Gene expression correlates of social evolution in coral reef butterflyfishes

Jessica P. Nowicki[1,2], Morgan S. Pratchett[1], Stefan P. W. Walker[1], Darren J. Coker[1,3], Lauren A. O'Connell[2]*

[1]ARC Centre of Excellence for Coral Reef Studies, James Cook University, Townsville, QLD 4810, Australia

[2]Department of Biology, Stanford University, Stanford, CA, 94305, USA

[3]Red Sea Research Center, Division of Biological and Environmental Science and Engineering, King Abdullah University of Science and Technology, Thuwal, 23955-6900, Saudi Arabia

Running title: butterflyfish social evolution mechanisms

Word count: Abstract: 199, Main text: 4,336

Key words: pair bond, sex differences, species differences, butterflyfish, nonapeptides

*To whom correspondence should be addressed:

Lauren O'Connell

Department of Biology

Stanford University

371 Jane Stanford Way

Stanford, CA 94305

loconnel@stanford.edu

Abstract

Animals display remarkable variation in social behavior. However, outside of rodents, little is known about the neural mechanisms of social variation, and whether they are shared across species and sexes, limiting our understanding of how sociality evolves. Using coral reef butterflyfishes, we examined gene expression correlates of social variation (i.e., pair bonding vs. solitary living) within and between species and sexes. In several brain regions, we quantified gene expression of receptors important for social variation in mammals: oxytocin (*OTR*), arginine vasopressin (*V1aR*), dopamine (*D1R, D2R*), and mu-opioid (*MOR*). We found that social variation across individuals of the oval butterflyfish, *Chaetodon lunulatus,* is linked to differences in *OTR*,*V1aR, D1R, D2R,* and *MOR* gene expression within several forebrain regions in a sexually dimorphic manner. However, this contrasted with social variation among six species representing a single evolutionary transition from pair bonded to solitary living. Here, *OTR* expression within the supracommissural part of the ventral telencephalon was higher in pair bonded than solitary species, specifically in males. These results contribute to the emerging idea that nonapeptide, dopamine, and opioid signaling is a central theme to the evolution of sociality across individuals, although the precise mechanism may be flexible across sexes and species.

Introduction

Animals display spectacular diversity in sociality (i.e., affiliative social behavior [1]) prompting fundamental questions about how it arises and evolves. A striking example that has garnered considerable research attention is pair bonded versus solitary living (herein, referred to as “social variation”, or “variation in sociality”), which varies on an individual and species level in several vertebrate lineages [2–6]. Understanding mechanisms of individual social variation within a species’ population is important, as this is the level on which natural selection acts to drive evolutionary change. Equally important understanding mechanisms of social variation across species because it can illuminate generalizable principles that might not be apparent in a single species. Most of what is known about the neural mechanisms of social variation comes from a single mammalian genus, *Microtus* rodents. Here, comparison of the pair bonded to solitary phenotype has revealed the involvement of oxytocin, arginine vasopressin, dopamine, and opioid neurochemical signaling. Differences in receptor density throughout specific regions of the brain are thought to gate social variation by tuning social recognition (nonapeptides), learned reward (dopamine), and positive affect (opioids) [2,4,7–9]. However, the extent to which neural patterns underlying social variation in voles translates to other taxa remains unclear.

In vertebrates, the neurochemical-receptor systems and brain regions that regulate social behavior are evolutionarily ancient and highly conserved [10,11]. It is therefore possible that similar patterns of neurochemical signaling within and among brain regions have been repeatedly recruited to facilitate social variation across different contexts. Nevertheless, sexual dimorphism in neural mechanisms of social variation may occur, due to sex differences in behavioral features of sociality and sex steroid hormones [12,13]. Sex differences may also arise from differences in pre-existing molecular and neural frameworks available for co-option during the development and/or evolution of sociality [13]. For example, in mammals, the evolutionary transition from solitude to pair bonding is hypothesized to have co-opted a pre-existing maternal-infant bond circuitry for females and territoriality circuitry for males [8]. These factors, in addition to phylogenetic distance, and the frequency and recency of evolutionary social transitions may also drive species differences in how the brain governs social variation [14]. Examining social variation

in non-mammalian species is needed to better understand if its repeated evolution has relied on similar or labile neural mechanisms.

