

Genetic Variation in Maternal Investment Patterns in Platyfish, *Xiphophorus maculatus*

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ABSTRACT

Maternal investment in offspring is constrained by a variety of trade-offs, and the investment pattern expressed by an individual can have important fitness consequences. The authors investigate two maternal investment trade-offs in the southern platyfish, *Xiphophorus maculatus*: investment in offspring size versus number, and investment in sons versus daughters. Variation in maternal age and size often confound measurements of investment patterns. Because allelic variation at a single locus in platyfish has a major effect on age and size at sexual maturation, investment patterns within a single age and size genotype could be examined, minimizing the influence of these factors on investment patterns. In addition, the platyfish sex determination system allows experimental investigation of relative investment in sons and daughters because different mating types produce different brood sex ratios. Total investment in broods did not differ among sibships, but the investment pattern did; some produced larger but fewer offspring, whereas others produced smaller but more offspring. In addition, there was no evidence of differential investment in sons and daughters; broods with all sons did not differ in size or weight from broods with an equal proportion of the sexes. This result provides direct support for the assumption of equal investment in sons and daughters that is common in discussions of sex-ratio evolution and sex allocation.

INTRODUCTION

Life-history traits are traits that have direct fitness consequences. The principal life-history traits are: age at maturity, size at maturity, age- and size-specific reproductive investment, age- and size-specific mortality, growth rate, lifespan, and the number, size, and sex ratio of offspring. Variation among individuals in each of these traits can affect survival and/or reproduction.¹⁻⁴ These life-history traits, however, often cannot evolve independently of each other because investment in one trait reduces the resources available for investment in other traits. Individuals thus must trade off investment among different life-history traits, and the pattern of allocation among traits also can have important fitness consequences.

One common life-history trade-off occurs between the number of offspring produced by a female, and how much is invested in each individual offspring.^{2,3,5} Increased investment in each offspring should positively affect offspring survival, growth rate, and ultimately, reproductive success. Similarly, an increase in the number of offspring should increase female reproductive success. However, given that a female has a fixed amount of energy to allocate to offspring, females may be constrained to produce either many smaller offspring or fewer larger offspring. Trade-offs between offspring size and offspring number have been examined in a diverse range of animals, and in many cases, a negative correlation has been found.³ However, in some species, either no correlation has been found, or offspring number and size

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are positively correlated. One of the best vertebrate examples of a trade-off between offspring size and number is in the Chinook salmon, *Oncorhynchus tshawytscha*. Heath et al.⁶ found a trade-off between offspring number and size, and that larger size equated to a higher offspring survivorship. They also found a shift to smaller offspring size in hatcheries, which they suggest is the result of young being more protected in hatcheries. This shift was mirrored by a shift in natural populations that were populated by hatchery fish, but not in populations that included few hatchery fish. The combined evidence indicated that variation in egg size and number has a genetic basis, and that artificial selection in hatcheries has favored those females that produce more offspring that are smaller.

Investment patterns may also vary with sex ratio. Fisherian sex ratio selection predicts that the population sex ratio will stabilize at the point at which the total investment by the population in female offspring equals the total investment by the population in male offspring.⁷ This equilibrium sex ratio is predicted whether all families in a population invest equally in sons and daughters, or there is variation across families in investment in the sexes. If sons and daughters are equally costly to produce, the numerical sex ratio at the termination of investment is predicted to equal the Fisherian sex ratio (i.e., equal investment in sons and daughter will result in an equal number of each sex). In contrast, if parents invest more in individuals of one sex, the more expensive sex will be rarer than the less expensive sex at the termination of investment (sex ratio selection equalizes the population-level investment in males and females, not the number of males and females). Thus, if parents invest more in each son than in each daughter, the numerical sex ratio at the termination of investment should be female biased. Similarly, if parents invest more in each daughter than each son, the numerical sex ratio at the termination of investment will be male biased. Differences in the relative costs of sons and daughters should be manifested in a trade-off between brood size and sex ratio. For example, if sons are more expensive to produce than daughters, broods that have a higher proportion of sons than daughters should be

smaller in size. Just as the production of larger offspring should result in the production of fewer offspring, the production of a higher proportion of the more expensive sex should result in the production of fewer offspring. Although many theoretical and empirical studies of sex ratio evolution assume that sons and daughters are equally costly, there is little direct experimental support for this assumption.

