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Cite this article: Barbasch TA, Behrens C, McLain M, Arredondo E, Bell AM. 2023 A distinct neurogenomic response to a trade-off between social challenge and opportunity in male sticklebacks (*Gasterosteus aculeatus*). *Biol. Lett.* **19**: 20230253.
https://doi.org/10.1098/rsbl.2023.0253

Received: 7 June 2023
Accepted: 20 October 2023

Subject Category:
Animal behaviour

Subject Areas:
behaviour, neuroscience

Keywords:
brain transcriptomics, decision-making, trade-offs, aggression, courtship, threespined sticklebacks

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Electronic supplementary material is available online at https://doi.org/10.6084/m9.figshare.c.6910734.

THE ROYAL SOCIETY
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A distinct neurogenomic response to a trade-off between social challenge and opportunity in male sticklebacks (*Gasterosteus aculeatus*)

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Animals frequently make adaptive decisions about what to prioritize when faced with multiple, competing demands simultaneously. However, the proximate mechanisms of decision-making in the face of competing demands are not well understood. We explored this question using brain transcriptomics in a classic model system: threespined sticklebacks, where males face conflict between courtship and territorial defence. We characterized the behaviour and brain gene expression profiles of males confronted by a trade-off between courtship and territorial defence by comparing them to males not confronted by this trade-off. When faced with the trade-off, males behaviourally prioritized defence over courtship, and this decision was reflected in their brain gene expression profiles. A distinct set of genes and biological processes was recruited in the brain when males faced a trade-off and these responses were largely non-overlapping across two brain regions. Combined, these results raise new questions about the interplay between the neural and molecular mechanisms involved in decision-making.

1. Introduction

Animals are frequently faced with competing demands on their time and energy. Decisions made by breeding individuals over whether to court mates or defend their territory against intruders reflect a fundamental and widespread trade-off between opportunities (e.g. mate choice, courtship, parental care) and challenges (e.g. mate competition, territorial defence) [1]. For example, in several fishes, males either prioritize one stimulus or divide their time between them when faced with a courtship opportunity and a territorial intruder simultaneously (e.g. [2–5]). While the outcome of decisions over courtship and territorial defence have been well-studied, how the brain responds to multiple, competing demands simultaneously remains an open question [1].

One-way mutually incompatible behaviours are managed is through differential activation of shared molecular mechanisms. For example, differential activation of populations of neurons can act as a ‘switch’ between courtship and aggression in mice (e.g. [6]) and fruit flies (e.g. [7,8]), and in sticklebacks, a set of shared genes was expressed in opposite directions in the brain in response to a territorial challenge versus a courtship opportunity [9]. Another possibility is that a distinct set of genes is recruited when an animal faces competing demands, reflecting the unique challenges associated with responding to competing stimuli [10]. Only by examining the behavioural and genomic responses of individuals faced with multiple demands *simultaneously* can we disentangle these hypotheses [11].

We used threespined sticklebacks (*Gasterosteus aculeatus*) to investigate whether distinct mechanisms are involved in managing competing demands. The conflict faced by breeding male sticklebacks between aggression and courtship is a now-classic example of the interaction between sex and aggression ‘drives’

[4,9,10,12,13]. Adult male sticklebacks defend territories against intruders, and at the same time attempt to secure mates by courting females using conspicuous 'zigzag' displays [13]. Here, we compared behavioural responses of male sticklebacks presented with a courtship opportunity and a territorial challenge simultaneously to when they were presented with a courtship opportunity or territorial challenge on its own. We predicted that males would compromise courtship and/or territorial defence behaviour when faced with this trade-off. Next, we compared gene expression profiles in two gross brain regions to identify shared and distinct mechanisms involved in managing trade-offs between courtship and territorial defence, and gain insight into whether the response differs across brain regions. Characterizing transcriptomic mechanisms recruited in the brain can identify candidate genes involved in decision-making and is a key first step to identifying their specific role in decision-making, how they interact with mechanisms at different spatial and temporal scales, and how they vary among individuals, populations, and species.

