Estradiol, reproductive cycle and preference behavior in a northern swordtail

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ABSTRACT

Estrogen is associated with female sexual behaviors, particularly receptive behaviors during the reproductive cycle. Less is known about the relationship between estrogen and female preference behaviors that may precede receptivity and copulation. Separating the mechanisms underlying preference from receptivity is often confounded by the tightly coupled cycle- or estrogen-dependent expression of female sexual behaviors. Here we utilize a live-bearing poeciliid (*Xiphophorus nigrensis*), a model species for studying the evolution of female mate choice that can store sperm over multiple brood cycles. We assayed estradiol along with preference, receptivity and locomotor behaviors in gestating females and then re-tested these females on days 1, 7, 14, 21, and 28 post-parturition. With *a posteriori* reproductive cycle assessment, we asked whether reproductive state predicts differences in (i) estradiol levels, and (ii) behaviors (preference, receptivity, and general locomotor activity). We then examined if estradiol levels (independent of reproductive state) explain any variation in these behaviors.

We found that endogenous estradiol levels vary across the reproductive cycle: gestating females had lower estradiol levels than those undergoing vitellogenesis/fertilization. In contrast, receptivity and preference behaviors did not vary over the reproductive cycle. Estradiol levels did not predict variation in receptive behavior, but were associated with increased locomotion. While individual female preference behaviors were consistent across the reproductive cycle, there was a small *negative* relationship between estradiol and preference behaviors explaining between 3% and 10% of the inter-female variation in preference behavior. Our data indicate *X. nigrensis* females may exhibit a facultatively dissociated reproductive system.

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1. Introduction

Female reproductive cycles exist to coordinate the expression of sexual behavior and can be regulated by a variety of external and internal cues (reviewed [36]). In many taxa, steroid hormones are associated with a reproductive cycle as a mechanism to coordinate the timing of female reproductive effort with a maximum chance for reproductive success. This classic paradigm is known as an associated reproductive system wherein ova production, steroid hormones and sexual behaviors cycle together [10]. For instance, in the rat, female sexual behaviors are strongly associated with reproductive cycle status, and behavioral estrus (i.e. the willingness to perform proceptive/solicitation and receptive behaviors towards males) is expressed primarily during the proestrus/estrus stages of the ovarian cycle when estrogen levels are high, ovulation is imminent, and the female is willing to accept copulation (reviewed in [15]). In other systems such as the red-sided garter snake, sexual behaviors and steroid hormones are decoupled. These systems display a dissociated reproductive pattern wherein

steroid hormone levels and ova production do not coincide with sexual behavior, and mating behavior can occur with very low levels of circulating steroid hormones (e.g. [10]).

The relationship between hormones and other critical behaviors leading to reproduction has been under-studied. Specifically, the role that hormones play in female mate discrimination or preferences for specific male phenotypes has only recently received attention [1]. In associative reproductive systems, changes in hormone levels have been shown to correlate with changes in receptivity, permissiveness and discrimination behaviors in female anurans [28,29], while in mice, peptide hormones may play a role in governing female response to dominant males [30]. However, in these systems the role of steroid hormones in female preferences may act primarily in a motivational aspect of mate choice: driving females to prefer to associate with opposite sex partners rather than mediating a discriminatory function amongst different males [1,7,37].

Little is known about the social and physiological influences underlying female preference in a dissociated reproductive system. The de-synchronization of reproductive behavior and gamete production can arise from physical constraints (e.g. in garter snakes, [16]; and sea perch, [54]) or social environment (e.g. sperm competition, see [4]). In many poeciliid fish species, a single mating bout

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can fertilize multiple subsequent broods, and broods with evidence of multiple paternity appear to be quite common [5,8,40,48]. Poeciliids are ovoviviparous and breed throughout the year in both natural and laboratory conditions [34]. Temporal patterns of brood production vary across species, but in swordtails (genus *Xiphophorus*), multiple ova cohorts at different levels of development are present in the ovary, although only one primary brood is fertilized and actively gestating at a time [50]. Whether driven by environment or social pressures, any physiological modification that allows gamete storage can then allow the de-coupling of the suites of behaviors associated with mate preference and sexual behavior from the steroid hormone levels necessary for gamete production. Such systems may favor expression of preference behaviors independent of reproductive cycle: allowing females to discriminate and mate amongst potential male partners at any stage of ova development.

In many spawning fish such as the goldfish, prostaglandins are the hormonal trigger for female sexual behavior (reviewed in [35]). In live-bearing fish, however, estrogen plays a key role in inducing receptivity. Much early poeciliid research focused on female reproductive cycling [24,40,47,50–52] and the endocrine factors underlying female receptivity [25,26]. Steroid hormone levels cycle along with brood development: estrogen is highest during fertilization/ parturition phases of the reproductive cycle and then declines during gestation [22,52]. In some poeciliids, female receptivity is tightly coupled to reproductive cycle, and females are generally most receptive during the brief period after parturition when the next brood of ova is ready to be fertilized [24,25]. However, it is less clear whether female preference behavior is necessarily confined to discrete phases of the reproductive cycle or is estrogen-dependent.