The expression of life-history traits can be affected by a variety of factors, including environmental conditions, resource availability, age, and size.¹⁻³ It is necessary to control experimentally for many of these factors when testing for genetically-based trade-offs between specific life-history traits. One vertebrate system, the southern platyfish, *Xiphophorus maculatus*, allows for the control of a number of these factors. Southern platyfish have been used in genetic research for over 60 years, including studies of sex determination,⁸⁻¹¹ color pattern variation,¹²⁻¹⁷ the genetic control of age and size at sexual maturation,^{18,19} and sex ratio evolution and sex ratio stability.^{20,21} A sex-linked locus, the pituitary locus (P-locus), has a major effect on the age at sexual maturation,^{18,19} which is resistant to environmental fluctuations.²² Variation at the P-locus in the southern platyfish has been well characterized, and genetic lines have been established with known variation at this locus. As a secondary effect, allelic variation at the P-locus also strongly influences the size at sexual maturation.¹⁸ For example, the age at which male homozygotes for the earliest allele, P1, mature is about 8 weeks of age and 21.0 mm in standard length, whereas the age at which male homozygotes for a second early allele, P2, mature is about 11-13.5 weeks of age and 25-29.0 mm in standard length.²³ In females, P-alleles have a stronger influence on size than age at sexual maturation.²³ Individuals with late maturation alleles can take well over a year to mature. It is possible to control for a variety of effects on offspring investment pattern (such as genetically-based differences among females in age and size at sexual maturation) by examining trade-offs within a single P-allele genotype.

A second sex-linked locus determines sexual genotype in *X. maculatus*; sex determination is controlled by a single locus, or a set of tightly

bound loci inherited as a single locus,^{10,13,19,24} with three commonly occurring factors, W, X, Y (synonymous to alleles for the purposes of this article). There are thus three female genotypes (XX, WX, and WY) and two male genotypes (XY and YY). Because different mating types result in different brood sex ratios (Fig. 1), this system is ideal for investigating the relative cost of sons and daughters. In fact, this is one of the few systems known in which sex chromosome allelic constitution affects sex ratios such that predictable deviations from 1/2 result. For example, a cross between an XX female and a YY male produces a brood consisting exclusively of sons (all offspring are XY males), while a cross between the same XX female and an XY male results in an average brood sex ratio of 1:1 (50% of the offspring are XX females and 50% of the offspring are XY males). If sons are more costly to produce than daughters, broods resulting from XX:YY matings, which contain only expensive males, should be smaller in number than broods resulting from XX:XY matings, which contain both expensive males and inexpensive females. Conversely, if daughters are more costly to produce than sons, broods resulting from XX:YY matings, which contain only inexpensive males, should be larger in number than broods resulting from XX:XY matings, which contain inexpensive males and expensive females.

The *X. maculatus* system provides the identifiable genetic variability necessary to test predictions about differential investment in young. The availability of genetic lines with known variation at the pituitary locus allows for a measure of control of age and size at sexual maturation not possible with most animal systems. In this study, we use a genetic line of the southern platyfish with known allelic variation at the pituitary locus to investigate whether there is

variation among different sibships in maternal allocation to offspring size, offspring number, and total brood investment. We also use the known genetic variation at the sex-determining locus to directly investigate the relative investment by females in sons and daughters at a much finer level than previous studies.

