Poeciliid Research

Physiological and anatomical changes in poeciliid fish produced by deprivation of heterosexual contact

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Abstract. Our study is a compilation of studies and discussions on how poeciliid individuals react when social factors change drastically. Such a drastic social factor is the deprivation of heterosexual contact. Physiological and anatomical changes in poeciliid fish produced by deprivation of heterosexual contact are sex reversal in females, on one side, and homosexual behavior in males, on the other side, among others. Spontaneous masculinization of females mediated by social factors was demonstrated in guppy (*Poecilia reticulata*), molly (*P. sphenops*) and swordtail (*Xiphophorus hellerii*). The sex reversal is very common in swordtail, even if the phenomenon is not frequently observed in guppy and molly. Homosexual behavior is frequently observed in several poecilid species. Most often, this behavior is the result of deprivation of heterosexual contact. At first sight, homosexual behavior does not seem to have a useful role in the survival of the population, if we look at homosexual behavior in the strict sense. When we consider that homosexual behavior is part of the behavior of atypical preference, or vaguely defined sexual preference, then the situation changes. Populations with variable preferences and individuals with wide preference may be those who will succeed interspecific crossings with related species. This is an important event for speciation. Moreover, the individuals with wide preference for mate choice can be the protagonists of mating with atypical conspecific individuals of opposite sex that can be more adapted to the latest conditions. Such atypical mate choice generates a new direction for evolution, according to the new conditions created.
Key Words: *Poecilia reticulata, Poecilia sphenops*, sex reversal, *Xiphophorus hellerii*.

Introduction. Perpetuation of vertebrates requires successful reproduction. Successful reproduction in turn requires the individual to be able to reproduce through superior qualities to competitors of the same sex, to find partners of the opposite sex best adapted to the given conditions, and, last but not least, to survive to be able to mate again.

The present study tries to explain that sometimes perpetuation can be ensured by individuals who are apparently the least adapted, because even adaptation is a relative term. That is, with the sudden change in the social conditions of the fish community, unadapted individuals can become the most adapted individuals to the new conditions created. Our study is a compilation of other studies, showing how individuals react when social factors change drastically. Such a social factor is deprivation of heterosexual contact.

Early studies on guppy in 2000s. Sex-ratio regulation is an important aspect for the survival of a population and it is an important issue of the species' ecology and evolution. Studies on guppy (*Poecilia reticulata*) sex-self-regulation within a population have been conducted by members of our team since the 2000s. Some parts of those studies have been the basis for the development of a doctoral thesis (Petrescu-Mag 2007a), and others have been published in the form of scientific articles (Mag-Mureșan & Bud 2004ab) or books (Petrescu-Mag 2007bc). The studies of those years focused on the possibilities of inducing the phenomenon of sex reversal in individuals in different ontogenetic stages: adult, juvenile, newborn, embryo and gamete, under the influence of social and environmental factors.

In previous research, we concluded that after certain age (after the adult stage is achieved) the individuals' chance to change their sex with the help of social factors is very low, close to zero (Petrescu-Mag 2007a). We can instead efficiently act on eggs, embryos, and then on fries and less on youthful individuals. This was confirmed by result analysis using the chi square test (Petrescu-Mag 2007bc).

We observed that, in the case of youthful guppy females and those under sex differentiation, fecundation seems to play a decisive role in defining their permanent sex. Youthful females close to adult age, once inseminated and fecundated, will remain females all their lives (Petrescu-Mag 2007bc). In fact, neither we, nor any scientist or known aquarist is aware of any female to male sex reversal after the female has given birth to a series of fries. Unlike guppy (*Poecilia reticulata*) (Figure 1, right), molly (*Poecilia sphenops*) (Figure 2, left), and platy (*Xiphophorus maculatus*), female to male sex reversal of adult individuals is very common in swordtails, *Xiphophorus helleri* (Figure 2, left). This is a personal observation in aquarium science as a hobbyist (unpublished data).

