Congenital predispositions and early social experience determine the courtship patterns of males of the Amarillo fish

ARTICLE *in* BEHAVIORAL ECOLOGY AND SOCIOBIOLOGY · APRIL 2014

Impact Factor: 2.35 · DOI: 10.1007/s00265-013-1678-3

Ornela De Gasperin
University of Cambridge

Behav Ecol Sociobiol (2014) 68:639–648
DOI 10.1007/s00265-013-1678-3

ORIGINAL PAPER

Congenital predispositions and early social experience determine the courtship patterns of males of the Amarillo fish

Ornela De Gasperin · Constantino Macías Garcia

Received: 11 August 2013 / Revised: 18 December 2013 / Accepted: 19 December 2013 / Published online: 11 January 2014

Abstract Mounting evidence indicates that learning shapes fish foraging and social behavior, which suggests that it may also underlie the frequently reported geographic variation in courtship. In this study, we analyzed how early social experience influences courtship patterns in males of the Amarillo fish (*Girardinichthys multiradiatus*). In a first experiment, we quantified the courtship of males from two allopatric populations grown in isolation and estimated the heritability of a key attribute of their courtship patterns. Then, we raised males from two allopatric populations with either sympatric or allopatric females and at two different densities, and registered their adult courtship patterns towards females from a third population. We found that the courtship pattern of males has a significant heritable component, yet in the second experiment, it was influenced by what type of females males were raised with, by their social rank (dominant/subordinate/singleton), and by the behavior of the female that they were courting. We conclude that courtship behavior in this fish has a congenital predisposition and is affected during development by different aspects of early experience and seemingly remains flexible throughout life; a complex ontogenetic trajectory that under natural conditions may have led to the locally stable dialect types observed in the courtship patterns of males from allopatric populations.

Keywords Early social experience · Learning · Allopatric populations · Courtship · Goodeid

Introduction

How behavior develops during the lifetime of an individual is the most neglected of the Tinbergen four questions (Tinbergen 1963; Stamps 2003). However, the social environment that an individual experiences during its development can shape many aspects of its adult behavior (Bateson 1983; Moore 2004). For instance, early social experience can modify social learning abilities and shoaling tendencies (Chapman et al. 2008), boldness and exploratory behavior (Chapman et al. 2010), nest building behavior (Margulis et al. 2005), sex recognition (Vos 1995), mate choice (Oetting et al. 1995; Kendrick et al. 1998; ten Cate and Vos 1999; Verzijden and ten Cate 2007; Tramm and Servedio 2008; Vyas et al. 2009), foraging tactics (Katsnelson et al. 2008), predator avoidance (Cook et al. 1985), aggressiveness (Arnold and Taborsky 2010), and singing styles (Clayton 1988; Hultsch 1991).

Although the social environment can have extensive effects on development, there are still relatively few studies that address how it can influence courtship patterns (White et al. 2002), and most studies addressing courtship development have focused on acoustic signals in birds (Freeberg 2000; Galef and Laland 2005). For example, exposure to strange songs during development can affect the song performed by adult birds (Eales 1985; Hultsch 1991), and brood parasites learn the singing style of their host species (Payne et al. 1998). Oscine birds seem to have an innate song template (Marler and Peters 1989) as young birds with no exposure to conspecific song can discriminate between conspecific and

---

Communicated by A. Pilastro

Electronic supplementary material The online version of this article (doi:10.1007/s00265-013-1678-3) contains supplementary material, which is available to authorized users.

C. Macías Garcia (✉)
Instituto de Ecología, Universidad Nacional Autónoma de México,
A.P. 70-275, C. P. Coyoacán, México City, D.F., Mexico
e-mail: maciasg@unam.mx

O. De Gasperin (✉)
Department of Zoology, University of Cambridge, Downing Street,
Cambridge CB2 3EJ, UK
e-mail: ornela.gasperin@gmail.com

heterospecific songs (Dooling and Searcy 1980; Nelson and Marler 1993) and prefer to listen at conspecific songs during sensitive periods (Braaten and Reynolds 1999). Therefore, both genetic and environmental factors seem to play important roles during song development in birds, and similar effects may occur in courtship patterns in different taxa. Yet, how social environment may influence the development of courtship and how these effects may interact with genetic factors in taxa other than birds has received considerably less interest.

Amongst fish, species-specific courtship signals can be visual (e.g., Maan et al. 2004), chemical (e.g., Hankison and Morris 2003; Wong et al. 2005), acoustic (e.g. Gerald 1971; Kihslinger and Klimley 2002), and/or electric (e.g., Hagedorn and Heiligenberg 1985), and their use can be genetically determined (including alternative mating strategies: Farr 1980; Zimmerer and Kallman 1989; Ryan et al. 1990). Notwithstanding, demonstrations of plasticity in the use of some courtship elements are increasingly common. For instance, Shaw et al. (2007) reported that ancestral courtship plasticity has been involved in the recurrent adaptive diversification of three-spined sticklebacks. In the Trinidadian guppy (*Poecilia reticulata*), reports on short-term modifications of courtship behavior include the effect of variation in sex ratio or predation risk on mating strategies (consensual or coercive; Jordan and Brooks 2010) or on the tendency to perform courtship (e.g., Magurran and Seghers 1990; Magnhagen 1991; Sih 1994; Long and Rosenqvist 1998), sometimes mediated by changes in female behavior (e.g., Evans et al. 2002). Recently, a long-term study on guppy courtship development showed that it can be influenced by the social environment (males, females, or males and females) which naïve male guppies have visual access to during development (Guevara-Fiore 2012), suggesting that early learning can have an important effect on adult courtship patterns of fish. Learned courtship behavior may reinforce the divergence of mating signals (Verzijden and ten Cate 2007; Tramm and Servedio 2008). Such divergence can influence the reproductive success of migrants dispersing or being introduced into new localities (Ritchie et al. 2007); thus, understanding how adult courtship may be shaped by the social environment experienced early on life can illuminate studies on reproductive isolation and speciation and may also inform programs of captive breeding and reintroduction of endangered species (see Kelley and Macías Garcia 2010).