2. Methods

(a) Behavioural trials

Experiments were conducted during the breeding season from June to August, 2020 using lab-reared individuals from a population of wild-caught threespined sticklebacks (*Gasterosteus aculeatus*) ([14,15]; see electronic supplementary material, Supplement 1A for details). Visual marks divided tanks (53 L × 33 W × 24 H cm) into three sections. Males built nests using sand and algae provided in the middle of the tank, while the outer two sections were used for stimuli. Behavioural trials consisted of four treatments: a courtship opportunity, a territorial challenge, simultaneous courtship opportunity and territorial challenge (hereafter: the 'trade-off' treatment), and a control. The focal male was acclimated to the presence of the observer for 5 min, then two flasks were introduced to either side of the tank for a 5-min trial. In the courtship opportunity treatment, a gravid female was confined to one of the flasks, in the territorial challenge treatment, a rival male displaying breeding coloration was confined to one of the flasks, and in the trade-off treatment, a gravid female to one and a rival male to the other flask. The control consisted of two empty flasks. This allowed us to control for the behavioural and transcriptomic responses to disturbance in response to presentation of the flasks; future studies should examine the extent to which the patterns observed here reflect the divided attention between two social stimuli versus the trade-off between courtship and aggression in particular. Rival and focal males were size-matched as much as possible, with an average ratio of intruder to focal standard length of 0.97 mm (+/− 0.02 s.d.). Gravid females were assigned randomly to focal males. The flasks allowed visual, but not olfactory, cues of the stimulus fish. Each male ($n = 17$) was exposed to all four treatments in random order, followed by all four treatments in random order a second time, for a total of eight trials per male. Each male was exposed to one treatment per day between the hours of 09.55 and 14.25. For each trial, the side of the tank with the flask containing a stimulus was randomized (excepting the control, which consisted of empty flasks). During each trial, the number of zigzags and number of bites performed by the male were scored using JWatcher [16].

(b) RNA extraction and sequencing

Sixty minutes after their final trial ($n = 4$ males after a courtship opportunity, $n = 5$ males after a territorial intrusion, $n = 4$ males after the trade-off treatment, and $n = 4$ males after the control treatment), the diencephalon and telencephalon (plus olfactory bulbs) were separated with a micro razor at the dorsal post-optic commissure by cutting vertically, following previous studies [9,17], preserved in RNAlater, and deep frozen at −80°C until extraction. RNA was extracted using Invitrogen PureLink RNA extraction kits (Invitrogen Corporation, Carlsbad, CA) and quality checked using Agilent Bioanalyzer chips. Sequencing was performed by the Functional Genomics Unit of the W.M. Keck Center (University of Illinois Urbana Champaign). Libraries were prepped using TruSeq Stranded mRNA sample prep kits and samples were sequenced to a depth of >30 M reads on a Novaseq 6000 S4 flow cell (Illumina). Reads were aligned to the stickleback reference genome (Ensembl release 95; [18–20]) using STAR [21] and read counts generated using HTSeq [22].

(c) Statistical analyses

Analyses were conducted using R version 4.2.2 [23]. To investigate behavioural responses to the trade-off between courtship and territorial defence, two generalized linear models were fit using the 'lme4' package: (1) number of zigzag displays when a female was present and (2) number of bites when a territorial intruder was present, with treatment as a fixed effect. In the model for zigzags, the treatment consisted of the courtship opportunity and trade-off treatments since the territorial challenge and control treatments did not contain a female stimulus. Similarly, in the model for bites, the treatment consisted of the territorial challenge and trade-off treatments. Individual ID was included as a random effect. Models were fit with Poisson error distributions with an observation level random effect to control for overdispersion [24]. *P*-values were calculated with the 'lmerTest' package. Order effects and repeatability were also investigated (electronic supplementary material, Supplement 1B).

Gene expression data were analysed separately for the diencephalon and telencephalon using edgeR v.30.40.2. Genes were filtered to include those with >0.5 count per million in four or more samples, resulting in 17 884 genes (80.2% of total) in the telencephalon and 18 278 (81.9% of total) in the diencephalon. Counts were TMM (trimmed mean of M-values) normalized using a tagwise dispersion estimate after computing common and trended dispersions. We identified differentially expressed genes (DEGs) in the three experimental treatments relative to the control using a 'glm' approach, with an empirical FDR *p*-value correction [25]. PCA was used on this set of DEGs in each of the two brain regions to identify principal components that best-separated treatments (electronic supplementary material, Supplement 1C). Genes were annotated using 'biomaRt' and GO analysis was performed using 'TopGo' to identify genes and biological processes recruited in response to each treatment relative to the control (electronic supplementary material, Supplement 1D).