Coral reef butterflyfishes (f. Chaetodontidae) offer a compelling opportunity for comparatively studying the evolution of sociality [3,15,16]. Chaetodontidae have undergone rapid and repeated species diversification since the mid-Miocene [17], resulting in at least 127 extant species that co-occur in coral reefs throughout the world [18]. Unlike other vertebrate clades, pair bonding is ancestral and moderately conserved (77 [~60 % of] species) [3], where it appears to have arisen due to the fitness benefits of cooperative territorial defense [19,20]. Pair bonds generally develop at the onset of reproductive maturity [21] and are characterized by a selective affiliation with one other individual, typically of the opposite sex [3]. Partners establish and defend stable coral feeding and refuge territories from neighbors using species-specific cooperative strategies [19,20]. Partnership endurance is critical for this function [19], and has been reported to last for up to seven years [22]. Yet, within the clade, several independent transitions to solitary living have occurred relatively recently [3]. Relative to pair bonders, “singletons” display low levels and indiscriminate affiliation with other conspecifics, although the stability of solitary living throughout these individuals’ life-time remains unresolved [3]. Moreover, many species display variation in sociality across sympatric individuals, providing opportunities for intra-specific comparisons. In a previous study, we utilized these contrasts to develop a framework for studying within and between species variation in sociality [3] (Fig. 1). Specifically, in a wild population located on Lizard Island, Australia, we verified social variation (i.e., pair bonded vs. solitary living) both on an individual level within *Chaetodon lunulatus,* and across six closely related species (~15 MYA divergence time) that represent a single evolutionary transition from pair bonded (*C. vagabundus, C. lunulatus,* and *C. baronessa*) to solitary (*C. trifascialis, C. rainfordii,* and *C. plebeius*) living. Importantly, these differences in sociality do not co-vary with other major ecological attributes, controlling for these potential confounds (Fig.1). Nor is it likely to be confounded with parental care, which is a common confound in mammalian male pair bonding systems that, although hasn't been studied in the focal populations, is reportedly absent in all butterflyfishes studied to date [20,23,24].

Here, we leveraged this butterflyfish system (Fig. 1) to compare brain gene expression associated with social variation (pair bonded versus solitary living) between sexes and within-versus-among species. We focused on oxytocin- (*OTR*), vasopressin- (*V1aR*), dopaminergic- (*D1R*, *D2R*), and mu-opioid- (*MOR*) receptor gene expression within eight brain regions of the vertebrate social decision-making network [10], facilitating comparisons to mammals. We tested the hypothesis that in butterflyfishes, mechanisms of social variation are similar across sexes, and within versus between species, and that neural patterns observed in butterflyfish are similar to those documented in *Microtus* voles.

