

## Looking for sexual selection in the female brain

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*Phil. Trans. R. Soc. B* 2012 **367**, 2348-2356

doi: 10.1098/rstb.2012.0105

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*Review*

# Looking for sexual selection in the female brain

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Female mate choice behaviour has significant evolutionary consequences, yet its mechanistic origins are not fully understood. Recent studies of female sensory systems have made great strides in identifying internal mechanisms governing female preferences. Only recently, however, have we begun to identify the dynamic genomic response associated with mate choice behaviour. Poeciliids provide a powerful comparative system to examine genomic responses governing mate choice and female preference behaviour, given the great range of mating systems: from female mate choice taxa with ornamental courting males to species lacking male ornamentation and exhibiting only male coercion. Furthermore, they exhibit laboratory-tractable preference responses without sexual contact that are decoupled from reproductive state, allowing investigators to isolate mechanisms in the brain without physiological confounds. Early investigations with poeciliid species (*Xiphophorus nigrensis* and *Gambusia affinis*) have identified putative candidate genes associated with female preference response and highlight a possible genomic pathway underlying female social interactions with males linked functionally with synaptic plasticity and learning processes. This network is positively correlated with female preference behaviour in the female mate choice species, but appears inhibited in the male coercive species. This behavioural genomics approach provides opportunity to elucidate the fundamental building blocks, and evolutionary dynamics, of sexual selection.

**Keywords:** behavioural genomics; mate choice; swordtails; synaptic plasticity; mating systems

## 1. INTRODUCTION

Female mate choice has been a subject of intense scientific study since the 1970s when Robert Trivers resurrected Darwin's idea of the role females play in sexual selection [1]. Since that time, studies have documented in abundance the tendency for females to attend to variation in male traits and variation in female response towards those traits [2–5]. As researchers tried to identify the internal processes regulating female responses, a number of mechanistic studies of female choice have examined sensory [6–10], endocrine [11,12] and neural [13,14] processes. Yet, we are only at the earliest stages of characterizing the dynamic genomic pathways associated with mate preference behaviour, including determining the coordinated expression of complex suites of genes in the brain while females are making mate choice decisions [15].

Genomic studies in *Drosophila* have elucidated patterns of sexual selection at the population level by characterizing genome-wide divergence in incipient speciation events [16], sexual antagonistic processes [17], as well as sex-specific differences in genomic expression [18]. These studies are powerful for identifying the differences that accrue as reproductive isolation proceeds, as well as identifying physiological processes associated with reproductive events and

responses to sexual conflict [17,19]. Genomic studies in other taxa have also been applied to investigate variation in behaviour at the individual level [20,21]. Applying genomic tools to individual variation at earlier steps in reproductive events, such as the mate choice decision itself, can identify key processes in the brain that initiate mate discrimination and reveal mechanisms underlying sexual selection via female mate choice.

We have begun to characterize genes in the brains of females that are active during mate choice conditions relative to other social encounters, to identify how sexual selection has shaped the vertebrate brain [15,22]. For this research, we work with a classic system in sexual selection, members of the fish family Poeciliidae, which encompasses both species with female mate choice (e.g. swordtails and guppies) as well as species where females lack choice owing to the prevalence of male coercion (e.g. mosquitofish). This diversity of mating systems coupled with the ease of studying mate preference behaviour in the laboratory and field makes this a powerful group to compare genomic and behavioural expression of mate choice in controlled laboratory settings, to isolate processes associated with female mate preference prior to copulation (figure 1).

In our early investigation into the genomic pathways of female mate choice, we have (i) identified brain-expressed candidate genes associated with mate choice conditions in a classic female mate choice species, *Xiphophorus nigrensis* [15], (ii) documented

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One contribution of 11 to a Theme Issue 'Sexual selection, social conflict and the female perspective'.