3. Results

(a) Behavioural responses

Males zigzagged 99% more on average when presented with a courtship opportunity, compared to the trade-off (Poisson glmm effect of treatment: coefficient = −0.69, z-value = −6.041, *p*-value = 1.54×10^{-9}, figure 1*a*). By contrast, on average males bit a similar number of times when a territorial challenge was presented alone compared to the trade-off (Poisson glmm: coefficient = 0.21, *z*-value = 1.304, *p*-value = 0.192, figure 1*b*). Bites, but not zigzags, were significantly repeatable across treatments, and order effects

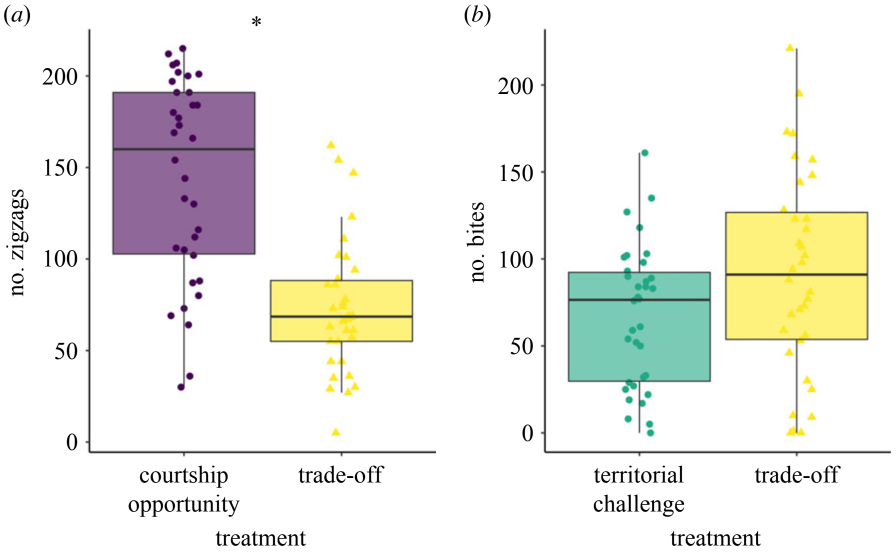

Figure 1. Male sticklebacks ($n = 17$) compromised courtship but not territorial defence when faced with a trade-off between them. (*a*) Males performed significantly fewer zigzag courtship displays per 5 min in the trade-off treatment compared to the courtship opportunity treatment. (*b*) Males performed a similar number of bites per 5 min in the trade-off and territorial challenge treatments. Points represent individual observations across trials and box plots show median, interquartile range, and 1.5*IQR. Asterisk indicates statistical significance.

were negligible relative to treatment effects (electronic supplementary material, Supplement 1B).

Differential gene expression analysis revealed the set of genes differentially expressed relative to the control in the two brain regions (electronic supplementary material, Supplement 2). Principal component analyses showed separation of the courtship opportunity from the territorial challenge and trade-off treatments along PC3 in the diencephalon and PC2 in the telencephalon (figure 2*a*; electronic supplementary material, Supplement 1C). While in both regions there was significant overlap of DEGs between treatments (hypergeometric overlap tests: all p-values < 0.0001), a unique set of genes was differentially expressed when males were confronted by a trade-off, and a majority of these genes were differentially expressed only in one brain region (figure 2*b*).