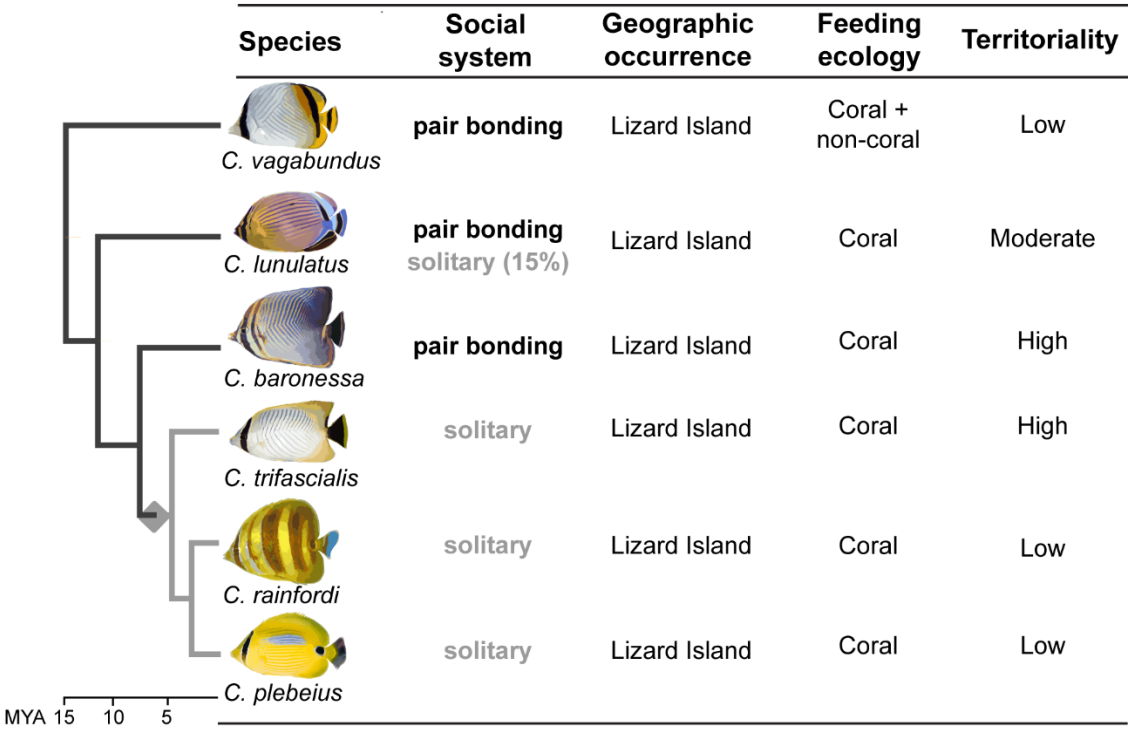

Figure 1. Study design comparing mechanisms of social variation in butterflyfishes. Within the populations, dichotomous social systems (pair bonded vs. solitary living) across individuals of *Chaetodon lunulatus* and across *Chaetodon* species do not co-vary with other attributes, offering more controlled examination of sociality. Grey diamond represents transition from pair bonded to solitary living that occurred in the last common ancestor of *C. trifascialis, C. rainfordi,* and *C. plebeius* ~7 MYA. Figure re-drawn from [3].

Methods

Field collections

Specimens were collected from wild populations at Lizard Island, in the northern Great Barrier Reef (GBR), Australia in 2013. Collections were made in May-July, outside of peak reproductive periods, capturing social behavior independent of reproductive activity. Since we were interested in adults, only individuals that were within 80% of the asymptotic size for each species were used, as these are more likely to be reproductively mature [21]. Individuals were haphazardly encountered on snorkel, allowed to acclimate to observer presence for 3 min, and level of selective affiliation with another conspecific was observed for 3 min. Individuals displaying a high level of affiliation selectively with another conspecific were considered pair bonded, while those displaying a low and indiscriminate level of affiliation with other conspecific were considered solitary. Collected sample sizes are as follows: *C. lunulatus* males = 11, females = 10; *C. baronessa* males = 7, females = 6; *C. vagabundus* males = 6, females = 6; *C. plebeius* females = 10; *C. rainfordii* males = 2, females = 11; *C. trifascialis* males = 2, females = 12. Behavioral characterization, collection, and sexing of focal fishes has been described previously (see [3]).

Brains were dissected, embedded in optimal cutting temperature compound (VWR), frozen and transported in liquid nitrogen, and stored at -80℃.