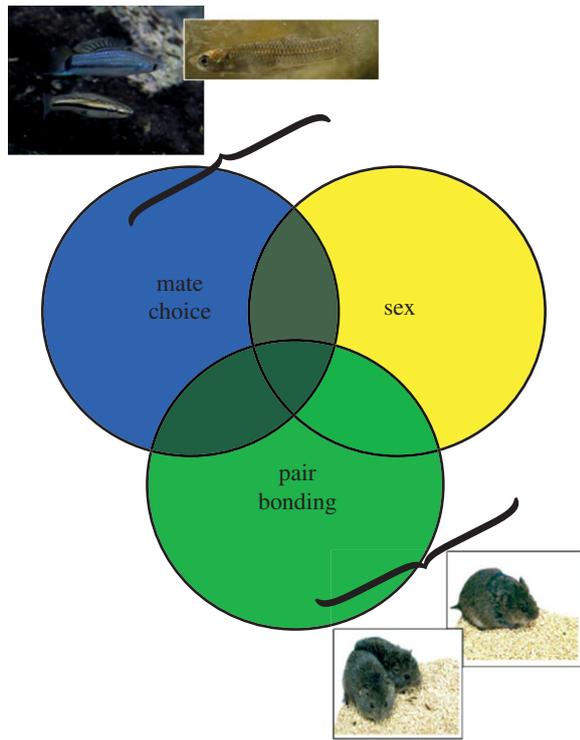


Figure 1. Different behavioural components of mating systems that influence individual reproductive success. Comparative approaches allow investigators to identify proximate mechanisms underlying differences in behaviour between species that exhibit different combinations of behaviours, e.g. researchers identified variation in vasopressin 1a receptor (V1AR) expression patterns as a critical determinant predicting differences in pair-bonding or affiliative behaviour between two congeneric vole species (the socially affiliative prairie vole, *Microtus ochrogaster*, and the solitary meadow vole, *Microtus pennsylvanicus*, photos from Lim *et al.* [23]). Candidate preference gene profile comparison in female brains between poeciliid species that display female mate choice/male courtship and species with minimal female choice/male coercion reveals contrasting expression and behavioural association patterns [22]; photos courtesy of Erich Schlegel. Note: other behavioural components that compromise mating systems that are not depicted in this Venn diagram representation but also have strong influences on sexual selection include male–male interactions and parental investment, see Alonzo [24].

correlative patterns between whole brain expression of some of these candidate genes and *X. nigrensis* female preference response towards males [15], and (iii) observed differential patterns of the relationship between gene expression and female association behaviour with males in another poeciliid species with a contrasting mating system (mosquitofish, *Gambusia affinis*, a male coercion species) [22]. The specific candidate preference genes we have investigated are functionally linked to synaptic plasticity and hence imply that the dynamic genomic pathway for female preference behaviour may be linked to learning pathways and information gathering behaviour that are inhibited in species with male coercion tactics. Current research is exploring how this pathway relates to other genetic pathways of social and affiliative behaviour (e.g. partner preference), as well as how these pathways differ by social context (varying levels of mate

choice complexity and differences between same sex versus opposite sex exposure).

## 2. THE SYSTEM

Swordtails (*Xiphophorus* sp.) and mosquitofish (*Gambusia* sp.) are members of a well-studied family of live-bearing freshwater fish, Poeciliidae. The family was first recognized for its power as an experimental system for studying the behaviour of female mate choice and the evolution of male secondary sexual traits with classic field and laboratory studies of the guppy, *Poecilia reticulata* [25–27]. Concurrent research in swordtails demonstrated that species in this family provided an excellent system for manipulative studies examining female mate choice processes such as sensory exploitation, pre-existing biases [28–30], mate choice copying [31] and testing evolutionary stable strategies across alternative male mating morphs [32,33].

As a system for studying genomic responses in the brain associated with mate preference, poeciliids have some key experimental and physiological advantages. Firstly, female preference behaviour is often measured in a choice environment where males and females are physically prevented from interacting, and quantified using behavioural assays validated as a proxy for reproductive intent (e.g. association time [34]). Preventing contact between the sexes isolates the mechanistic responses in the brain from the confound of sexual contact and the physiological responses associated with it (e.g. *Drosophila*, where sex elicits an immune response [17,19]). Secondly, to identify genomic processes of preference during a mate choice encounter, we need to isolate preference mechanisms from the endocrine response associated with physiological receptivity. The swordtails provide a promising system to tease these two processes apart, as research with *X. nigrensis* shows that preference behaviour is not influenced by reproductive state [35]. The likely reason for the ability to uncouple hormonal state and mating behaviour is the sperm storage capabilities of poeciliids [36], which allow females to engage in mating regardless of reproductive state. Thus, genomic preference responses measured in the brains of poeciliid fish where preference can be decoupled from reproductive state should provide a more stable system to explore preference genomics than taxa where reproductive state dictates preference response [12]. This is borne out in behavioural examinations of female preference, in which individuals exhibit highly repeatable preference scores [37] and do not significantly change their preference behaviour over the course of their reproductive cycle [35].