The possibility of changing the guppy sex ratio by the modification of some social factors was explained by the authors of our team in two ways. In fact, these were two hypotheses, as long as there was no physiological, biochemical or molecular investigation to support any of them.

The first hypothesis refers to "the capacity of the female body to respond to the absence of courtship and/or fecundation by releasing increased quantities of androgenic hormones, and these hormones can influence the evolution of its own gonads (more rarely), or the primary bipotent embryonic gonads of the progeny (most frequently)" (Petrescu-Mag 2007a). This hypothesis emitted at that time could also explain the sex reversal noticed sometimes in cases of youthful females. We stated further that although there is no solid anatomical and morphological connection between the mother and the embryos (no placenta, Petrescu-Mag et al 2019) and maternal blood does not wash the embryonic tissues, androgenic hormones can reach the target tissues of the embryos due to their steroidal nature.

The second hypothesis assumes that a "postcopulatory selection of sperm cells by the participation of cryptic females" is the explanation for unexpected ratios observed during the experiments (Petrescu-Mag 2007a). "The female body could favor the male producing gametes to the prejudice of the female producing ones in the absence of males". However, "the experiments conducted on guppy fries show that besides the genetic component involved in sex differentiation, there is also a factor related to the interaction between individuals, factor that seems to be able to act on the individual even after parturition" (Petrescu-Mag 2007bc). This last statement returns to the first hypothesis and supports it in theory.

Figure 1. Aquarium, colorful varieties of poecilid fish. Molly (black marble variety), swordtail (red albino variety) in left picture, guppy (tuxedo variety) in the right picture (original pictures).

Raw and unpublished data on swordtail masculinization. In 2008, we performed an experiment that has not been published so far. It aimed to understand the differences in terms of masculinization in *X. hellerii* and *P. reticulata* from birth to 1 year after maturity. The working method is well-known among guppy breeders, namely, the extraction of each male from the culture tank immediately after sexual differentiation. Thus, the culture tank permanently contains only females and sexually undifferentiated individuals. The results obtained are presented in Table 1.

Table 1

Masculinization in poeciliid fish produced by deprivation of heterosexual contact

Species	*Lot*	*Initial numbers*	*Results**	
			Males	*Females*
P. reticulata	Experimental	100	54	40
	Control‡	100	52	45
X. hellerii	Experimental	100	89	0
	Control‡	100	61	37

Note: ‡Control - no extraction was made until the end of the experiment; * - missing numbers are due to mortality during the experiment. Source: original data (unpublished).

We can see that, in the case of guppy fish, there is a probability that the small differences were caused by the sex change, forced by social factors. However, these differences are minor and statistically unverified. In the case of swordtail, there is no doubt and no additional statistics are required: it is clear that complete masculinization is caused and forced by social factors, namely the lack of females in the pool.

Thus, in the case of swordtail, the deprivation of heterosexual contact induces masculinization, as a self-regulatory effect in the population. This is explicable from an ecological and evolutionary point of view.

The latest study sheds light. In 2015, a study conducted by a team of Polish researchers explained through anatomical and biochemical investigations what are the substances that underlie the late sex change in poeciliid fish (Kulczykowska et al 2015). They provided the first insight into the endocrine background of two phenomena that frequently occur in experimental mono-sex lots of black molly: 1) masculinization in females and 2) male homosexual behavior.

They say that in socially controlled situations, brain neurohormones affect both the phenotypic sex determination and sexual behavior. To list the most important known neurohormonal candidates involved in sex determination and sexual behavior, these hormones are the nonapeptides arginine vasotocin and isotocin, counterparts of the well-

known mammalian arginine vasopressin and oxytocin, respectively (Kulczykowska et al 2015).

In order to reveal potential hormone interactions, the authors of the aforementioned paper measured the concentrations of bioactive vasotocin and isotocin in the brain, along with those of the sex steroids 11-ketotestosterone and 17b-estradiol in the ovary of females, gonads of masculinized females, testes of males displaying homosexual behavior and testes of males with no homosexual behavior (Kulczykowska et al 2015). This information was completed by morphological and histological analyses of the gonads. Correlations between brain nonapeptides and gonadal steroids strongly suggest a cross talk between hormonal systems (Kulczykowska et al 2015). In the black molly, the masculinization process was associated with the production of brain vasotocin and gonadal steroids, whereas homosexual behavior involves both brain nonapeptides, but neither of the sex steroids (Kulczykowska et al 2015).

