loss of inhibition and consequent maturation of the juvenile. This point may be reached while the juvenile is still smaller than the mature male.

#### *Maintenance of variation in age and size at maturity*

Early maturation can confer high fitness in continuously breeding populations, provided the population is growing (Stearns, 1976; Caswell and Hastings, 1980). In seasonal breeders, size at maturity can have a greater impact on fitness than age at maturity, whereas in continuously breeding species both size and age are important (Cole, 1954; Lewontin, 1965; Stearns, 1976). Although many poeciliids are aseasonal breeders, some species are seasonal and show seasonal fluctuations in size at maturity (Krumholz, 1948; Snelson, 1984; Hughes, 1986; Reznick and Braun, 1987).

In a Costa Rican population of *Phallichthys amates* female reproductive condition peaks during the wet season, but reproducing females can be found year-

round, suggesting year-round breeding activity (Winemiller, 1993). Congeneric *P. quadripunctatus* is found in the same environment, so it is likely that it also breeds throughout the year and that both the age and size at which maturity is attained remain important components of male fitness.

If reproduction is continuous, small male *P. quadripunctatus* gain a substantial advantage by maturing in about two-thirds the time that large males do, a benefit which must be countered in some way by the late-maturing large males. There is evidence that large males in this species have higher reproductive activity than small males (Kolluru, 1994). Increased success in acquiring mates or siring offspring may therefore compensate for the delay in reproduction. In addition, several factors affecting male size distributions in nature, including size-selective mortality (Trexler et al., 1992) and predation (Britton and Moser, 1982; Reznick, 1982; Trexler et al., 1994), size-specific swimming ability (Ryan, 1988), and sexual selection (Farr, 1989; Ryan et al., 1990; McPeck, 1992) have been demonstrated in other poeciliids and may affect the relative fitness of different size classes of *P. quadripunctatus*.

The demonstration of a Y-linked trait controlling age and size at maturity in *P. latipinna* (Travis, 1994) suggests that this phenomenon is not limited to *Xiphophorus*. Our study is the first formal investigation of mature male size variation in the tribe Heterandriini and supports the idea that genetic control of maturation may be widespread in poeciliids (Kallman, 1989). Further studies on the sex-determining system, genetic and environmental components of maturation schedules, and demography of natural populations of *P. quadripunctatus* are necessary to establish the genetic mechanism controlling male age and size at maturity in this species.

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## Appendix A

Sizes of sires and corresponding sons, categorized by rearing treatment, from Experiment One. Bold-faced numbers indicate the weight of the mature male present in the tank with the experimental male.

| Sire | Litter | Standard length at maturity (mm) |      |             |             |             |             |
|------|--------|----------------------------------|------|-------------|-------------|-------------|-------------|
|      |        | Individually reared              |      |             | Pair reared |             |             |
| 1(L) | 1      | 14.8                             | 14.9 | 15.3        | 13.6        | 18.5        | 18.9        |
|      |        |                                  |      |             | <b>17.6</b> | <b>18.3</b> | <b>18.1</b> |
| 2(L) | 1      | 19.0                             | 19.5 | 20.0        | 19.4        | 19.7        | 20.1        |
|      |        |                                  |      |             | <b>18.2</b> | <b>18.2</b> | <b>17.7</b> |
|      | 2      | 15.5                             | 15.6 | 19.0        | 18.8        | 19.6        | 20.0        |
|      |        |                                  |      |             | <b>15.1</b> | <b>16.1</b> | <b>19.3</b> |
|      | 3      | 18.8                             | 18.9 | 19.5        | 19.1        | 19.7        | 20.2        |
|      |        |                                  |      | <b>14.8</b> | <b>15.4</b> | <b>19.4</b> |             |
|      | 4      | 15.0                             | 19.7 | 19.8        | 14.6        | 17.5        | 18.5        |
|      |        |                                  |      |             | <b>18.8</b> | <b>19.6</b> | <b>15.3</b> |
|      | 5      | 16.2                             | 19.8 | 19.9        | 18.1        | 19.1        | 19.2        |
|      |        |                                  |      |             | <b>19.5</b> | <b>19.0</b> | <b>15.7</b> |
| 3(L) | 1      | 15.1                             | 15.2 | 19.4        | 15.1        | 17.9        | 18.7        |
|      |        |                                  |      |             | <b>18.7</b> | <b>18.7</b> | <b>18.5</b> |
|      | 2      | 15.1                             | 18.4 | 19.1        | 15.5        | 18.2        | 19.0        |
|      |        |                                  |      | <b>15.4</b> | <b>19.7</b> | <b>18.9</b> |             |
|      | 3      | 14.8                             | 15.2 | 15.4        | 15.2        | 15.2        | 18.4        |
|      |        |                                  |      |             | <b>18.4</b> | <b>18.7</b> | <b>18.8</b> |
| 4(L) | 1      | 15.5                             | 18.5 | 18.5        | 18.6        | 18.7        | 18.9        |
|      |        |                                  |      |             | <b>18.7</b> | <b>18.6</b> | <b>18.5</b> |
|      | 2      | 15.4                             | 18.8 | 19.1        | 19.0        | 19.1        | 19.3        |
|      |        |                                  |      | <b>19.0</b> | <b>18.4</b> | <b>18.1</b> |             |
|      | 3      | 17.9                             | 19.1 | 19.1        | 17.5        | 17.8        | 18.3        |
|      |        |                                  |      |             | <b>19.8</b> | <b>15.2</b> | <b>18.0</b> |
| 5(S) | 1      | 18.7                             | 18.9 | 19.2        |             | N/A         |             |
| 6(S) | 1      | 18.4                             | 19.0 | 19.1        | 14.8        | 17.7        | 18.9        |
|      |        |                                  |      |             | <b>15.4</b> | <b>18.9</b> | <b>18.3</b> |