4. Discussion

Animals must often make decisions that are vital to their reproductive success, yet competing demands are rarely presented in isolation. Our study investigated how breeding male sticklebacks respond to the simultaneous demands of courtship and territorial defence. Behaviourally, males prioritized territorial defence over courtship when faced with a trade-off between them: they reduced courtship behaviour but maintained similar levels of territorial defence behaviour in the trade-off treatment. In nature, females may be drawn to territorial disputes and display a preference for aggressive males [26–28], thus prioritizing territory defence behaviours over courtship behaviours may mitigate the conflict between them. Furthermore, aggression (bites) was repeatable but not courtship behaviour (zigzags), perhaps because courtship behaviour is more malleable and responsive to the immediate environment (e.g. female interest, male quality) than territorial aggression. Previous studies also found evidence for a trade-off between courtship and territorial defence, however there was variation in how males responded. Candolin [3] found that male sticklebacks decreased some courtship behaviours, but *increased* zigzags toward dummy females in the presence of a competitor, and Dzieweczynski *et al.* [4] found that males presented with a dummy male and female simultaneously either exclusively interacted with one stimulus or divided their time between them. Population and methodological differences, including our use of live, rather than dummy, stimulus fish may help account for this variation, because how males assign value to courtship and territorial defence may depend on many factors, including female interest and intruder behaviour.

Brain gene expression profiles reflected the behavioural overlap between the territorial challenge and trade-off treatments. Additionally, there was a large set of differentially expressed genes and gene functions that were unique to the trade-off treatment, suggesting that the need to manage courtship and territorial defence elicits a distinctive response in the brain that is more than an additive response to a courtship opportunity and a territorial challenge alone. The list of differentially expressed genes unique to the trade-off treatment included some interesting candidates such as dopamine receptor D2 like (drd2l) and vasoactive intestinal peptide receptor 1a (vipr1a) in the telencephalon. The dopamine pathway is predicted to modulate reward and motivation [29], and thus may be important in assigning value when faced with competing stimuli. VIP signalling has been implicated in the trade-off between affiliative and aggressive behaviour in birds [30], and in sticklebacks [9]. Several additional genes of interest are discussed in electronic supplementary material, Supplement 1D. Characterizing this set of genes opens the way for studies into their involvement in gene regulatory networks, how their patterns of expression vary, and how mechanisms interact across spatial and temporal scales.

The set of differentially expressed genes and enriched biological processes were largely non-overlapping between telencephalon and diencephalon, suggesting that managing competing demands could be a brain-wide phenomenon rather than being restricted to a few key nuclei. Investigating finer spatial and temporal scales would provide insight into what specific regions and cell types are involved, and how they are interrelated. Combined, these results raise novel