Perhaps the most potent poeciliid feature for investigations of sexual selection and the female brain arises from their dramatic interspecific variation in mate choice behaviour. While many poeciliid fishes are gold standards for studying female mate choice processes [29,38–40], in nearly half of all poeciliids sexual selection is mediated by male coercion rather than female mate choice [41–43]. In species with female choice, females choose to associate with large, courting males and can readily exhibit preference and receptivity displays [28]. Meanwhile in species dominated by male coercion, females tend to avoid copulation with males

Table 1. Mate choice associated genes as identified via our microarray experiment with *X. nigrensis*. These genes were differentially expressed in female brains when exposed to a large versus small male (LS) pairing relative to all other social conditions (two females (FF), two small males (SS), two empty compartments (AA); from the electronic supplementary material, table S1 in Cummings *et al.* [15]). An additional gene, *Ras-related protein O-RAL* (CN47180), showed significant differential expression in LS and FF conditions relative to other social exposures. The ESTs representing the cDNAs on the microarray are available through NCBI GenBank (accession numbers CN468542–CN472211; dbEST\_Id 22642169–22645838) and contig information from TIGR gene indices (<http://www.tigr.org/tdb/tgi/>).

unique ID	annotation
TC194	<i>neuroserpin precursor (axonin-2)</i>
CN469026	<i>neuroligin-3</i>
CN470870	<i>importin <math>\beta_1</math> subunit</i>
TC383	$\beta_1$ adrenergic receptor ( $\beta_T$ )
CN471713	<i>apyrase</i>
CN471295	<i><math>\beta</math>-soluble NSF attachment protein</i>
CN471287	<i>nicolin 1</i>
CN470026	<i>integrin <math>\beta_5</math> subunit precursor protein</i>
CN468829	<i>seven-pass transmembrane receptor precursor</i>
TC104	<i>Pex5p protein</i>
TC119	<i>stathmin 2 (SCG10 protein) (superior cervical ganglion-10 protein)</i>
CN471139	<i>long-chain-fatty-acid-CoA ligase 4</i>
TC143	<i>glutamine synthetase</i>
TC162	<i>gi 1127551 gb AAA83744.1  (U18939) orf2</i>
TC350	<i>similar to complement C8 gamma chain precursor</i>
CN469010	<i>ENSMUSP00000005277</i>
CN469518	<i>C-14 sterol reductase</i>
CN469990	<i>splicing factor 3b, subunit 1, 155kD</i>
hh_Ab_Brain2000_000001497	<i>NMDA receptor subunit</i>

and show little receptive behaviour [44]. An example of the latter is *Gambusia holbrooki*, or the eastern mosquitofish, where males do not court but gain mating success through forced copulations, and female avoidance of males is strong [45,46].

We started our exploration of the genes underlying mate choice by using a behavioural genomics approach with a northern swordtail, *X. nigrensis*. We examined the genomic responses in the brains of female swordtails to various social interactions, including social exposure to males of various attractivity and male mating strategy. The El Abra pygmy swordtail, *X. nigrensis*, is a species that has genotypically derived male size classes [47] that exhibit a courtship and an ornamentation strategy (large males), a force-copulation unornamented strategy (small males) or a mixed strategy (intermediate-sized males have ornamentation but can exhibit either courtship or force-copulation behaviour). Females in this species strongly prefer large, courting, ornamented males over small males that do not court but use force copulation for mating success [37,48]. The complexity of male phenotypes in this species allows us to expose females to a gradient of mate choice conditions: e.g. salient (or simple choice of a large, courting male paired with a small, non-courting male), minimal (two small males that lack ornaments and rely on coercive mating tactics), or complex mate choice (two large, ornamented males).

### 3. THE SEARCH FOR CANDIDATE GENES FOR MATE PREFERENCE

The main candidate genes associated with social affiliation in vertebrates are the neuropeptides arginine vasopressin and oxytocin, each playing sex-specific roles

in regulating partner preference behaviour in some rodent species following copulation [23,49,50]. Pair-bonding can play a large role in the reproductive success of each partner [24]; however, it is not the initial decision involved in the reproductive process (figure 1). Mate discrimination or mate preference is the first critical step in determining differential reproductive success for many species (i.e. sexual selection via female mate choice).