Gene expression

Frozen brains were coronally sectioned at 100 µm on a cryostat, and brain regions were identified using a *Chaetodon* brain atlas [25,26] and isolated using micropunches (Fig. 2 inset). RNA from each brain region was extracted and reverse transcribed to cDNA, purified, and stored at -20 °C. The *Chaetodon lunulatus* transcriptome was sequenced, from which cloning and exon-exon flanking qPCR primers for target genes and 18S endogenous control were designed and optimized (See Supplementary Materials). Quantitative PCR was then performed on each sample using a reaction mixture and qPCR cycling instrument (CFX380) that was recommended by the enzyme manufacturer (see Supplementary Methods S1 for detailed methods, Table S1 for primer sequences, and Table S2 for cycling parameters). Not all regions of each brain were measured for gene expression due to insufficient tissue available.

Statistical analysis

Gene expression differences were analyzed for the *C. lunulatus* (intra-) and the *Chaetodon* (inter-) species comparison separately. Each brain region was analyzed separately using the MCMC.qpcr model (MCMC.qpcr R package, version 1.2.3 [27]), specifying the following parameter levels to optimize chain mixing: Cq1 = 49, number of iterations = 510000, thinning interval = 500, and burnin = 10000 (see Supplementary Methods S2 for R script). For each analysis, gene expression differences were analyzed by examining the main and interactive effects of sex and social system, with individual as a random factor. Results are reported as natural log fold changes of posterior mean from an *a priori* reference state of male pair bonding. The *Chaetodon* species comparison revealed that for males, oxytocin receptor expression within the supracommissural part of the ventral telencephalon (Vs) varied significantly between social systems (see Results). In order to determine whether this difference was consistent across all species, we then examined the main effect of species exclusively for this brain region, gene, and sex. Results are reported as natural log fold changes in posterior mean from the *a priori* reference state of *C. baronessa.* Male *C. plebeius* were omitted from this analysis, as none were sampled. To approximate p-values based on a limited sample size, in all analyses, Bayesian two-sided p-values were calculated. [27]. Pair-wise differences between factor levels were explored using Tukey *post hoc* analysis.

Results

Pair bonded versus solitary Chaetodon lunulatus

In *C. lunulatus,* brain region-specific receptor expression differed between social systems in sex specific ways (Table S3; Fig. 2a). In *C. lunulatus* males, *D1R* within the ventral portion of the dorsal telencephalon (Vd, putative homolog of the mammalian nucleus accumbens) (ln(fold change) = -5.25; p = 0.01) and the preoptic area (POA) (ln(fold change) = -4.74; p = 0.02) was lower in solitary individuals than pair bonded counterparts. *MOR* expression within the medial part of the dorsal telencephalon (Dm) was lower in solitary individuals than pair bonded counterparts (ln(fold change) = -4.17, p = 0.02). Finally, *V1aR* expression within the ventral and lateral parts of the ventral telencephalon (Vv/Vl) appeared lower in solitary compared to pair bonded

individuals (ln(fold change) = -4.39); however, this was to a statistically non-significant extent (p = 0.059). In females, by contrast, *D1R* expression within the Dm (ln(fold change) = -3.91; p = 0.03) and Vd (ln(fold change) = -5.96; $p < 0.001$) was lower in solitary than pair bonded individuals. Finally, *D2R* expression within the Vd was lower in solitary than pair bonded individuals (ln(fold change) = - 3.96; p = 0.04), and while Vd *OTR* showed a similar trend (ln(fold change) = -114.79), this was to a statistically non-significant extent (p = 0.07). (See Table S3 for detailed statistical results.)

Pair bonded versus solitary Chaetodon *species*

Receptor expression patterning of individual differences in social systems found in *C. lunulatus* was inconsistent with that of species differences in social system (Fig. 2ab; Tables S3,4). Across sexes, genes, and brain regions; only *OTR* expression within the supracommissural part of the ventral telencephalon (Vs, putative homologue of the mammalian medial amygdala/bed nucleus of the stria terminalis) varied consistently across all pair bonded and solitary species in males, where expression was lower in solitary than pair bonded males (ln(fold change) = -90.59; p = 0.02) (Fig. 2b). We found no brain region gene expression differences for *MOR* or dopamine receptors between pair bonded and solitary species in either sex. (See Tables S4,S5 for detailed statistical results.)