In this study, we investigated whether the courtship patterns of adult males is influenced by their congenital underpinning and by the social environment experienced during development in two experiments using a fish whose complex courtship pattern differs between allopatric populations and is influenced by female behavior (González Zuarth and Macías Garcia 2006; González-Zuarth et al. 2011). We first raised males in isolation and quantified the heritability of their courtship behavior. Afterwards, we raised males with either sympatric or allopatric females and compared their adult courtship patterns towards a novel female. Throughout the text, we use the terms “congenital” to refer to both genetic and prenatal (i.e., during gestation) developmental influences on courtship patterns, as opposed to postnatal influences, which we refer to as “effect of social environment”.

Girardinichthys multiradiatus is a member of the Goodeinae, a subfamily of viviparous fish endemic to Central Mexico (Webb et al. 2004). Female mate choice is based on the size and color of the male sexually dimorphic dorsal and anal fins, and on the courtship behavior, which includes elaborate dances (Macías Garcia et al. 1994; Macías-Garcia and Saborío 2004). Goodeid males lack an intermittent gonopodium and require female cooperation to transfer sperm during a copulatory embrace. They cannot force copulations; thus, females decide whom to mate with (Macías Garcia et al. 1994; Macías-Garcia and Saborío 2004). During courtship, males perform a variety of displays that can be grouped into two categories: dynamic and static. Dynamic displays are dances such as the figure-of-eight dance, fin folding, and flagging, performed in front or besides the females. These displays are exclusive of courtship encounters and may involve energetic wavering of the fins and rapid displacement describing the figure of the numeral “8”. Static displays are common to agonistic (both inter- and intrasexual) and courtship encounters; the fish stop in mid water, either besides or in front of the other fish, and spread their dorsal and anal fins. These displays can escalate into overt aggression, waving their bodies, followed by attempts at biting the rival. Females and subordinate males often respond to static displays with body vibration (energetic and costly body shaking in a static position) which functions as an appeasement signal (see descriptions by Macías-Garcia and Valero 2001; Valero et al. 2005; González Zuarth and Macías Garcia 2006) and also influences the subsequent behavior of the courting male (González-Zuarth et al. 2011; Martínez Medina et al. 2013).

The distribution of *G. multiradiatus* is fragmented; its populations have diverged genetically, behaviorally, and morphologically, and these differences have been linked to premating isolation (González Zuarth and Macías Garcia 2006; but see Macías Garcia et al. 2012). Although all populations share all the elements of the courtship displays (they perform all the dynamic and static behaviors indicated above), the tendency to perform them vary between populations, leading to distinct local courtship patterns that can be used to classify males by their population of origin (González Zuarth and Macías Garcia 2006; González-Zuarth et al. 2011). These differences in courtship seem to be influenced by the immediate social environment as field-grown males confronted to allopatric females perform courtship in a way that is quantitatively different from their population-typical courtship pattern, but also from the pattern of the population of origin of the allopatric female (González Zuarth and Macías Garcia 2006;

González-Zuarth et al. 2011). These findings demonstrate short-term plasticity in the courtship of *G. multiradiatus* and suggest that the observed population differences are at least, in part, the consequence of differences in female behavior. Here, we first evaluated the extent to which the population-specific courtship patterns are inherited; then, we investigated whether the exposure to allopatric females during development can lead to long-term modifications of the way males respond to encounters with novel females.

Materials and methods

Experiment 1: congenital influence of courtship patterns

Origin of fish Detailed behavioral records from the field indicate that males from Zempoala (19°03″ N, 99°18″ W) spend relatively long periods directing fin displays to the females and only rarely perform elaborate flagging and figure-of-eight dances; thus, their courtship encounters are prolonged but seldom escalate. By contrast, males at Acambay (19°57′ N, 99°46′ W) shift rapidly from fin displays to flagging and figure-of-eight dance; thus, their courtship encounters are short and often involve escalation (Macías Garcia 1994). We used fish from Acambay and from Zempoala to evaluate to what extent the differences in courtship patterns are congenital. We measured the behavior of 116-day-old male *G. multiradiatus* reared in individual 2.3-L containers since the age of 60 days. A total of 100 males from 28 broods were used; 54 males (14 broods) were born of females from Acambay (A: 3.86±1.2 males/brood; mean± sd are used throughout unless specified otherwise), and 46 (14 broods) of females from Zempoala (Z: 3.29±1.2 males/brood). Mothers were collected with dip nets from four outdoor ponds set up 6 months earlier at the National Autonomous University of Mexico (UNAM) and populated with 100 adult fish (55 females and 45 males) from either of the two focal populations (two ponds per population; see Macías Garcia et al. 1998 for details). Ponds for each population were replicated, to avoid the coincidence between original population source and pond. Each mother was kept in a 40-L (27×30×50-cm-deep) maternity tank until parturition and fed Daphnia or *Artemia* sp daily at noon. Temperature was 26–28 °C throughout the experiment, and photoperiod was 12/12-h light/dark. Upon parturition, each female was returned to its pond, and the brood was kept in the natal aquarium until the age of 60 days, when the fin size and associated body shape dimorphism began developing, permitting the majority of fish to be sexed. Up to five males from each brood were then transferred to individual 20×13×9-cm-deep opaque plastic containers and raised in total isolation (visual, physical, and chemical) from other fish. However, males from Acambay can reach larger sizes in the field as compared to Z males (Macías Garcia 1994).