Without candidate genes for the initial discriminating step of a reproductive interaction (mate choice), we began our search for mate choice candidate genes with the use of a cichlid-based microarray that showed high rates of heterologous hybridization for a wide range of fish species including *Xiphophorus* sp. [51]. To isolate genes that were uniquely expressed in mate choice social encounters, we socially isolated 28 reproductively mature female *X. nigrensis* for two weeks. We then exposed each female to a 30 min trial in one of four possible dichotomous choice environments immediately followed by whole brain removal for RNA extraction [15]. Females were randomly exposed in a non-contact experimental chamber to (i) simple mate choice (a large versus small male), (ii) female control (two size matched females), (iii) minimal mate choice control (two small males, the coercive male phenotype), or (iv) asocial control (empty stimulus compartments).

Microarray analysis of whole brain gene expression from these four conditions identified 77 genes (19 of which were annotated, table 1) that were uniquely expressed in the 'simple mate choice' condition when females were exposed to a large and small male pairing [15]. To examine the relationship between these genes and individual variation in behaviour, we cloned five of these candidate genes in *X. nigrensis*, and repeated the experiment with 30 more *X. nigrensis* females using

Table 2. Context-specific relationships between gene expression and behaviour. Pearson correlation coefficients,  $r$ , between behaviour and whole brain gene expression for female *X. nigrens* exposed to males (LS + SS,  $n = 12$ ), females (FF,  $n = 5$ ) and asocial (AA,  $n = 8$ ) conditions from Cummings *et al.* [15]. Note *egr-1* was not present on the initial microarray, however we included it in our qPCR experiment. Significant correlations ( $p_{1\text{-tail}}$  values  $< 0.05$ ) are shown in bold.

	locomotor activity (transits through tank)	preference behaviour
<i>neuroserpin</i>		
male exposure, (SS only)	-0.22	<b>0.76<sup>a,b</sup> (0.99<sup>a</sup>)</b>
FF	-0.07	0.32
AA	-0.40	0.06
<i>neurologin-3</i>		
male exposed (SS only)	-0.18	<b>0.77<sup>a,b</sup> (0.99<sup>a</sup>)</b>
FF	0.23	0.48
AA	-0.27	-0.11
<i>importin</i>		
male exposed (SS only)	-0.16	<b>0.79<sup>a,b</sup> (0.99)</b>
FF	0.13	0.43
AA	-0.26	0.03
<i>apyrase</i>		
male exposed (SS only)	-0.22	<b>0.72 (0.94)</b>
FF	0.02	0.36
AA	-0.52	0.16
$\beta_1$ adrenergic receptor <sup>c</sup>		
male exposed (SS only)	-0.06	0.44 (0.80)
FF	-0.05	-0.17
AA	-0.53	0.52
<i>egr-1</i>		
male exposed (SS only)	-0.12	<b>0.75<sup>a,b</sup> (0.93)</b>
FF	-0.03	0.34
AA	-0.20	-0.30

<sup>a</sup>Significance remains after Bonferroni correction (where  $p_B = 0.05/12 = 0.004$ ).

<sup>b</sup>Significance remains after removing the female with the highest gene expression value.

<sup>c</sup>Significant relationship between gene expression and receptivity (glide displays) during LS trials ( $\beta_1$  expression versus log-transformed glides,  $r = -0.79$ ;  $t = 3.20$ ,  $p = 0.009$ ).

quantitative real-time polymerase chain reaction (qPCR) analyses. These subsequent experiments revealed significant correlations between whole brain expression of four of the five mate choice associated genes and female mate preference behaviour only in male-exposed environments (table 2). These correlations were specific to female mate preference and not other behaviours (e.g. receptivity displays or locomotor activity) and were observed even with females exhibiting preference for one of the small males in the two small male 'control' (figure 2). This latter result provides strong evidence that the association between some of these candidate genes and preference is context- (and not stimuli- or size-) specific [15].

#### 4. COMPARATIVE STUDIES

To provide corroborative support that this genomic response observed in male-exposed *X. nigrens* females