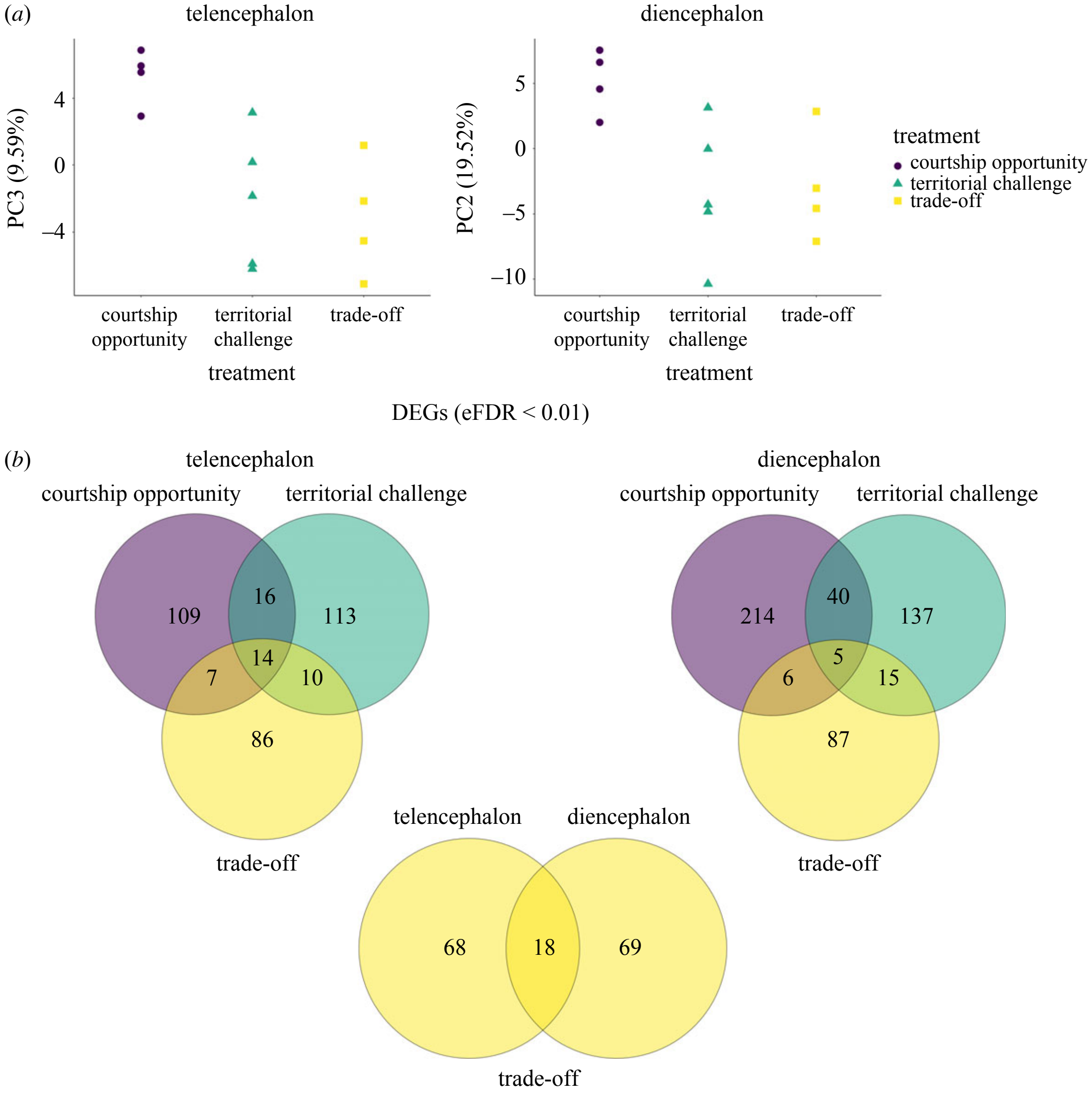

Figure 2. Differentially expressed genes (DEGs) in the telencephalon (left) and diencephalon (right) of males confronted with a courtship opportunity, a territorial challenge, and the trade-off between them, relative to a control (eFDR < 0.01). (*a*) Principal component analysis (PCA) revealed that the gene expression profiles of males faced with a trade-off more closely resembled the profile of males confronted by a territorial challenge than a courtship opportunity. These patterns were statistically significant (electronic supplementary material, Supplement 1C). Points represent individuals sampled for brain gene expression after a courtship opportunity (purple), a territorial challenge (green), or the trade-off (yellow). (*b*) Venn diagrams show unique and overlapping DEGs in the three experimental groups relative to the control group (top) and across brain regions for the DEGs unique to the trade-off treatment (bottom). Overlaps are statistically significant (hypergeometric tests: p-values < 0.0001).

questions about the interplay between the neural and molecular mechanisms involved in managing competing demands.

Ethics. All experiments were conducted with the approval of the Institutional Animal Care and Use Committee at the University of Illinois Urbana Champaign (protocol no. 21031).

Data accessibility. Raw sequence and processed count data are available in the GEO repository (accession no. GSE233058 [31]). Behavioural data and code for all statistical methods and figures are available from the Dryad Digital Repository: https://doi.org/10.5061/dryad.g79cnp5w0 [32].

Lists of differentially expressed genes and significantly enriched GO terms are provided in the electronic supplementary material (Supplement 2, 3) [33].

Declaration of AI use. We have not used AI-assisted technologies in creating this article.

Authors' contributions. T.A.B.: conceptualization, data curation, formal analysis, funding acquisition, investigation, supervision, validation, visualization, writing—original draft, writing—review and editing; C.B.: conceptualization, data curation, formal analysis, investigation, methodology, supervision, writing—review and editing; M.M.: conceptualization, data curation, formal analysis, investigation, writing—original draft; E.A.: investigation, methodology, writing—review and editing; A.M.B.: conceptualization, funding acquisition, project administration, supervision, writing—review and editing.

All authors gave final approval for publication and agreed to be held accountable for the work performed therein.

