Supplementary Materials – Additional Methods

(A) Tank conditions and maintenance of *X. maculatus* for laboratory evolution study

Experimental tanks were connected by a flow-through water system that allowed the intermixing of water between populations but prevented the movement of fish. A 12:12 light:dark schedule, with simulated dusk and dawn, was maintained throughout the study. The platyfish were fed ad libitum with live brine shrimp, fish flakes and/or liver paste 2-3 times daily. Tanks were matched for water depth and temperature (24+/-1˚C), and all tanks had a high level of microhabitat complexity, including vegetation, refuge cages, boulders, and heterogeneity in background coloration, simulating field conditions.

(B) Experimental *X. maculatus* P-genotypes for laboratory evolution study

The southern platyfish, *X. maculatus*, is a small livebearer occurring in streams, ponds and lakes in Central America. Genetic lines of these platyfish with known P-allele genotypes were used for this laboratory evolution study. The sequence of the nine described platyfish P-alleles, from earliest to latest maturation, is: $P_1 < P_2 < P_7 < P_3 < P_9 < P_8 < P_6 < P_4 < P_5$ (Kallman 1989). Genetic lines with a XP_1, YP_2, and YP_6 were purchased from the *Xiphophorus* Genetic Stock Center, San Marcus, TX, and bred. The YP_6 was derived from the Coatzacoalcos River (Cp) and the YP_2 was derived from the Jamapa River (Jp), both in the state of Vera Cruz, MX. Dr. K. Kallman (American Museum of Natural History and the Genetics Laboratory at the New York Aquarium), and more recently, the Stock Center, maintained the allelic combinations via controlled crosses, thus allowing phenotypic identification of P-genotypes. I mated males with either a YP_6 or a YP_2 allelic combination to XP_1XP_1 females (Jp) for 3.5-5.5 years in my laboratory to produce sufficient numbers of fish for the experiment. Therefore, there were two original lines, and by the time the experiment started, the fish should have had a mixed Cp-Jp genetic background.

In the genetic lines, the pigmentary allele spot-sided (Sp: melanin speckling along flank), was linked to the X sex-determining factor (for the purposes of this study, factor is synonymous to allele: Bull 1984) and the P_1-allele. Both male genotypes shared the XP_1Sp combination with females, but the male genotypes differed in the P-allele and color marker on the Y chromosome. One male had an earlier P-allele (P_2) linked to the Y sex-determining factor and the pigmentary alleles anal red (Ar: red coloration of male gonopodium, the structure used to transfer sperm to females) and stripe-sided (Sr: melanin speckling along scale edges on flank), resulting in the genotype XP_1SpYP_2ArSr. The other male had a later maturation P-allele (P_6) linked to the Y sex-determining factor and the pigmentary allele anal spot red (Asr: red coloration on the body immediately above the gonopodium), resulting in the genotype XP_1SpYP_6Asr. Thus, both male genotypes had melanin coloration along the flank, and red coloration at the ventrum. The pigmentary alleles are co-dominant (Schreibman & Kallman 1977), and were chosen such that there were minimal differences in coloration between the two male genotypes. (For additional information about the pigmentary alleles, see Basolo 2006). In addition, several autosomal melanin alleles that were not in genetic linkage with the P-alleles varied across the three genotypes. In these genetic lines, the recombination rates between the P-alleles and red pigmentary alleles is < 0.002, and for P-alleles and sex-linked melanin pigmentary alleles, it is < 0.01 (Kallman 1970, 1975).

Although tested for, no difference between the sexes in growth rate prior to the onset of sexual maturation has been found (Kallman 1975). In addition, no difference among P-genotypes in growth rate prior to the onset of sexual maturation has been detected when

tested under a variety of conditions (McKenzie et al. 1983). A re-analysis of these findings concluded that P-genotypes remained "detectable despite a strong attempt to exaggerate phenotypic plasticity" (Stearns & Koella 1986). Under controlled laboratory conditions, the average time to maturation for P_1P_1 individuals is eight weeks. Heterozygotes mature at a time that is intermediate to homozygotes (Kallman 1989); based on this, the average time to maturation for P_1P_2 genotypes would be 11 weeks, and for P_1P_6 genotypes, 16 weeks. Thus, the relative generation time advantage for the small male genotype was 145%.

(C) Replicate experimental populations for the laboratory evolution study

To establish eight replicate populations, XP_1SpXP_1Sp females were mated to either a XP_1SpYP_2ArSr or a XP_1SpYP_6Asr male, and the offspring were raised in group tanks until they were transferred to the experimental tanks. For the XP_1SpYP_2ArSr males, 181 sub-adults (sexual maturation initiated) and adults, and two juveniles were divided across the eight tanks. (The initiation of sexual maturation in males is identified by distinct changes during the development of the gonopodium, the structure used to transfer sperm to females, from the anal fin.) For the XP_1SpYP_6Asr males, 175 sub-adults and adults, and 10 juveniles were divided across the eight tanks. This made a total of 183 XP_1SpYP_2ArSr males and 185 XP_1SpYP_6Asr males. For the XP_1SpXP_1Sp females, 247 adults, 109 sub-adults, and 68 juveniles were divided across the eight experimental tanks. This made a total of 356 adult and sub-adult males, 356 and sub-adult and adult females, and 80 juveniles divided across the eight tanks at T0. A 38-week equilibration period followed the establishment of the populations. During this period, the populations and genotype frequencies were assessed twice, at 12 and 26 weeks post-establishment. At both times, senescent or sick fish were removed with replacement (matching for genotype), and adult and sub-adult males and females added to return the genotype frequencies and the sex ratios to values equivalent to those at the time of establishment of the populations (T0). Babies and juveniles were not adjusted. After the second adjustment with replacement, 12 weeks elapsed before the T1 scoring.

At the end of the equilibration period, the frequencies of the male genotypes in the eight populations were quantified (T1). A cichlid, *C. octofasciatum*, that was within the upper end of the size range found in wild platyfish populations (100—108 mm standard length) was then placed in each of four populations. Over the next 33 weeks, the genotype frequencies were quantified twice by removing the fish from each population tank and scoring them: once after 15 weeks (T2), and again after 18 weeks (T3). At T2, size measurements were also taken. For two predator-present replicates, some early maturing males were inadvertently added following the T2 scoring. However, if the addition of the early maturing males biased the results at T3, it would have made the predator-present and predator-absent replicates less different rather than more different; the statistical comparisons are thus conservative.

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