Procedure Behavioral records were made starting when the males were 116 days old. On the day of the trial, the individual container was transferred to an observation room, placed under a 20-W fluorescent lamp, and left to habituate for 10 min. Then, the male was gently driven to one extreme of its container and enclosed between the container wall and a parallel opaque partition that was placed ca. 2 cm from the wall. A transparent partition was then introduced in the middle of the container, parallel to the opaque partition, and one female of two used throughout of the same cohort as the fish and originating in Zempoala was placed in the empty half of the container. Only two, very similar females from one of the populations were used, so as to standardize the stimulus to which males from both populations were exposed. After a further 5 min, the opaque partition confining the male was removed, and the timing and frequency of behavior patterns was recorded using a computer event recorder (The Observer™). This experimental setup restricted the variety of dynamic displays that could be performed by the males. Specifically, the figure-of-eight dance and overtaking cannot be performed when the male and the female are separated by a transparent divider. We focused, consequently, on the frequency with which the males performed flagging (dynamic courtship) in their first visual encounter with a female after having matured in isolation. Trials lasted 10 min, and the fish containers were then returned to the housing room.

Experiment 2: effect of social environment on courtship patterns

Origin of fish We used males from two allopatric populations (Zempoala and El Porvenir [19°40′ N, 100°38′ W]) which are genetically differentiated (F_{ST}=0.4291, D_{ST}=0.4; Macías Garcia et al. 2012) and whose courtship patterns differ significantly from each other. The Acambay population used to calculate the heritability of some aspects of courtship had unfortunately dried out, and we used Porvenir males instead. The courtship patterns of Porvenir males also include a high frequency of dynamic displays (González Zuarth and Macías Garcia 2006; González-Zuarth et al. 2011). We recorded their courtship behavior towards females from a third population (San Juanico; 19°55′ N, 99°43′ W), which is genetically and geographically distant from both Zempoala and El Porvenir (Zempoala-San Juanico F_{ST}=0.45, Porvenir-San Juanico F_{ST}= 0.2; Macías Garcia et al. 2012), and whose courtship behavior is intermediate between that of Zempoala and El Porvenir (field-grown males from this population use dynamic behaviors more frequently than Zempoala males, but not as frequently as El Porvenir males; see González-Zuarth et al. 2011).We collected 30 pregnant females from the distant locations of

Zempoala (Z) and El Porvenir (P; see map in Macías Garcia et al. 2012). Each female was kept in a 20-L tank at an ambient temperature (ca. 21 °C; see below), with a 12/12-h light cycle, and fed commercial fish food flakes twice daily until 1 day after it gave birth, when she was removed, leaving her brood visually and physically isolated from any other fish until the sex of the young could be discerned. This occurred at between 60 and 125 days of age (100±16 days old), a later and more variable age than in the previous experiment, very likely due to the difference in thermal conditions during development. We did not keep track of either total brood size or sex ratio. Males that could not be paired with females of a similar age were promptly moved to community tanks.

Experimental rearing conditions Once we separated the young fish by sex, we began the treatments according to the following procedure. Depending on availability, either one or two males per brood were allocated to each treatment: sympatric and allopatric. Each pair of sibling males was placed with a pair of females (sisters whenever possible) of about their same age, from another brood of either their same (sympatric) or the alternative (allopatric) population. When only one pair of male siblings could be completed, we placed one brother with one allopatric female and another brother with one sympatric female, thus maintaining the sex ratio in all treatments but with two different density levels: one male and one female per tank, or two males and two females per tank. We followed the same procedure with both populations, thus generating four treatments: Zempoala control (Zempoala males growing up with Zempoala females; ZZ) and experimental (Zempoala males growing up with El Porvenir females; ZP), and El Porvenir control (El Porvenir males growing up with El Porvenir females; PP) and experimental (El Porvenir males growing up with Zempoala females; PZ). We had replicates with two brothers per treatment and with one brother per treatment from both Zempoala and El Porvenir (see Table 1).

Males from the same brood but in alternative treatments were housed, together with the corresponding females, in adjacent compartments created by introducing a longitudinal opaque polycarbonate partition in a 40-L tank, the side in which each treatment was placed being decided randomly. Consequently, all the fish grew up in 20-L compartments (13.5×30×50), and they only had visual and physical contact with the fish within their compartment, which was fitted with an air-powered foam filter and natural and artificial aquatic plants. The tanks were maintained inside a room with windows, therefore having access to natural light. We also had light bulbs with a 12-h light/dark cycle. Fish were fed daily with commercial fish food flakes (SeraVipan™) and kept at a water temperature closely fluctuating around 21 °C. We cleaned and made partial water changes to all tanks once every other week.

Courtship recording When the fish were between 210 and 275 days of age (Zempoala, 261±12 days old; El Porvenir, 252±18 days old) we recorded their courtship behavior towards females from a third population (San Juanico). The typical courtship pattern of males from this population is intermediate between that of Zempoala and El Porvenir. Field-grown males from this population use dynamic behaviors more frequently than Zempoala males, but not as frequently as El Porvenir males (see González-Zuarth et al. 2011). Contrasting the behavior of the males from our four treatments towards females from a third population allowed evaluating the question of whether early social interactions influence courtship patterns in subsequent encounters with novel females. The alternative procedure of testing the males with females from the focal populations would have required testing them repeatedly (to include appropriate controls) and increasing the sample size to compensate the concomitant reduction in statistical power. Courtship patterns were quantified as follows.

For 10 days before the trial, at feeding times, we observed the behavior of siblings sharing a tank in order to annotate morphological/color differences to determine which one was the dominant; this information was used during trials and incorporated in the analyses (see below). Male *G. multiradiatus* are very pugnacious, both in the field (Macías Garcia 1994) and in aquaria (Macías-Garcia and Valero 2001), where clear dominance hierarchies that

Table 1 Distribution of males per treatment in the experiment to assess the effect of social environment on courtship patterns

Origin	t	Fish at the onset of the experiment				Fish recorded				
		Replicates			Males	Males				
		n	Twins	Sing	n	Registered	Dom	Sub	Sing	Dead
Zempoala	ZZ	19	10	9	29	16	2	2	12	13
	ZP	19	10	9	29	16	4	4	8	13
El Porvenir	PP	19	15	4	34	14	4	7	3	16
	PZ	19	15	4	34	16	5	6	5	18

t treatment, *twins* replicates consisting of two siblings raised together, *Sing* singletons, *Dom* dominant, *Sub* subordinate, *Dead* died before trials began (i.e., the total number of males at the onset of the experiment equals the number of males registered plus the number of fish which died) determine priority of access to resources always emerge between males. We noted which male directed bites and chased the other away from the food; this was usually the larger and more colorful of the pair, and these behaviors were consistent from day to day (in this species, winning a fight makes it more likely that a male will win a subsequent encounter; Macías Garcia and Valero 2010). Since some males grew without brothers, our setup generated three categories of males: dominant, subordinate, and singletons.