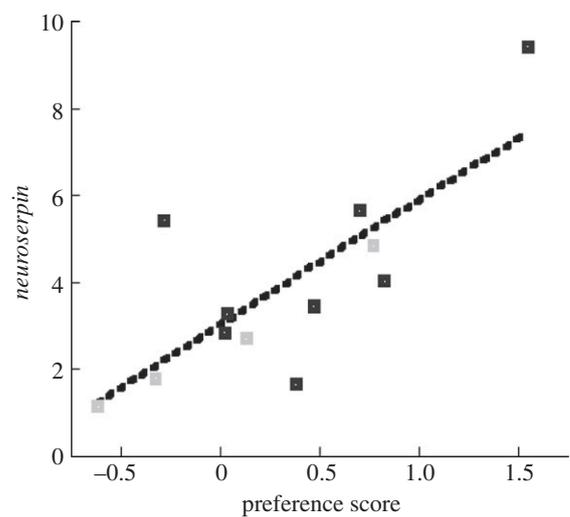


Figure 2. *Xiphophorus nigrens* female preference behaviour and whole brain expression of *neuroserpin* precursor in male-exposed conditions [15]. Females exposed to the LS condition (large versus small male) are represented by dark grey squares; SS (two small males) are light grey squares. Preference scores are defined as (proportion of association time with individual 1 +  $\log(1 + \text{glide displays towards individual 1/transits through centre of tank})$ ), where association time with individual 1 is greater than that with individual 2. Dashed line indicates significant correlation at  $p < 0.05$ . Whole brain expression of *neuroserpin* precursor ( $\times 10^9$ ) was normalized by  $E^{\text{avg CT}}/\text{cDNA RiboGreen}$ .

was shaped by sexual selection and linked to female mate choice discrimination, we conducted a comparative analysis with another poeciliid species, *G. affinis*, in which females show limited or no mate preferences for conspecific males [22]. *G. affinis* males lack courtship or ornamentation and rely solely on coercive tactics for mating success. To this end, we repeated the behavioural paradigm with female *G. affinis* exposed to large and small male *G. affinis* and examined whole brain gene expression patterns of three mate choice-associated genes (*neuroserpin*, *neurologin-3* and *N-methyl-D-aspartate (NMDA)* receptor). If the genomic pattern we found in *X. nigrens* was mirrored in a system without strong female preference responses (characterized by male coercion rather than female choice), then the genomic pathway we characterized in *X. nigrens* may have captured brain responses associated with male exposure independent of mating systems rather than a pathway of preference *per se*. Hence, our *a priori* predictions were to find: (i) behavioural differences between the species reflecting courtship versus coercive systems—specifically, we expected to observe a tendency to associate with males in the courting species and male avoidance in the coercive species; and (ii) species-level differences in 'preference candidate gene' responses and their relationship to female behaviour when females were exposed to males—specifically, we expected to find the positive relationship between gene expression and female preference behaviour in male-exposed *X. nigrens* females to be absent or dramatically muted in *G. affinis*.

Our behavioural comparisons between the two species met our predictions: *G. affinis* exhibited signs of male avoidance (e.g. spent less time associating with

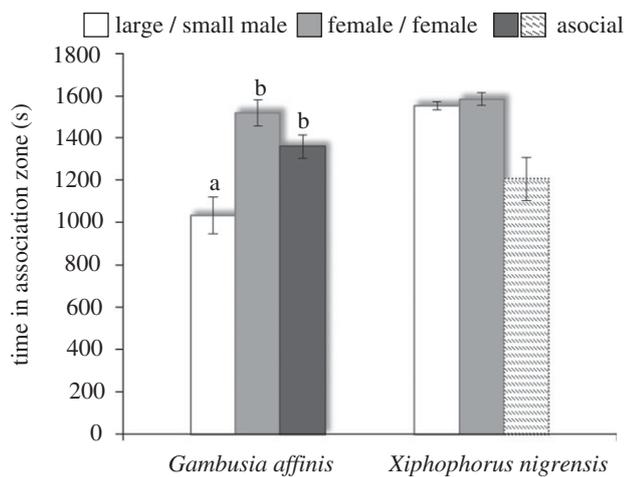


Figure 3. Comparison of the total time female *G. affinis* and *X. nigrensis* spent in the association zones in male-exposed (LS), female-exposed (FF) or empty chamber-exposed (AA) environments from Lynch *et al.* [22]. Note the asocial-exposure group represented here for swordtails is data reported by Cummings *et al.* [15]. Different letters above the bars indicate statistically significant differences.

males than females), whereas *X. nigrensis* were just as likely to spend time associating with males as they were females (figure 3). In our gene expression comparisons, *G. affinis* exhibited a pattern that was statistically distinct from *X. nigrensis* in the relationship between candidate gene expression (*neuroserpin* and *neuroligin-3*) and female preference behaviour (figure 4), supporting the hypothesis that our candidate preference-associated genes are related to mate preference rather than male exposure. However, rather than an absence of pattern in the male coercive species, we observed a negative relationship between whole brain expression of these candidate genes and female association bias when compared with the mate choice taxon. *X. nigrensis* females that displayed high levels of preference (association bias) exhibited higher whole brain expression levels of *neuroserpin* and *neuroligin-3* than females performing more muted behavioural preference displays (figure 4). In contrast, *G. affinis* females with a high association bias for males exhibited lower expression levels of these genes than females with minimal or no association bias. Furthermore, candidate preference-associated gene expression was context-specific, as neither species exhibited significant relationships between whole brain *neuroserpin* or *neuroligin-3* expression and association bias in the female control exposure condition [22]. Interestingly, however, *G. affinis* females did show a positive relationship between *neuroserpin* expression and total activity (swims through the experimental tank) in the asocial environment, whereas *X. nigrensis* did not.