MATERIALS AND METHODS

The Southern Platyfish *Xiphophorus maculatus*

The southern platyfish is a small, live-bearing, freshwater fish that occurs in eastern river drainages from Veracruz, Mexico to Belize and Guatemala. Females mate with multiple males, and as a result, offspring in a given brood may have different fathers.¹³ In addition, females retain sperm, and as a result, they can produce a series of broods from a single mating. Paternal investment in offspring ends with insemination, and maternal investment ends at birth.

Genetic lines of the southern platyfish were established at the Genetics Laboratories, New York Aquarium, NY, by M. Gordon and K. Kallman. Different strains have been bred and maintained for over 30 years, creating inbred lines that are highly homogenous that are used for a variety of research purposes. The strains are now located at the *Xiphophorus* Genetic Stock Center, Texas State University, San Marcos, TX. Genetic variation at several sex-linked loci in these genetic lines has been identified, including variation at the sex determination locus and at the pituitary locus.

Experimental design

Three genotypes from genetic lines of *X. maculatus* were obtained and used to establish breeding stocks for this study: (1) XP1XP1 females, (2) XP1YP2 males, and (3) YP2YP2 males. Thus, all females were homozygous for the P1 allele, one of the nine known *X. maculatus* P-alleles that assort at the pituitary locus and determine age and size at sexual maturity.^{19,23} Although the males differed in one P-allele, both were early maturation alleles. The three genotypes were selected to minimize variation within and among sibships in P-allele genotypes (these sibships will be referred to as