Trials began by gently maneuvring one male into the front of its home tank and isolating this (observation) enclosure from the rest of the tank by introducing an opaque polycarbonate sheet in the tank. After a 5-min habituation period, we introduced a female from San Juanico to the observation enclosure, and an observer acquainted with Goodeid behavior but naïve with respect to our experimental procedures recorded the frequency and duration of all the courtship behaviors displayed by the male, including dynamic (frequency of overtaking, frequency and duration of fin folding, frequency and duration of flagging, and frequency and duration of figure-of-eight dances) and static courtship (frequency and duration of lateral and frontal fin displays), as well as biting and mating attempts that the male directed to the female (the female never tried to bite the male), and whether the female responded with body vibrations (see Valero et al. 2005). At the end of 10 min, the female was removed from the observation enclosure, and if there was a sibling, it was gently maneuvred into the observation enclosure while maneuvring the used male out of it. After another 5 min of habituation, the female was introduced again, and their behavior was recorded as before. The San Juanico females used were selected to be as similar in size as possible to the males that courted them (see Macías Garcia 1994). We used one female (of a total of eight available) for an entire day, when she was presented to between eight and ten males. Presentation order to females was balanced by male identity (dominant/subordinate/singleton) and by treatment (ZZ, ZP, PP, PZ). We recorded in total 32 males from Zempoala (16 ZZ and 16 ZP) and 30 from El Porvenir (14 PP and 16 PZ; see Table 1).

Statistical analyses

Experiment 1: congenital influence of courtship patterns

Heritability calculations For Acambay and Zempoala males reared in isolation, we calculated the heritability (h^2) of mean frequency of flagging displays (the one element of dynamic courtship that can be performed in the experimental conditions), as two times the interclass correlation between siblings (= the ratio of among family/total variance for each population; see Table 2), therefore regarding males within a family as full siblings (Falconer 1960). Although there is experimental evidence that broods in the Amarillo can be sired by more than one male, in most cases, the majority of the siblings are sired by only one male (Macías-Garcia and Saborío 2004). This procedure may lead to an overestimation of h^2 since (1) males within a brood shared a common maternal environment and (2) because courtship displays are only expressed by males and their inheritance may be sex-linked. On the other hand, we do not expect that our standardized procedure should generate interpopulation differences in courtship, which may then be regarded as genetically determined. Additionally, we set a general linear model to compare the differences in courtship bout duration, and the frequency of flagging displays using population as fixed factor, brood nested on population, and individuals nested on brood.

Experiment 2: effect of social environment

Before testing for differences in the use of courtship displays between treatments, we evaluated whether any of our measures of courtship were redundant. Frequency and duration were invariably correlated (Table 3); thus, we retained only the latter whenever the two were recorded. We then standardized (subtracting the mean and dividing by the sd) the remaining variables and built a measure of static (= duration of frontal+lateral fin displays), and a measure of dynamic courtship (= duration of fin folding+flagging+figure-of-eight dance+frequency of overtaking). We used repeated measures ANOVAs to evaluate the effect of the treatments on static and on dynamic courtship (Table 4). The ANOVAS included male family as the subject variable, male population of origin (fixed, two levels: Zempoala/ Porvenir) and whether the female responded to courtship with vibrations or not (fixed, two levels: 1/0; see Valero et al. 2005; González-Zuarth et al. 2011) as between-subject effects, and the origin of the female males were raised with (hereafter “social female”; fixed, two levels: Zempoala/ Porvenir) and social rank (fixed; dominant/subordinate/singleton) as within subject effects. The latter also controlled for the effects of different densities during development, as the two levels of density are included in the three rank levels (Table 1). We tested for two- and three-level interactions between these four factors. A simpler model using the latency to court and excluding female vibration and male social rank as explanatory variables was used to verify that males in the four treatments were similarly motivated to court during trials. Analyses were performed using NCSS™ v.7.1.21.

Results

Experiment 1: congenital influence of courtship patterns

Heritability of courtship patterns

Forty-nine of the 54 males from Acambay performed flagging (this is the majority of males from all families), whereas only

Table 2 ANOVA for unbalanced full-sib design. Families were represented by two to five males raised in isolated containers until adulthood. Note that the calculation of among-families *SS* differs from that normally used in full-sib designs (e.g., Lynch and Walsh 1998) to account for differences in number of sibs per family. It is likely that h^2 is overestimated because of sex-linkage and common maternal effects

Variance partition	Pop	df		*SS*		*MS*		$h^2 \simeq 2t\text{FS}$[a]	se (h^2)[b]
Among families	Acambay	N − 1	13	$SS_{\text{f}} = \sum_{\text{i}=1}^{N} n_{\text{i}}(z_{\text{i}}-z)$	9,063.2	SSf/(N − 1)	697	0.18	0.07
	Zempoala		13		21.87		1.68	0.32	0.11
Within families	Acambay	T − N	40	$SS_{\text{w}} = \sum_{\text{i}=1}^{N} \sum_{\text{j}=1}^{n} \left(z_{\text{ij}}-z_{\text{i}}\right)^2$	20,273.6	SSw/(T − N)	506.8		
	Zempoala		32		33.33		1.04		

Pop population, *SS* sum of squares, *df* degrees of freedom, *MS* mean squares, h^2 upper bond estimate of heritability, t_{FS} interclass correlation for full sibs, σ variance, *f* effect of family on phenotype, *z* phentypic value, *w* residual error, *n* number of sibs in a family, *N* number of families