In recent decades, poeciliids have also become a model system in sexual selection for female mate choice studies [2,12,18, 32,42,43], and preferences for discrete male phenotypes are very tractable in laboratory conditions. Preference (e.g. association bias, [33,53]) and receptivity (e.g. glides, [11]) behavioral measures in laboratory dichotomous mate choice experiments have proven to be reliable indicators of reproductive choices and copulation events, respectively. In *Xiphophorus nigrensis*, males come in genotypically-determined size classes with larger male classes that develop swords, colorful ornamentation, and court females, and small class non-ornamented males that use a female chase strategy to acquire matings [19,41]. Most females show a strong and consistent preference for the largest size class males over the smallest size class [11,43].

Here we ask if preference behavior is dependent on reproductive cycle stage in *X. nigrensis* females. We also test whether individual variation in the expression of preference, receptivity or general activity is linked to endogenous estrogen (17β-estradiol) levels in intact *X. nigrensis* females.

2. Materials and methods

We measured estradiol levels and quantified male-oriented behaviors in gestating female *X. nigrensis* fish and then at weekly intervals post-parturition. We then analyzed the association between reproductive cycle status and/or estradiol levels on individual variation in preference, receptive and general locomotor behaviors.

2.1. Collection and monitoring of test animals

Pregnant *X. nigrensis* females ($N = 21$) were obtained from semi-wild populations held at Brackenridge Field Laboratories (University of Texas) or from populations originally wild-caught at the Nacimento de Rio Choy in San Luis Potosi, Mexico. In the laboratory, fish were isolated and kept at a 12:12 photoperiod in a temperature-controlled ($\sim$27 °C) room. Initial pregnancy status was estimated by visual assessment of the size of the distended abdomen and brood patch (a visible pregnancy sign; [3,39]), and females were checked daily for parturition. Female standard lengths (SL; body length from the tip of the snout to the anterior edge of the caudal fin) ranged from 21.1 to 44.3 mm. Female estradiol (E_2) levels and preference behaviors were tested before parturition, and then tested again on Days 1, 7, 14, 21, and 28 post-parturition (parturition = Day 0). Three females gave birth before they could be given a pre-parturition trial, so those three had a total of 5 trials (all post-parturition), while the rest ($N = 18$) had a total of 6 trials. All trials took place between August 2007 and June 2008.

2.2. Reproductive cycle assessments

The presence or absence of stored sperm in poeciliids appears to determine whether sexually isolated females follow parturition with another brood development cycle (red circle in Fig. 1A) or enter an indeterminate stage with intermittent phases of vitellogenesis and unfertilized egg reabsorption (blue circle in Fig. 1A). With no *a priori* knowledge of whether females had stored sperm in this study, we could only assess reproductive cycle status *a posteriori*. Therefore, we monitored all 21 females for a second parturition. All trials in which females were known to be actively gestating a brood were assigned as gestation trials, and so include not only all the original pre-parturition trials but also those trials taking place during the gestation period for females who produced a second brood ($N = 6$; referred to as 'sperm-present' females, Fig. 1B). We estimated the gestation period as 27 days per our temperature and lighting regimen (see also [47]). Therefore, we subtracted 27 days from any second parturition dates and assigned trials taking place during this period as gestation category trials. Trials taking place in the window following the initial parturition date and prior to the 27-day gestation period were assigned to a vitellogenesis/fertilization category because the vitellogenesis/egg maturation and fertilization phases cannot be distinguished *a posteriori*. The cycle lengths for the 6 "sperm-present" category females were 28, 39, 42, 41, 56, and 62 days. The variation in cycle length is assumed to occur with the timing of egg maturation and fertilization, not gestational length [8,47]. The remaining 15 females did not produce a second brood during the experiment and were assigned to a putatively sperm-depleted 'indeterminate' category (Fig. 1B). The indeterminate group is likely to be heterogeneous in that it represents females undergoing vitellogenesis as well as the reabsorption stages, and so this group contains females at very different reproductive conditions.

2.3. Sexual isolation

Of the 21 females in the study, most ($N = 17$) were isolated from males <7 days prior to onset of the experiment and were termed 'short-term isolates' (including all 6 sperm-present females). Four females were isolated from males >60 days prior to the onset of the experiment and were labeled 'long-term isolates'. Long-term isolation had no effect on our behavioral measures, but did have a significant effect on E_2 levels ($t = 2.577$; $p = .019$; data not shown). Therefore, the long-term isolate females were removed from the reproductive cycle status and individual variation in E_2 and behavior analyses. However, including them (full data set $N = 21$ versus $N = 17$ short-term isolate data set) produces similar results in both reproductive status and individual variation analyses (data not shown).