Conflict of interest declaration. We declare we have no competing interests.

Funding. This work was supported by an NSF postdoctoral research fellowship to T.A.B. (award ID: 2109619). This material is based upon work supported by the National Science Foundation (grant no. IOS 1645170). Research reported in this publication was supported by NIGMS of the National Institutes of Health (grant no. 1R35GM139597).

Acknowledgements. Many thanks to Abbas Bukhari, Usan Dan, Facundo Fernandez-Duque, Katie Julkowski, Meghan Maciejewski, Kevin Neumann and Meg Tucker for helpful comments during the preparation of this manuscript. Thanks also to Eva Fischer and A. Bukhari for valuable input on statistical analysis and K. Julkowski for logistical support in the laboratory.

References

1. O'Connell LA, Hofmann HA. 2011 Genes, hormones, and circuits: an integrative approach to study the evolution of social behavior. *Front Neuroendocrinol.* **32**, 320–335. (doi:10.1016/j.yfrne.2010.12.004)
2. Santangelo N, Itzkowitz M, Richter M, Haley MP. 2002 Resource attractiveness of the male beaugregory damselfish and his decision to court or defend. *Behav. Ecol.* **13**, 676–681. (doi:10.1093/beheco/13.5.676)
3. Candolin U. 1997 Predation risk affects courtship and attractiveness of competing threespine stickleback males. *Behav. Ecol. Sociobiol.* **41**, 81–87. (doi:10.1007/s002650050367)
4. Dzieweczynski TL, Mack CL, Granquist RM. 2009 Lovers and fighters: male stickleback, *Gasterosteus aculeatus*, differ in their responses to conflicting stimuli. *Anim. Behav.* **78**, 399–406. (doi:10.1016/j.anbehav.2009.04.025)
5. Dzieweczynski TL, Gill CE, Walsh MM. 2010 The nest matters: the influence of reproductive status on decision-making to conflicting stimuli in male Siamese fighting fish. *Behaviour* **147**, 805–823. (doi:10.1163/000579510X493142)
6. Lin D, Boyle MP, Dollar P, Lee H, Lein ES, Perona P, Anderson DJ. 2011 Functional identification of an aggression locus in the mouse hypothalamus. *Nature* **470**, 221–226. (doi:10.1038/nature09736)
7. Certel SJ, Savella MG, Schlegel DC, Kravitz EA. 2007 Modulation of *Drosophila* male behavioral choice. *Proc. Natl Acad. Sci. USA* **104**, 4706–4711. (doi:10.1073/pnas.0700328104)
8. Koganezawa M, Kimura KI, Yamamoto D. 2016 The neural circuitry that functions as a switch for courtship versus aggression in *Drosophila* males. *Curr. Biol.* **26**, 1395–1403. (doi:10.1016/j.cub.2016.04.017)
9. Sanogo YO, Bell AM. 2016 Molecular mechanisms and the conflict between courtship and aggression in three-spined sticklebacks. *Mol. Ecol.* **25**, 4368–4376. (doi:10.1111/mec.13766)
10. McNamara JM, Houston AI. 2009 Integrating function and mechanism. *Trends Ecol. Evol.* **24**, 670–675. (doi:10.1016/j.tree.2009.05.011)
11. Bell AM. 2023 The evolution of mechanisms for decision-making. *Trends Ecol. Evol.*, S0169-5347(23)00238-0. (doi:10.1016/j.tree.2023.09.007)
12. Tinbergen N. 1951 *The study of instinct*. Oxford, UK: Oxford University Press.
13. Van Iersel JJA. 1953 An analysis of the parental behaviour of the male three-spined stickleback (*Gasterosteus aculeatus* L.). *Behaviour Supplement* **3**, 1–159.
14. Blouw DM, Hagen DW. 1990 Breeding ecology and evidence of reproductive isolation of a widespread stickleback fish (Gasterosteidae) in Nova Scotia, Canada. *Biol. J. Linn. Soc.* **39**, 195–217. (doi:10.1111/j.1095-8312.1990.tb00512.x)
15. Samuk KM. 2016 The evolutionary genomics of adaptation and speciation in the threespine stickleback. PhD thesis, University of British Columbia, Vancouver, BC, Canada.