[a] $t_{\text{FS}} = \frac{\sigma_{\text{f}}}{\sigma_{\text{z}}}$, where $\sigma_{\text{f}} = \frac{MS_{\text{f}}-MS_{\text{w}}}{n}, \sigma_{\text{w}} = MS_{\text{w}}, \sigma_{\text{z}} = \sigma_{\text{f}} + \sigma_{\text{w}}$, and $n = T/N$

[b] $\text{se}\left(h^2\right) \simeq 2(1-t_{\text{FS}})[1+(n-1)t_{\text{FS}}]\sqrt{2/[Nn(n-1)]}$

11 of 46 males from Zempoala did (belonging to only half of the families from Zempoala; X^2=9.33, p=0.002). Acambay males performed significantly more flagging during the trials than Zempoala males ($F_{(1,\ 98)}$=27.66, p<0.001; see supplementary online material Table S1), and the frequency of flagging had significant heritable components in both populations (flagging: Acambay h^2=0.18±0.07 [se]; Zempoala, h^2= 0.32±0.11 [se]; see Table 2).

Experiment 2: effect of early social environment on courtship patterns

Latency to initiate courtship was similar for males from the two populations (El Porvenir, 105.6±30.7 s; Zempoala, 134.2 ±29.8 s; $F_{(1,\ 26)}$=0.45, p=0.5), regardless of the population of origin of the social female ($F_{(1,\ 26)}$=1.02, p=0.49), and the interaction was not significant ($F_{(1,\ 26)}$=0.1, p=0.76), suggesting that males in all treatments had a similar motivation to court.

The amount of static courtship performed by the males was the same for all treatments and male social ranks and was unaffected by whether the female responded to courtship with body vibration (all p>0.15). As suggested before (see above), static courtship was also linked to aggression; it was positively correlated with the number of times the male directed bites to the female (r^2=0.26, $F_{(1,\ 59)}$=21.8, p<0.001; Table 5). In what follows, we will refer only to effects on the amount of dynamic courtship.

Social experience and male courtship patterns: effect of female origin

As previously reported for their wild-caught counterparts, males from El Porvenir performed more dynamic courtship than males from Zempoala ($F_{(1,\ 30)}$=4.67, p=0.04; Table 4). Origin of the social female on its own had no effect on the amount of dynamic courtship performed, but it had a strong impact on whether males courted dynamically when the

Table 3 The duration (D) with which a courtship display was performed was invariably correlated (r≥0.65 in all cases; in italics) with how often it was performed (its frequency, F), and it was not correlated with either frequency or duration of other displays, except for flagging, whose duration was correlated (but r<0.6) with the two measures of figure-of-eight dance

Courtship display		Fin folding	Flagging		Frontal fin display		Lateral fin display		Figure-of-eight dance	
		F	D	F	D	F	D	F	D	F
Fin folding	D	*0.65*	0.09	0.16	−0.11	−0.11	−0.29	−0.24	0.18	0.17
	F		0.22	0.29	−0.13	−0.13	−0.21	−0.03	0.33	0.30
Flagging	D			*0.90*	−0.11	0.00	−0.15	0.10	0.45	0.42
	F				−0.08	−0.02	−0.19	0.05	0.59	0.59
Frontal fin display	D					*0.84*	0.29	0.09	−0.07	−0.07
	F						0.29	0.17	−0.06	−0.07
Lateral fin display	D							*0.66*	−0.12	−0.14
	F								−0.01	−0.06
Figure-of-eight dance	D									*0.99*

Table 4 ANOVA table of dynamic courtship performed by males from two populations raised with either sympatric or allopatric social females. The larger significant effects (in italics) were found in the two- and three-level interactions involving male origin (the only significant main effect), suggesting that congenital population differences in courtship patterns are influenced in complex ways by the early social environment (social female phenotype and male social rank) and current interactions (vibration behavior of courted female; see text)

Term	*df*	*SS*	*MS*	*F*	*p*	Power*
A: male origin	1	32.01	32.01	4.67	*0.039*	0.55
B: vibration (1/0)	1	10.05	10.05	1.47	0.235	0.22
AB	1	10.54	10.54	1.54	0.224	0.22
C(AB): male family	30	205.47	6.85	1.68	0.195	
D: female origin	1	4.59	4.59	1.13	0.313	0.16
AD	1	10.96	10.96	2.69	0.132	0.32
BD	1	31.76	31.76	7.80	*0.019*	0.71
ABD	1	0.32	0.32	0.08	0.784	0.06
E: social rank	2	2.68	1.34	0.33	0.727	0.09
AE	2	68.38	34.19	8.40	*0.007*	0.89
BE	2	58.22	29.11	7.15	*0.012*	0.83
ABE	2	47.75	23.88	5.86	*0.021*	0.75
DE	2	21.27	10.64	2.61	0.122	0.40
ADE	2	60.12	30.06	7.38	*0.011*	0.83
BDE	2	88.12	44.06	10.82	*0.003*	0.95
S	8	40.72	4.07			
Total (adjusted)	61	492.10				
Total	62					

Column labels as in Table 2

F F ratio, *p* probability

*α=0.05

female responded with body vibrations; males raised with El Porvenir females displayed dynamic courtship for longer periods of time if the female they were courting did not vibrate than if she did, the opposite being the case for males that grew up with Zempoala females (social female × female vibration, $F_{(1,\ 10)}$=7.8, p=0.02; Table 4).

Table 5 A multiple regression analysis of frequency of bites directed to the female confirmed that static, but not dynamic courtship, can be a prelude to aggression, as the vast majority of the variance (r^2) explained by the model is due to the variation in static courtship. See text for the calculation of the explanatory variables (labels as in Tables 2 and 4)

Term	df	r^2	*SS*	*MS*	*F*	*p*
Intercept	1		1.45−19	1.45−19		
Model	2	0.30	18.41	9.21	12.755	0.00
Dynamic courtship	1	0.01	0.44	0.44	0.615	0.44
Static courtship	1	0.26	15.71	15.71	21.760	0.00
Error	59	0.70	42.59	0.72		
Total (adjusted)	61	1.00	61	1		

Social experience and male courtship patterns: effect of male social rank

Male social rank differentially influenced the amount of dynamic courtship displayed by males from the two populations. Subordinate males from El Porvenir performed dynamic courtship for longer than males in any other category, whereas subordinate males from Zempoala performed the least (male origin × social rank; $F_{(2,\ 10)}$=8.40, p=0.007; Table 4).