Sex reversal expected, but homosexual behavior for what? As we have established above, the spontaneous change of the sex of supernumerary females is explicable and useful for the survival of the population. It is a mechanism that favors perpetuation when males are too few or even absent (see the case of populations of poecilids at a high risk of predation). However, homosexual behavior does not seem at first sight to have a useful role in the survival of the population if we look at homosexual behavior in the strict sense. When we consider that homosexual behavior is part of the behavior of atypical preference, or vaguely defined sexual preference, then the situation changes, as we will see below.

Same-sex sexual behavior has been observed in many animals, including guppy males (Field & Waite 2004). Apparently, this behavioral sequence does not bring the individual any selective advantage, and is detrimental because it does not bring a greater number of descendants. Therefore, we ask why such a detrimental behavior does not disappear by natural selection. Same-sex sexual behavior should be considered an atypical preference of the individual for choosing a mate. Why does the typical preference not disappear? Because, surprisingly, from time to time, this atypical preference plays an important role in the survival and evolution of a population in nodal moments (Petrescu-Mag 2018).

Guppy fish tend to be numerous in smaller streams and pools than in large, deep, or fast-flowing rivers (Magurran & Philip 2001). Let us imagine small sized guppy fish populations. In cases of reproductive isolation (such as captive individuals) on the islands or after the partial drying of the waters where guppy fish live, the fish would be doomed if all the individuals would have elevated preferences for a potential mate (Petrescu-Mag 2018). When the population number decreases, those individuals willing to make compromises in choosing the partner become decisive in the perpetuation of the species. In such situations where nature offers you mates with a lot of stinginess, individuals with "high pretensions and fine tastes" are not winners, but losers. Populations with variable preferences and individuals with wide preference may be those who will succeed interspecific crossings with related species. See occasional interspecific attempts of hybridization presented in papers like: Valero et al (2009), Bias (2013), Franchini et al (2018). On the other hand, the individuals with wide preference for mate choice can be the protagonists of mating with atypical conspecific individuals that can be more adapted to the latest conditions (Petrescu-Mag 2018).

The importance of interspecific hybridization for poeciliid speciation has been stated or discussed in Păsărin & Petrescu-Mag (2011), Oroian (2015) and Franchini et al (2018). Furthermore, interspecific hybridization played an important role for superior vertebrates, including hominids (Ackermann et al 2016).

Thus, atypical preference plays a particularly important role in the production of interspecific hybrids and later in introgressions, which in turn can be very important in species evolution. This is why the atypical preference for mate choice does not disappear from the poeciliid population (Petrescu-Mag 2018).

Conclusions. Physiological and anatomical changes in poeciliid fish produced by deprivation of heterosexual contact are sex reversal in females, and same-sex sexual behavior in males. Spontaneous masculinization of females mediated by social factors was demonstrated in guppy (*Poecilia reticulata*), molly (*P. sphenops*) and swordtail (*Xiphophorus hellerii)*. If in guppy and molly the phenomenon is seldom observed, the sex change is very common for swordtails. Homosexual behavior is frequently observed in several poecilid species. Most often, this behavior is the result of deprivation of heterosexual contact. Homosexual behavior does not seem at first sight to have a useful role in the survival of the population, if we look at homosexual behavior in the strict sense. When we consider that homosexual behavior is part of the behavior of atypical preference, or vaguely defined sexual preference, then the situation changes. Populations with variable preferences and individuals with wide preference may be those who will succeed interspecific crossings with related species, on one hand. This is an important event for speciation. On the other hand, the individuals with wide preference for mate choice can be the protagonists of mating with atypical conspecific individuals of opposite sex that can be more adapted to the latest conditions. Such matings generates a new direction for evolution, according to the new conditions created.

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