The difference in the gene by behaviour patterns exhibited between the species may be a product of species-specific motivation differences in interacting with large males. While *G. affinis* females exhibit greater avoidance of males in general than *X. nigrensis* (figure 3), they also show a significant association bias towards large males, although it is weaker than female *X. nigrensis* [22]. The difficulty is determining whether

these behavioural biases reflect the same motivation for each species. Large males in the *X. nigrensis* species are ornamented and perform courtship displays, and females prefer to mate with this phenotype over the unornamented, non-courting small class males [48]. Importantly, association time has been validated as a predictor of mating probability in a congeneric species, *X. helleri*, with large, ornamented, courting males [34]. Meanwhile, in *G. affinis*, both large and small males are unornamented (see figure 1 for a typical *G. affinis* male) and use forced copulation as a reproductive tactic. Evidence suggests that large male *G. affinis*, more than small males, may provide some protection to the female from further harassment via mate guarding [52]. Hence, it is not clear whether the behavioural bias towards large males in *G. affinis* reflects a weaker, more muted preference for large males as sexual partners as demonstrated in *X. nigrensis*, or if this is a social preference mediated by harassment avoidance. Nonetheless, the dramatically different gene expression patterns in *G. affinis* as they exhibit large male association biases suggests that the response towards males is distinctly different from that exhibited by *X. nigrensis*.

## 5. A PUTATIVE GENOMIC PATHWAY FOR MATE CHOICE

The contrasting gene by behaviour pattern suggests that the putative genomic pathway leading to male discrimination in the female mate choice taxon (*X. nigrensis*) is different or inhibited in the taxon with high male coercion (*G. affinis*). Our initial studies point towards a hypothesis suggesting that taxa engaging in female mate choice express genomic pathways mediating an information-gathering process in the brain that is inhibited in taxa with male coercion systems [15,22]. This hypothesis stems from both the functional and behavioural implications of some of the candidate preference genes and is consistent with observations of learned mate preference behaviour across a number of animal species.

At the functional level, many of the potential candidate preference genes identified in our microarray experiment (table 1), including *neuroserpin* and *neuroligin-3*, are linked to synaptic plasticity and synaptogenesis, which are the neural underpinnings for learning and memory events. For instance, *neuroserpin* is an extracellular serine protease inhibitor, which has been implicated in synaptic-remodelling [53], while *neuroligins* are post-synaptic proteins important for synaptic maturation [54,55]. Manipulation studies using transgenic mice have demonstrated that changes in expression of *neuroserpin* can lead to changes in exploratory behaviour and neophobia [56]; but the molecular mechanism for how this is achieved is still unknown. However, a role in influencing behaviour via cognitive function has been suggested given that *neuroserpin* is highly expressed in brain regions associated with learning and memory [53] and regulates synaptic morphology in these areas [57].

The functional role that many of these synaptic plasticity genes may play in a mate choice context could relate to modulating, refining or reinforcement of synaptic connections as females assess males.