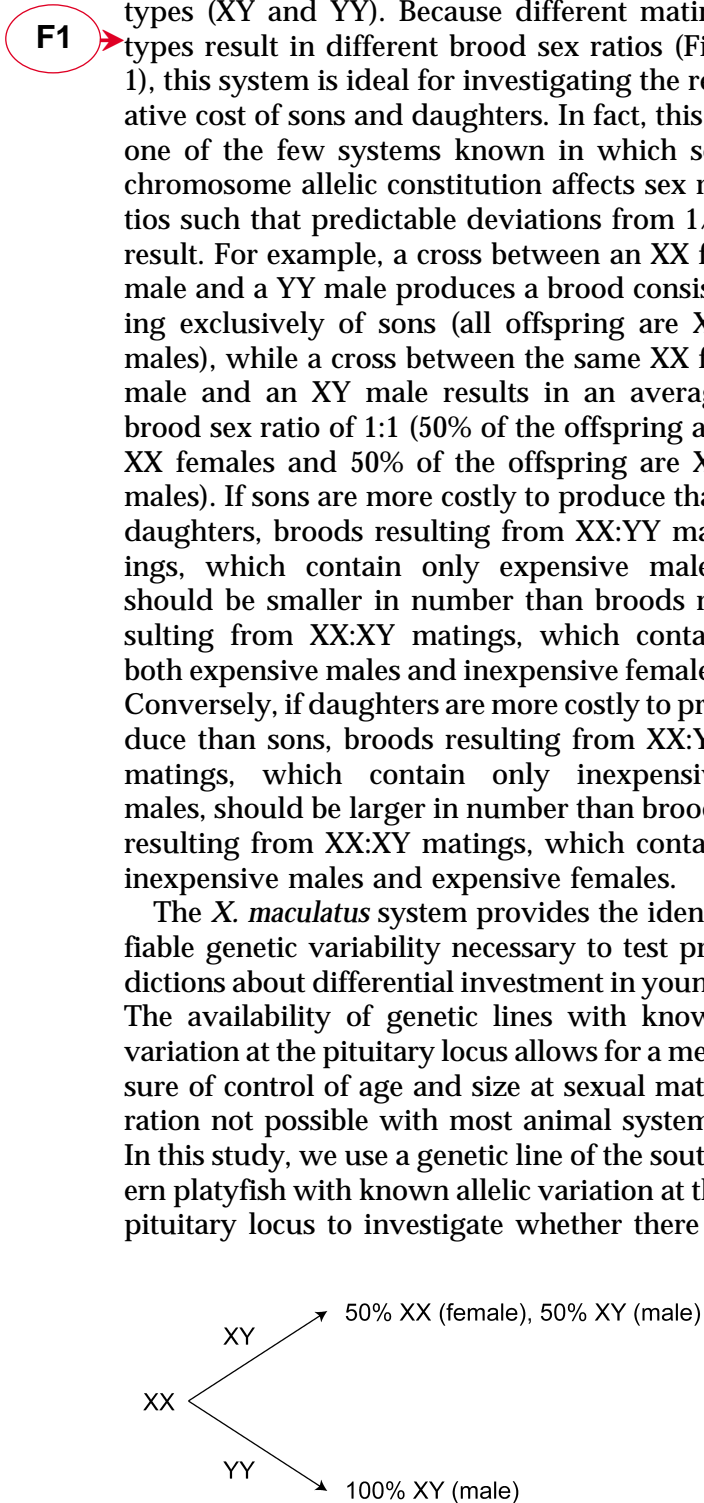


FIG. 1. Predicted brood sex ratios for XX female southern platyfish mated with an XY male versus an YY male.

maternal sibships), and thus to minimize any effects of P-allele genotype on offspring investment patterns.

To obtain test individuals for this study, pairs of XX females were placed in divided 10 gallon tanks, one on each side of the divider. Each female was provided with a single mate (XY male) and each resulting brood was collected within 24 h of birth and the brood was divided between several tanks. Juvenile males were removed from these sibship tanks as they were detected based on differences in male and female anal fin morphology. Females from the same full-sibling broods are designated as members of the same maternal sibship. These sibships were raised under standardized conditions. The tanks were housed in a room with overhead lighting on a 16/8 light-dark schedule, and natural sunlight was provided. Room temperature was maintained at an average of 26°C. All fish received four *ad libitum* feedings daily: liver paste in the morning, live brine shrimp twice in the afternoon, and commercial fish flakes in the evening.

Sexually mature, virgin XX females from eight maternal sibships were each randomly assigned either an XY or an YY mate, such that each sibship would produce both broods with males and females, and all male broods (Fig 1). The parental pairs were each placed in separate 19.8 L tanks for 12–20 days, after which time the males were removed. If young were not produced from the initial matings, a new male mate of the same genotype was introduced into the tank for an additional mating period. The broods of 41 females were removed from the mother's tank within 24 h of birth (sibship 1 = 10 mothers; sibship 2 = 3 mothers; sibship 3 = 8 mothers; sibship 4 = 4 mothers; sibship 5 = 5 mothers; sibship 6 = 4 mothers; sibship 7 = 4 mothers; sibship 8 = 3 mothers). The age at reproduction was available for females in sibships 1–5, but not for females in sibships 6–8. Twenty-two of the mothers had been mated with an XY male and 19 had been mated with an YY male.

For maternal sibships 1 and 2 ($n = 13$ females), the offspring were counted only, therefore birth weights from these broods are not available. Offspring from the remaining six maternal sibships (sibships 3–8: $n = 28$ females) were removed from the mother's tank,

counted, and frozen within 24 h following birth for subsequent weight measurement. Tanks with females giving birth were not fed until all of the babies had been collected; therefore, individual offspring weights and brood weights were not affected by postparturition feeding by offspring. To determine the weight of the offspring, an entire brood, or a random subset of the frozen offspring from each brood, was thawed and placed in a drying oven for 12 h. The dry weight of each individual baby was then measured to the nearest 0.01 mg using a Fisher Scientific XA-200 DS analytical balance.

Data were analyzed with the SYSTAT statistical package.²⁵ Two-factor ANOVA was used to examine the effects of maternal sibship and sire genotype (XY or YY) on brood size, average offspring weight, and total brood weight.