Additionally, there were significant three-way interactions between male population of origin, origin of social female, male social rank, and whether the courted female responded with vibration (Table 4; see also supplementary online material Fig. S1).

Discussion

The substantial and significant differences in courtship behavior of males on their first exposure to females indicate that the previously reported population differences in courtship patterns have a congenital influence. Although maternal effects cannot be discounted, it is unlikely that systematic population differences arose as a consequence of the uniform handling of females through pregnancy. Common maternal effects may have inflated our measures of heritability, which may also be devaluated because of sex linkage. A half-sib/full-sib design would be necessary to obtain an unbiased (narrow sense) estimate of heritability and to assess sex linkage. Assuming that the frequency of flagging is at least partly equivalent to the more complete measure of dynamic courtship taken in the experiment on social environment, it seems that a significant heritable component (for Acambay and Zempoala, and presumably also for El Porvenir males) that predisposes *G. multiradiatus* males to court in particular ways, can be modified by social upbringing.

Several aspects of the male courtship patterns were influenced by the behavior of the female that they courted during the trials. This agrees with previous studies showing that *G. multiradiatus* males change their courtship behavior when placed with allopatric females, seemingly because the latter responds in subtly different population-specific ways to the different male displays (González Zuarth and Macías Garcia 2006; González-Zuarth et al. 2011). This suggests that courtship behavior remains flexible throughout the lifetime of an individual, and that males use information from the female that they are courting to modify their courtship patterns. Some aspects of courtship communication in this species may remain similar across populations, as we could not discern differences in the way males from both populations responded to the behavior (vibrations) of the female (although we had little power to detect such effect; see Table 4). Yet most of the remaining interaction involving the male population of origin were significant, which suggests that some aspects of the reproductive communication in this species have diverged between populations, and that the female response to the elements of the male courtship may have different meanings in different populations.

Males from El Porvenir showed higher frequency of dynamic courtship than males from Zempoala, independently of the origin of the female they grew up with. This mirrors previous findings using field-grown fish and suggests that this difference has a congenital component. Growing up with females of different origin influenced how males reacted to the behavior of the females they court when adults. Interestingly, female vibration discouraged dynamic courtship of males that grew exposed to females from a population where dynamic displays are frequent. This may be a common pattern given the finding of a recent study in the same species where female perception (visual and/or mechanical) of approaching males was manipulated, which also found that vibration discourages males (from another population; San Lázaro) from courting. It would be interesting to test whether the reverse pattern is confined to Zempoala or whether it occurs at other places (Martínez Medina et al. 2013). Regardless of the direction of the effect, this finding adds to an increasing literature, showing that females from different taxa can influence male courtship development. For example, female brown-headed cowbirds reinforce preferred male signals using body movements and social interactions (Miller et al. 2008). A similar phenomenon could happen in our fish, as females may reinforce certain courtship behaviors in males by either allowing them to mate with them (positive reinforcement) or by punishing males when performing the wrong courtship patterns (through biting, escaping, or vibrating).

The social rank of the male influenced his courtship behavior, and it did so in opposite ways according to the origin of the male (Zempoala males perform less dynamic courtship as their social rank decreases, the opposite being true for males from El Porvenir). This may provide a mechanism of rapid population divergence, since the females in separate populations would derive different benefits from choosing males on the basis of their courtship patterns.

Subordinate males from Zempoala performed more dynamic courtship when the female that they were courting responded with body vibrations to their display, and they performed less dynamic courtship when the female did not respond with body vibrations. As subordinate males that perform displays risk attracting punishment by dominant males (Macías-Garcia and Valero 2001)—who may themselves be attracted by vibration—they are probably trained to only court when encouraged. Future studies should address how differences in density during upbringing may influence the development of male courtship patterns and his aggressiveness.

It seems that the stable courtship patterns that characterize the populations of *G. multiradiatus* (cf. Zempoala parsimonious courtship patterns, first observed in 1990) may be the consequence of processes akin to those responsible for the maintenance of bird dialects (see Slater et al. 1984; Wilczynski and Ryan 1999): males are born with a predisposition to use certain aspects of their courtship repertoire (a possible analogue to the auditory template model of passeriform birds; e.g., Marler and Tamura 1962); then, during development, they are exposed to adult males—and females—socially and sexually interacting (just as growing birds are exposed to the song of tutors; see Slater 1983), which, together with their own performance and feedback from local females, may lead to the production of the population-specific courtship "dialect." Indeed, some fish are known to be attentive to—and to learn from—adult males (e.g., the guppy; Guevara-Fiore 2012), although the sensitive period when this learning takes place may be somewhat short (<6 weeks; Barbosa et al. 2013). This latter type of learning, which is involved in the maintenance of bird dialects (e.g., McGregor 1983), was not allowed in our study. Instead, males received feedback from females, and just as in birds that female song preferences influence the development of bird dialects (see Riebel 2003), our experimental setup shows that female population of origin (and presumably their behavior), together with the social status determined by the presence or absence of a competing sibling, can influence the development of courtship patterns.

Overall, our results show that male courtship behavior in *G. multiradiatus* has a sizable heritable component, but it is also strongly influenced by different aspects of the social environment in which males develop, as well as by the immediate response that it evokes in females, thus retaining a degree of flexibility throughout life.

Acknowledgments We thank S. Herce Castañón, E. Ávila, J. Aguirre, and P. Gesundheit for considerable help in the field; SEMARNAT for the fishing permission (01290/13); M. Méndez Janovitz for recording the fish behavior; and S. Herce Castañón and A. Ojeda Laguna for filming the fish. C. Berea de la Rosa collected the data on heritability, which was part of a larger project funded with CONACyT grant 1616-N9208 to CMG. We thank two anonymous referees and the Associate Editor for very valuable comments and criticism.