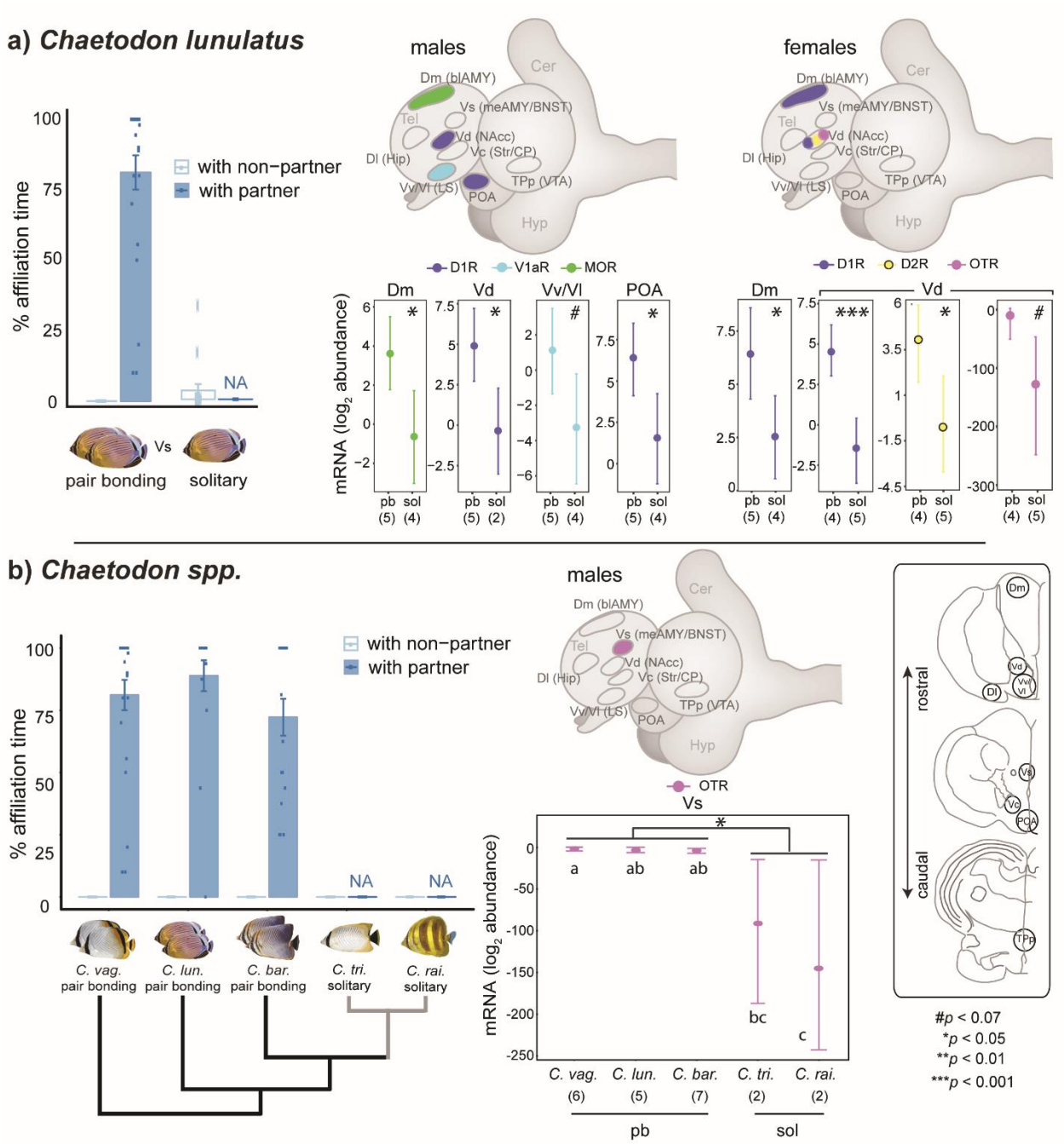

Figure 2. Neurochemical receptor gene expression in specific brain regions differs between pair bonded (pb) and solitary (sol) butterflyfishes in a context-specific manner. Gene expression of nonapeptide, dopamine, and mu-opioid receptors correlates with social variation, yet patterns are distinct between sexes and within **(a)** versus between **(b)** species. Bar plots show pair bonding fish displaying high levels of selective affiliation with a partner (blue) vs. non-partner (white), and solitary fish displaying low levels, throughout a 3 min observation period immediately before collection. Behavioral values represent means +/- standard error, and dots represent individual data points. Point plots show receptor gene expression within brain regions that differ significantly between social systems, where values represent means +/- 95% credible

intervals of the posterior distribution. Schematics show brain regions where receptor gene expression (light blue = arginine vasopressin *V1aR*, purple = oxytocin *OTR*, dark blue = dopamine *D1R*, yellow = dopamine *D2R*, green = mu-opioid *MOR*) differs between social systems. *Inset*: brain regions micro-dissected for gene expression analysis. *Brain region abbreviations:* teleost: telen. = telencephalon, Dm = medial part of the dorsal telen., Vd = dorsal part of the ventral telen., Dl = lateral part of the dorsal telen., Vv/Vl = lateral and ventral part of the ventral telen., Vs = supracommissural part of the ventral telen., Vc = central part of the ventral telen., POA = pre optic area, TPp = periventricular part of the posterior tuberculum. Putative mammalian homolog: blAMY = basolateral amygdala, NAcc = nucleus accumbens, HIP = hippocampus, LS = lateral septum, meAMY/BNST = medial amygdala/bed nucleus of the stria terminalis, Str = Striatum, CP = caudate putamen, VTA = ventral tegmental area.

Discussion