RESULTS

On average, females were 258.1 days of age at the birth of their first brood ($n = 30$, SE = 12.0), and they produced young, on average, 44.4 days from the time males were introduced into their tanks ($n = 41$, SE = 3.5). The brood sizes of females ranged from 4 to 68 offspring ($n = 41$, $x = 24.4$, SE = 18.6). For the six maternal sibships in which offspring weight was quantified, the average weight per individual offspring was 0.64 mg ($n = 28$, SE = 0.045). The average brood weight was 10.80 mg ($n = 28$, SE = 1.48).

There was not a significant correlation of the time from mating to parturition (an interval that represents the maximum possible length of gestation for a female) with brood size ($n = 41$, $r = -0.050$, $P > 0.05$), average offspring weight ($n = 28$, $r = 0.332$, $P > 0.05$), or total brood weight ($n = 28$, $r = 0.042$, $P > 0.05$).

We used two-way ANOVA to examine the influence of maternal sibship and sire genotype (XY or YY) on investment in offspring. There was significant variation among maternal sibships in brood size and average offspring weight, but not in total brood weight (Table 1). These results suggest that there is variation among full-sibling families in offspring number and offspring size, but not variation among full-sibling families in total brood investment.

TABLE 1. TWO-WAY ANOVAS EXAMINING THE INFLUENCE OF SIRE AND MATERNAL SIBSHIP ON INVESTMENT IN OFFSPRING

Variables	Effect	df	F	P
Brood size	Sire genotype	1	0.22	0.641
	Maternal sibship	7	3.05	0.019
	Sire* maternal sibship	7	0.49	0.832
	Error	25		
Offspring weight	Sire genotype	1	0.16	0.690
	Maternal sibship	5	8.88	<0.001
	Sire* maternal sibship	5	1.46	0.257
	Error	16		
Brood weight	Sire genotype	1	0.60	0.450
	Maternal sibship	5	0.97	0.463
	Sire* maternal sibship	5	1.26	0.329
	Error	16		

F2

Sire genotype (XY versus YY) did not have a significant effect on brood size, average offspring weight, or total brood weight (Table 1; Fig. 2). These results suggest that individual offspring from all-male broods do not weigh more or less than offspring from broods with both male and female offspring.

The interaction of sire and maternal sibship was also examined (Table 1). There was no effect of the interaction on any of the three measures of offspring investment. These results suggest that the effect of sire genotype on offspring investment pattern does not differ for females from different maternal sibships.

To examine the correlation among families between offspring number and offspring size, we used correlation and partial correlation analysis. There was a nearly significant negative correlation among families between brood size and offspring weight ($r = -0.78$, $df = 4$, $P = 0.07$); families with females that produced more offspring tended to produce smaller offspring. Controlling for variation in brood weight, there was a significant negative correlation among families between brood size and offspring weight ($r = -0.94$, $df = 3$, $P < 0.02$); families with females that produced more offspring produced smaller offspring.

DISCUSSION

In genetic lines of the southern platyfish, we found no significant variation among full-sibling families in total brood weight. As a result,

total investment in reproduction did not appear to differ. There was, however, significant variation among full-sibling families in brood size and average offspring weight. There thus appears to be genetic variation for these two life-history traits, even within these inbred lines. Furthermore, there was a negative correlation among sibships between offspring number and offspring size. Females from some sibships produced fewer offspring, but invested more in each individual offspring, whereas females from other sibships produced more offspring, but invested less in each individual offspring. There thus appears to be a genetically-based trade-off between these two life-history traits. Together, these results suggest that P1P1 females do not show genetic varia-



FIG. 2. Mean number of offspring per brood (+SE) for females mated to XY and YY males. Matings with XY males are expected to produce, on average, 50:50 brood sex ratios, whereas matings with YY males are expected to produce broods with only male offspring.

tion for total brood investment, but do vary in how this investment is allocated between brood size and offspring size.