Ethical standards In the design and conduct of the experiments, we followed the ethical guidelines for the use of animals in research published by ASAB/ABS. All the experiments complied with Mexican legislation.

References

Arnold C, Taborsky B (2010) Social experience in early ontogeny has lasting effects on social skills in cooperatively breeding cichlids. Anim Behav 79:621–630

Barbosa M, Ojanguren AF, Magurran AE (2013) Courtship display persists despite early social deprivation. Ethology 119:1–7

Bateson P (1983) Genes, environment and the development of behavior. In: Halliday TH, Slater PJB (eds) Animal behavior, vol 3, Genes, development and learning. Blackwell, Oxford, pp 52–81

Braaten RF, Reynolds K (1999) Auditory preference for conspecific song in isolation-reared zebra finches. Anim Behav 58:105–111

Chapman BB, Ward AJW, Krause J (2008) Schooling and learning: early social environment predicts social learning ability in the guppy *Poecilia reticulata*. Anim Behav 76:923–929

Chapman BB, Morell LJ, Krause J (2010) Unpredictability in food supply during early life influences boldness in fish. Behav Ecol 21:501–506

Clayton NS (1988) Song learning and mate choice in estrildid finches raised by two species. Anim Behav 36:1589–1600

Cook M, Mineka S, Wolkenstein B, Laitsch K (1985) Observational conditioning of snake fear in unrelated rhesus monkeys. J Abnorm Psychol 94:591–610

Dooling R, Searcy M (1980) Early perceptual selectivity in the swamp sparrow. Develop Psych 13:499–506

Eales LA (1985) Song learning in zebra finches: some effects of song model availability on what is learnt and when. Anim Behav 33: 1293–1300

Evans JP, Kelley JL, Ramnarine IW, Pilastro A (2002) Female behavior mediates male courtship under predation risk in the guppy (*Poecilia reticulata*). Behav Ecol Sociobiol 52:496–502

Falconer DS (1960) Introduction to quantitative genetics. Ronald, New York

Farr JA (1980) Social behaviour patterns as determinants of reproductive success in the guppy, *Poecilia reticulata* Peters (Pisces: Poeciliidae): an experimental study of the effects of intermale competition, female choice, and sexual selection. Behaviour 74:38–91

Freeberg TM (2000) Culture and courtship in vertebrates: a review of social learning and transmission of courtship systems and mating patterns. Behav Process 51:177–192

Galef BG Jr, Laland KN (2005) Social learning in animals: empirical studies and theoretical models. Bioscience 55:489–499

Gerald JW (1971) Sound production during courtship in six species of sunfish (Centrarchidae). Evolution 25:75–87

González Zuarth C, Macías Garcia C (2006) Phenotypic differentiation and pre-mating isolation between allopatric populations of *Girardinichthys multiradiatus*. Proc R Soc Lond B 273:301–307

González-Zuarth CA, Vallarino A, Macías Garcia C (2011) Female responsiveness underlies the evolution of geographic variation in male courtship patterns between allopatric populations of the fish *Girardinichthys multiradiatus*. Evol Ecol 25:831–843

Guevara-Fiore P (2012) Early social experience significantly affects sexual behavior in male guppies. Anim Behav 84:191–195

Hagedorn M, Heiligenberg W (1985) Court and spark: electric signals in the courtship and mating of gymnotoid fish. Anim Behav 33:254–265

Hankison SJ, Morris MR (2003) Avoiding a compromise between sexual selection and species recognition: female swordtail fish assess multiple species-specific cues. Behav Ecol 14:282–287

Hultsch H (1991) Early experience can modify singing styles: evidence from experiments with nightingales, *Luscinia megarhynchos*. Anim Behav 42:883–889

Jordan LA, Brooks RC (2010) The lifetime costs of increased male reproductive effort: courtship, copulation and the Coolidge effect. J Evol Biol 23:2403–2409

Katsnelson E, Motro U, Feldman MW, Lotem A (2008) Early experience affects producer-scrounger foraging tendencies in the house sparrow. Anim Behav 75:1465–1472

Kelley JL, Macías Garcia C (2010) Ontogenetic effects of captive breeding. In: Breed MD, Moore J (eds) Encyclopedia of animal behavior 2. Academic, San Diego, pp 589–595

Kendrick KM, Hinton MR, Atkins K, Haupt MA, Skinner JD (1998) Mothers determine male sexual preferences. Nature 395:229–230

Kihslinger RL, Klimley AP (2002) Species identity and the temporal characteristics of fish acoustic signals. J Comp Psychol 116:210–214

Long KD, Rosenqvist G (1998) Changes in male guppy courting distance in response to a fluctuating light environment. Behav Ecol Sociobiol 44:77–83

Lynch M, Walsh B (1998) Genetics and analysis of quantitative traits. Sinauer Associates, New York

Maan ME, Seehausen O, Söderberg L, Johnson L, Ripmeester EAP, Mrosso HDJ, Taylor MI, van Dooren TJM, van Alphen JJM (2004) Intraspecific sexual selection on a speciation trait, male coloration, in the Lake Victoria cichlid *Pundamilia nyererei*. Proc R Soc Lond B 271:2445–2452

Macías Garcia C (1994) Social behavior and operational sex ratios in the viviparous fish *Girardinichthys multiradiatus*. Copeia 1994:919–925

Macías Garcia C, Jiménez G, Contreras B (1994) Correlational evidence of a sexually-selected handicap. Behav Ecol Sociobiol 35:253–259

Macías-Garcia C, Saborío E (2004) Sperm competition in a viviparous fish. Environ Biol Fish 70:211–217

Macías Garcia C, Saborío E, Berea C (1998) Does male-biased predation lead to male scarcity in a viviparous fish? J Fish Biol 53:104–117