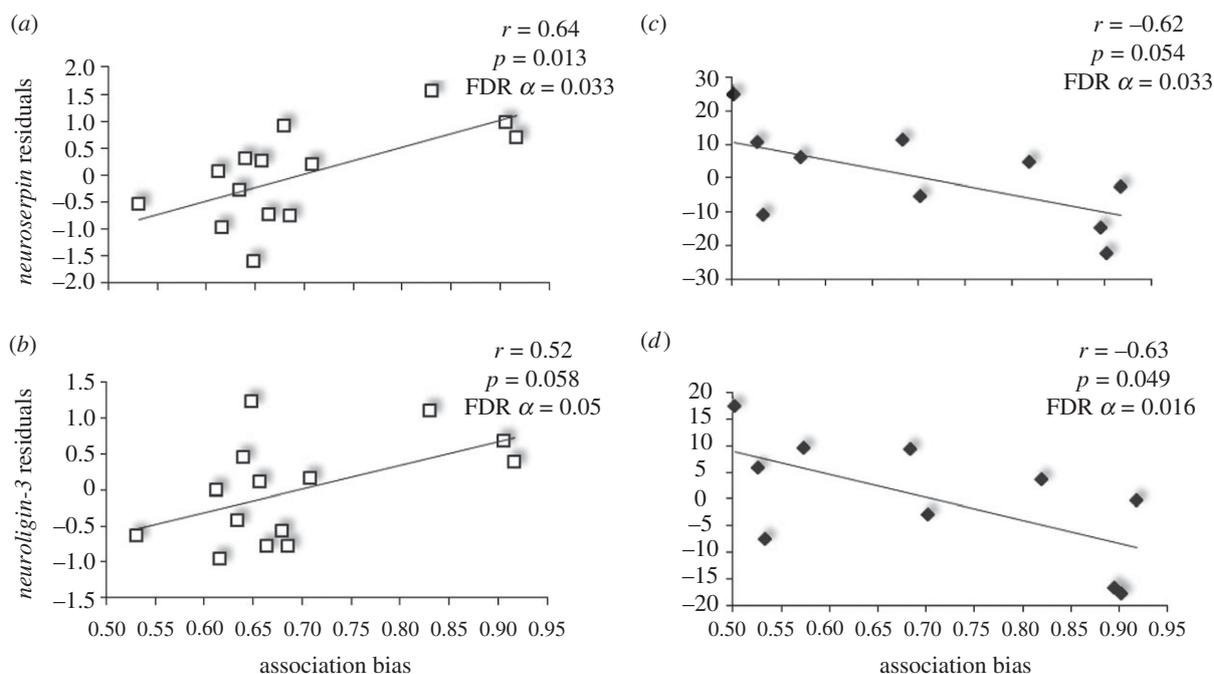


Figure 4. The relationship between preference behaviour (association bias) and gene expression for female (a,b) *X. nigrens* and (c,d) *G. affinis* exposed to large versus small males. Estimated whole brain expression of (a,c) *neuroserpin precursor* and (b,d) *neurologigin-3* was evaluated as the residuals from a linear regression of mean quantity of three qPCR replicates and the initial cDNA used in the assay (as measured by RiboGreen) [22]. Planned post hoc comparisons using an analysis of covariance (ANCOVA) revealed a significant interaction between the species in the *neuroserpin*-behaviour relationship ( $t = 2.43$ ;  $p = 0.024$ ) and *neurologigin-3*-behaviour relationship ( $t = 2.46$ ;  $p = 0.023$ ).

Importantly, many of these genes are highly conserved across the animal kingdom [53], from vertebrates to invertebrates [58], and hence the mate choice behavioural genomic response we are finding in the swordtails may be a conserved pathway across a number of taxa exhibiting female mate choice. Female swordtails and other taxa with ornamented, courting males may integrate learning and memory components into preference pathways as they assess the combination of phenotypic and behavioural features of a courting male's display. The implication for sexual selection as a whole is that it suggests female preference functions may exhibit an environmental (learned) component. In other words, male courtship does not stimulate a fixed preference pathway, but rather each experience in which females assess (and potentially choose) mates results in modulation of synaptic connections in important brain areas related to behaviour. Learned or experience-dependent preference functions would result in great variation in female preference within a population that would mirror the range of variation in female experience with males. Variation in female preference provides a mechanism for maintaining diversity of male signals within populations and is also a critical component to models of incipient reproductive isolation between populations.

Behavioural evidence for experience-dependent mate choice is found across a number of taxa from birds [59–61], to mammals [62], to fish including several poeciliids [63–65]. Many animals rely on learning their mate choice cues via different processes, such as sexual imprinting at early developmental stages, as well as adult-based discrimination learning [61,66,67].

One form of discrimination learning that has been demonstrated to influence sexual imprinting is 'peak shift'—a phenomenon by which preferences arise in a direction beyond the properties of the rewarding stimulus and away from the negative stimulus [60]. Peak shift discrimination learning relies on the comparison of two phenotypes of differential rewards (e.g. male versus female, conspecific versus heterospecific), and as such has been proposed to be a key feature in avian sexual selection given the preponderance of biparental care and reinforcement processes [67,68].