Females in our study had the same P-allele genotype and thus had a restricted range of variation in age and size. As a result, the effects we report may not be generalizable to other P-allele genotypes. In addition, we examined female investment under a single environmental condition. It is possible that we might see different patterns in different environments. Finally, since XY and YY sires had different P-allele genotypes, it is possible that offspring investment could vary with the P-allele genotypes of the sons. All sons, and daughters, however, had the same P-allele genotype, regardless of sire (XP1XP1 and XP1YP2).

Why might there be variation in female allocation patterns in southern platyfish? At many coastal plain locations where *X. maculatus* occur, there is extreme temporal variation in environmental conditions due to variation in rainfall among the seasons. During the dry season, bodies of water in which platyfish are found (ponds, streams, wetland areas, and small rivers) steadily shrink in size and depth. When this occurs, platyfish populations are at high densities. Once the rainy season begins, the same bodies of water expand dramatically, allowing platyfish to spread out to new areas. However, food availability can often be low in these newly colonized areas for a time. Such extreme environmental changes across seasons could favor the maintenance of variation in allocation patterns; females who produce many offspring but invest less in each individual may be favored at certain times while females who produce fewer offspring, but provision each to a greater degree, may be favored at other times. Similarly, there can be extreme variation among platyfish populations in environmental conditions; some sites have highly turbid water and a great deal of aquatic vegetation, whereas other locations have nearly clear water and little aquatic vegetation. It remains to be determined whether temporally or spatially variable selection can at least partially explain the maintenance of the types of variation in female reproductive allocation patterns detected in this study.

Because of the three-factor sex determining system of platyfish, we were able to arrange

matings that produced either all-male broods or mixed-sex broods. This allowed us to test whether sons and daughters are equally costly to produce, an underlying assumption of many theoretical and empirical studies of sex ratio evolution. Variation in the relative costs of sons and daughters should result in a correlation between family size and family sex ratio; assuming that parents have a fixed amount of resources to invest in offspring, when one sex is more costly to produce, families with a preponderance of the more costly sex should be smaller than families with a preponderance of the less costly sex. Female platyfish do not appear to invest more in either sex: neither brood size nor brood weight differed between the all male broods sired by YY males and the mixed sex broods sired by XY males. Because the relative cost of producing a son was not found to differ from that of producing a daughter, the numerical sex ratio appears to be a valid measure of Fisherian sex ratio in the southern platyfish.

CONCLUSIONS

When investigating trade-offs between specific life-history traits, it is often difficult to control for the effects of other life-history traits. The P-allele system in southern platyfish, along with the availability of genetic lines with known P-genotypes, allows a level of experimental control of two life-history traits, age and size at sexual maturation, that is not possible to control in other vertebrate systems. While there was no variation among full sibships in total reproductive effort, as measured by brood weight, we did find variation among full sibships in how this effort was allocated; some families produced more but smaller offspring while other families produced fewer but larger offspring. This suggests that there may be heritable variation in female allocation patterns. In addition, the three-factor sex-determination system in platyfish allowed testing of the relative cost of sons and daughters. Neither brood size nor brood weight was correlated with brood sex ratio. As a result, there was no evidence that either sex is more costly to produce. Although much is known about the genetic ba-

sis of several life-history traits in southern platyfish, particularly the genetic basis of age and size at sexual maturation, this knowledge has not been broadly applied to the study of life-history evolution. The platyfish system, however, would be an excellent model for further studies of life-history evolution, including studies of how female investment patterns differ among P-allele genotypes.