Macías Garcia C, Smith G, González Zuarth C, Graves JA, Ritchie MG (2012) Variation in sexual dimorphism and assortative mating do not predict genetic divergence in the sexually dimorphic Goodeid fish *Girardinichthys multiradiatus*. Curr Zool 58:440–452

Macías-Garcia C, Valero A (2001) Context-dependent sexual mimicry in the viviparous fish *Girardinichthys multiradiatus*. Ethol Ecol Evol 13:331–339

Macías Garcia C, Valero A (2010) Sexual conflict and sexual selection in the Goodeinae, a clade of viviparous fish with effective female mate choice. Adv Stud Behav 42:1–54

Magnhagen C (1991) Predation risk as a cost of reproduction. Trends Ecol Evol 6:183–186

Magurran AE, Seghers BH (1990) Risk sensitive courtship in the guppy (*Poecilia reticulata*). Behaviour 112:194–201

Margulis SW, Nabong M, Alaks G, Walsh A, Lacy RC (2005) Effects of early experience on subsequent parental behavior and reproductive success in oldfield mice, *Peromyscus polionotus*. Anim Behav 69: 627–634

Marler P, Peters S (1989) Species differences in auditory responsiveness in early vocal learning. In: Dooling RJ, Hulse SH (eds) The comparative psychology of audition: perceiving complex sounds. L. Erlbaum, Hillsdale, pp 243–273

Marler P, Tamura M (1962) Song 'dialects' in three populations of white-crowned sparrows. Condor 64:368–377

Martínez Medina L, Macías Garcia C, Flores Urbina A, Manjarres J, Moyaho A (2013) Female vibration discourages male courtship behaviour in the Amarillo fish (*Girardinichthys multiradiatus*). Behav Process 100:163–168

McGregor PK (1983) The response of corn buntings to playback of dialects. Z Tierpsychol 62:256–260

Miller JL, King AP, West MJ (2008) Female social networks influence male vocal development in brown-headed cowbirds, *Molothrus ater*. Anim Behav 76:931–941

Moore BR (2004) The evolution of learning. Biol Rev 79:301–335

Nelson DA, Marler P (1993) Innate recognition of song in white-crowned sparrows: a role in selective vocal learning? Anim Behav 46:806–808

Oetting S, Proeve E, Bischof H-J (1995) Sexual imprinting as a two-stage process: mechanisms of information storage and stabilization. Anim Behav 50:393–403

Payne RB, Payne LL, Woods JL (1998) Song learning in brood-parasitic indigo birds *Vidua chalybeata*: song mimicry of the host species. Anim Behav 55:1537–1553

Riebel K (2003) The ‘mute’ sex revisited: vocal production and perception learning in female songbirds. Adv Stud Behav 33:49–86

Ritchie MG, Hamill RM, Graves JA, Magurran AE, Webb SA, Macías Garcia C (2007) Sex and differentiation: population genetic divergence and sexual dimorphism in Mexican goodeid fish. J Evol Biol 20:2048–2055

Ryan MJ, Hews DK, Wagner WE Jr (1990) Sexual selection on alleles that determine body size in the swordtail *Xiphophorus nigrensis*. Behav Ecol Sociobiol 26:231–237

Shaw KA, Scotti ML, Foster SA (2007) Ancestral plasticity and the evolutionary diversification of courtship behaviour in threespine sticklebacks. Anim Behav 73:415–422

Sih A (1994) Predation risk and the evolutionary ecology of reproductive behavior. J Fish Biol 45:111–130

Slater PJB (1983) The study of communication. In: Haliday TR, Slater PJB (eds) Animal behaviour, vol 2. Blackwell Scientific Publications, Oxford, pp 9–42

Slater PJB, Clements FA, Goodfellow DG (1984) Local and regional variations in chaffinch song and the question of dialects. Behaviour 88:76–97

Stamps J (2003) Behavioral processes affecting development: Tinbergen’s fourth question comes to age. Anim Behav 66:1–13

Tinbergen N (1963) On aims and methods in ethology. Z Tierpsychol 20:410–433

ten Cate C, Vos DR (1999) Sexual imprinting and evolutionary processes in birds: a reassessment. Adv Stud Behav 28:1–31

Tramm NA, Servedio MR (2008) Evolution of mate-choice imprinting: competing strategies. Evolution 62:1991–2003

Valero A, Hudson R, Ávila Luna E, Macías Garcia C (2005) A cost worth paying: energetically expensive interactions with males protect females from intrasexual aggression. Behav Ecol Sociobiol 59:262–269

Verzijden MN, ten Cate C (2007) Early learning influences species assortative mating preferences in Lake Victoria cichlid fish. Biol Lett 2:134–136

Vos DR (1995) The role of sexual imprinting for sex recognition in zebra finches: a difference between males and females. Anim Behav 50:645–653

Vyas A, Harding C, Borg L, Bogdan D (2009) Acoustic characteristics, early experience, and endocrine status interact to modulate female zebra finches’ behavioral responses to song. Horm Behav 55:50–59

Webb SA, Graves JA, Macías-Garcia C, Magurran AE, Ó Foighil DO, Ritchie MG (2004) Molecular phylogeny of the livebearing Goodeidae (Cyprinodontiformes). Mol Phylogenet Evol 30:527–544

White DJ, King AP, West MJ (2002) Facultative development of courtship and communication in juvenile male cowbirds, *Molothrus ater*. Behav Ecol 13:487–496

Wilczynski W, Ryan MJ (1999) Geographic variation in animal communication systems. In: Foster SA, Endler JA (eds) Geographic variation in behavior: perspectives on evolutionary mechanisms. Oxford University Press, Oxford, pp 234–261

Wong BBM, Fisher HS, Rosenthal GG (2005) Species recognition by male swordtails via chemical cues. Behav Ecol 16:818–822

Zimmerer EJ, Kallman KD (1989) Genetic basis for alternative reproductive tactics in the pygmy swordtail, *Xiphophorus nigrensis*. Evolution 43:1298–1307