16. Blumstein DT, Daniel JC. 2007 *Quantifying behavior the JWatcher way*. Sunderland, MA: Sinauer Associates, Incorporated.
17. Sanogo YO, Band M, Blatti C, Sinha S, Bell AM. 2012 Transcriptional regulation of brain gene expression in response to territorial aggression. *Proc. R. Soc. Lond. B* **279**, 4929–4938.
18. Jones FC *et al.* 2012 The genomic basis of adaptive evolution in threespine sticklebacks. *Nature* **484**, 55–61. (doi:10.1038/nature10944)
19. Nath S, Shaw DE, White MA. 2021 Improved contiguity of the threespine stickleback genome using long-read sequencing. *G3* **11**, jkab007. (doi:10.1093/g3journal/jkab007)
20. Peichel CL *et al.* 2020 Assembly of the threespine stickleback Y chromosome reveals convergent signatures of sex chromosome evolution. *Genome Biol.* **21**, 1–31. (doi:10.1186/s13059-020-02097-x)
21. Dobin A, Davis CA, Schlesinger F, Drenkow J, Zaleski C, Jha S, Batut P, Chaisson M, Gingeras TR. 2013 STAR: ultrafast universal RNA-seq aligner. *Bioinformatics* **29**, 15–21. (doi:10.1093/bioinformatics/bts635)
22. Anders S, Pyl PT, Huber W. 2015 HTSeq—a Python framework to work with high-throughput sequencing data. *Bioinformatics* **31**, 166–169. (doi:10.1093/bioinformatics/btu638)
23. R Core Development Team. 2022 *R: a language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing. See http://www.R-project.org/.
24. Harrison XA. 2014 Using observation-level random effects to model overdispersion in count data in ecology and evolution. *PeerJ* **2**, e616. (doi:10.7717/peerj.616)
25. Bukhari SA, Saul MC, James N, Bensky MK, Stein LR, Trapp R, Bell AM. 2019 Neurogenomic insights into paternal care and its relation to territorial aggression. *Nat. Commun.* **10**, 4437. (doi:10.1038/s41467-019-12212-7)
26. Barker TC, Milinski M. 1993 The advantages of being red: sexual selection in the stickleback. *Mar. Freshw. Behav. Physiol.* **23**, 287–300. (doi:10.1080/10236249309378870)
27. Candolin U. 1999 Male–male competition facilitates female choice in sticklebacks. *Proc. R. Soc. Lond. B* **266**, 785–789. (doi:10.1098/rspb.1999.0706)
28. Candolin U. 2000 Male–male competition ensures honest signaling of male parental ability in the three-spined stickleback (*Gasterosteus aculeatus*). *Behav. Ecol. Sociobiol.* **49**, 57–61. (doi:10.1007/s002650000267)
29. Baik JH. 2013 Dopamine signaling in reward-related behaviors. *Front. Neural Circuits* **7**, 152.
30. Kingsbury MA, Wilson LC. 2016 The role of VIP in social behavior: neural hotspots for the modulation of affiliation, aggression, and parental care. *Integr. Comp. Biol.* **56**, 1238–1249. (doi:10.1093/icb/icw122)
31. Barbasch TA, Behrens CB, McLain M, Arredondo ED, Bell AM. 2023 Data from: A distinct neurogenomic response to a tradeoff between social challenge and opportunity in male sticklebacks (*Gasterosteus aculeatus*). Gene Expression Omnibus (accession number: GSE233058) and GitHub. See https://github.com/tbarbasch/Tradeoffs.
32. Barbasch TA, Behrens CB, McLain M, Arredondo ED, Bell AM. 2023 Data from: A distinct neurogenomic response to a trade-off between social challenge and opportunity in male sticklebacks (*Gasterosteus aculeatus*). Digital Repository. (doi:10.5061/dryad.g79cnp5w0)
33. Barbasch TA, Behrens CB, McLain M, Arredondo ED, Bell AM. 2023 A distinct neurogenomic response to a trade-off between social challenge and opportunity in male sticklebacks (*Gasterosteus aculeatus*). Figshare. (doi:10.6084/m9.figshare.c.6910734)