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REFERENCES

1. Stearns SC. The evolution of life history traits; a critique of the theory and a review of the data. *Ann Rev Ecol Syst* 1977;8:145–171.
2. Stearns SC. *The Evolution of Life Histories*. Oxford University Press, England, 1992.
3. Roff DA. *The Evolution of Life Histories: Theory and Analysis*. Chapman & Hall, London, 1992.
4. Charlesworth B. *Evolution in Age-Structured Populations*. Cambridge University Press, Cambridge, England, 1994.
5. Smith CC, Fretwell SS. The optimal balance between size and number of offspring. *Am Nat* 1974;108:499–506.
6. Heath DD, Heath JW, Bryden CA, Johnson RM, Fox CW. Rapid evolution of egg size in captive salmon. *Science* 2003;299:1738–1740.
7. Fisher RA. *The Genetical Theory of Natural Selection*. Dover, New York, 1930 (reprinted 1958).
8. Gordon M. Genetics of *Platypoecilus*. III. Inheritance of sex and crossing over of the sex chromosomes in the platyfish. *Genetics* 1937;22:376–392.
9. Gordon M. Genetics of *Platypoecilus maculatus*. IV. The sex determining mechanism in two wild populations of the Mexican platyfish. *Genetics* 1947;32:8–17.
10. Gordon M. Genetics of *Platypoecilus maculatus*. V. Heterogametic sex-determining mechanism in females of a domesticated stock originally from British Honduras. *Zoologica* 1952;36:91–100.
11. Kallman KD. The sex-determining mechanism of the platyfish. In: *Genetics and Mutagenesis of Fish*. Schroder JH (ed), pp. 19–28. Springer, New York, 1973.
12. Gordon M. The genetics of a viviparous top-minnow *Platypoecilus*; the inheritance of two types of melanophores. *Genetics* 1927;12:253–283.
13. Kallman KD. Genetics and geography of sex determination in the poeciliid fish, *X. maculatus*. *Zoologica* 1965;50:151–190.
14. Kallman KD. Sex determination and the restriction of pigment patterns to the X and the Y chromosomes in populations of the poeciliid fish, *Xiphophorus maculatus*, from the Belize and Sibun Rivers of British Honduras. *Zoologica* 1970;55:1–16.
15. Kallman KD, Schreibman MP. The origin and possible genetic control of new, stable pigment patterns in the poeciliid fish, *Xiphophorus maculatus*. *J Exp Zool* 1971;176:147–168.
16. Valenti RJ, Kallman KD. Effects of gene dosage and hormones on the expression of Dr in the platyfish, *Xiphophorus maculatus* (Poeciliidae). *Genet Res Camb* 1973;22:79–89.
17. Basolo AL. Genetic linkage and color polymorphism in the southern platyfish (*Xiphophorus maculatus*): a model system for studies of color pattern evolution. *Zebrafish* 2006;3:in press.
18. Kallman KD. The platyfish, *Xiphophorus maculatus*. In: *Handbook of Genetics*, Volume 4. King RC (ed), pp. 81–132. Plenum Publishing Corporation, New York, 1975.
19. Kallman KD. Genetic control of size at maturity in *Xiphophorus*. In: *Ecology and Evolution of Livebearing Fish (Poeciliidae)*. Meffe GK, Snelson FF (eds), pp. 163–185. Prentice-Hall, Englewood Cliffs, NJ, 1989.
20. Basolo AL. The dynamics of Fisherian sex ratio evolution: theoretical and experimental investigations. *Am Nat* 1994;144:471–487.
21. Basolo AL. The effect of intrasexual fitness differences on genotype frequency stability at Fisherian sex ratio equilibrium. *Ann Zool Fenn* 2001;38:297–304.
22. McKenzie QD Jr, Crews D, Kallman KD, Policansky D, Sohn JJ. Age, weight and the genetics of the platyfish, *X. maculatus*. *Copeia* 1983;1983:770–774.
23. Kallman KD, Borkoski V. A sex-linked gene controlling the onset of sexual maturation in female and male platyfish (*Xiphophorus maculatus*); fecundity in females and adult size in males. *Genetics* 1978;89:79–119.
24. Bellamy AW, Queal ML. Heterosomal inheritance and sex determination in *Platypoecilus maculatus*. *Genetics* 1951;36:93–107.
25. Wilkinson L. *SYSTAT: The System for Statistics*. Evanston, IL: SYSTAT, Inc, Evanston, IL, 1987.

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