Learning to discriminate between favourable and unfavourable stimuli may also be a feature of species in which females experience alternative male phenotypes. In these species, females must learn to discriminate between males that sneak and males that provide a potential reward (e.g. territorial feeding opportunities; protection from other males). In contrast, species with only force-copulating males, such as *G. affinis*, may not have the opportunity for discrimination learning as they lack the combination of both a rewarding and a non-rewarding stimulus. Learning and memory processes, however, would seem important for females in coercive mating systems as well. If aversion learning pathways differ from those of reward or appetitive learning processes, it would be interesting to examine the molecular substrates of aversive learning pathways in the female *G. affinis* brain during male-exposure behaviour trials.

While some of these preference candidate genes are linked to synaptic plasticity at the cellular level, they are also linked to exploration and social interactions at the behavioural level. *Neuroserpin* knock-out mice, for

instance, show reduced exploratory behaviour and increased neophobia in new environments [56]. It is reasonable to hypothesize that mate choice behaviour invokes the same pathways required for exploration and requires a reduction in neophobia. If neuroserpin is part of a neophobia-modulating pathway, this would be consistent with our behaviour and gene expression interactions across our two poeciliid species with contrasting mating systems. A reduction in neophobia as mediated by neuroserpin would modulate the relationship between *neuroserpin* and preference behaviour in the mate choice species *X. nigrensis* and favour an inhibition of this pathway in male coercive species where greater association with males (even for mate guarding purposes) may involve harm. Consistent with this hypothesis, *G. affinis* females showed a positive relationship between movement in the experimental tank (total transits) and whole brain *neuroserpin* expression in the asocial condition when females were without any stimuli [22]. These data are consistent with the hypothesis that exploratory processes are inhibited in the presence of coercive males in this species.

While sexual selection is a possible mechanism for the dynamic differential genetic expression between females of these two species when interacting with males, it is important to note that natural selection processes are just as likely. Species-specific ecological features may promote differential genomic responses towards conspecific males, such as foraging ecology or predation pressures that vary by social or asocial conditions. Furthermore, natural selection may promote species-specific differences in contexts that elicit learning/exploratory pathways in the brain. In other words, synaptic plasticity networks are likely to evolve for basic decision-making processes that species employing female mate choice co-opt for a specific kind of decision.

## 6. CURRENT AND FUTURE STUDIES

We have shown that female *X. nigrensis* brains exhibit a dynamic genomic response in mate choice conditions involving suites of genes differentially expressed in a short period of time (30 min). Specific genes showed significant correlated patterns of expression with preference behaviour across females [15], yet differed significantly in species with a coercive mating system [22]. While these results suggest that we have captured parts of the genomic response coordinating preference, they do not necessarily show that these specific genes are the fundamental cause of this behaviour or that they represent conserved behavioural genomic differences between species with contrasting mate preference systems. We are currently conducting further work to characterize this pathway in terms of neural circuitry [69], as well as how these genomic pathways are distinct from pathways associated with general social affiliation [70]. These studies have demonstrated that circulating oestradiol levels do not correlate with changes in female preference behaviour nor the expression levels of various preference candidate genes at the whole brain level [70] or in specific brain regions [69]. These results provide further

demonstration of the power of the swordtail system to explore the behavioural genomics of mate preference dissociated from reproductive state and hormonal effects.

An important next step in our laboratory is to broaden our phylogenetic exploration of the pathways for mate preference across the Poeciliidae family. We are currently taking advantage of next-generation sequencing techniques (RNA-Seq) to characterize and to quantify conserved and divergent patterns of transcriptomes across four species with divergent mating systems. This will be the first step to expand the comparative approach that will lay the groundwork for more comprehensive sampling across poeciliid and other vertebrate groups that show diversity in mating systems. In addition, we need to address the causality versus correlative role of these genes and determine whether specific candidate genes for preference play direct roles in regulating preference behaviour. As a start, we have conducted pharmacological experiments that block specific components of the synaptic plasticity pathway (e.g. MK-801 and an NMDA receptor antagonist) to give us functional insight into the some of the neural pathways underlying preference behaviour. Our long-term goal is to use gene knockdown and over-expression techniques to manipulate expression levels of candidate genes *in vivo* and determine their functional interactions within networks underlying mate choice behaviour.

The author would like to acknowledge the Columbia University Seminar in Population Biology which provided the inspiration for this article. Furthermore, I would like to thank M. Ramsey, K. Lynch and two anonymous reviewers for helpful suggestions on earlier versions of this manuscript, the Mexican government for continued support for animal collection permits, and UT's Brackenridge Field Laboratories for husbandry and infrastructure necessary for large-scale behavioural research with poeciliids.

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