Our work suggests that in *Chaetodon* butterflyfishes, nonapeptide, dopaminergic, and mu-opioid receptor gene expression is important for mediating social variation. However, the brain regions in which signaling occurs differs by sex and within versus between species. mirroring previous findings in *Microtus* voles [4,7,29,30]. These results contribute to the emerging theme that the convergence of social variation across sexes and species has relied on repeated co-option of evolutionarily ancient nonapeptide, dopaminergic, and opioid systems, though the precise signaling mechanisms can be evolutionarily labile.

Previous studies examining the involvement of receptor abundance patterns throughout the brain in individual social variation are largely limited to nonapeptide receptors in *Microtus ochrogaster* prairie voles, where patterns appear sexually dimorphic [4,7,29]. We found a similar trend in the oval butterflyfish, where individual differences in sociality appear linked to differential *V1aR* expression in the ventral portion of the ventral telencephalon (Vv/vl) in males, and differential *OTR* expression in the dorsal portion of the ventral telencephalon (Vd) in females. These sex-specific patterns of individual social variation are distinct from voles and instead more closely mirror *species* differences, where monogamous species exhibit higher V1aR density within the lateral septum (LS, the putative mammalian homologue of the teleost Vv/Vl) in males [33], and higher OTR density within the nucleus accumbens (NAcc, the putative mammalian homologue of the teleost Vd) in females and males [34]. We also examined the link between dopamine *D1R* and *D2R,* and *MOR* receptor expression and individual social variation, discovering that all three systems also appear involved in sexually dimorphic manner. In females, forebrain *D1R* and *D2R* expression is involved; whereas in males, forebrain *D1R* and *MOR* expression is involved. Differential dopamine and MOR patterning within the brain is also linked to species variation in sociality in *Microtus* voles [34–36]. When compared to our current findings in more detail, two themes emerge: firstly, that NAcc-like D1R signaling is repeatedly involved; and secondly, the brain regions involved in MOR patterning are distinct. Taken together, these results suggest that lateral septum-like V1aR, nucleus accumbens-like OTR and D1R, and MOR signaling might have converged between rodents and fishes to mediate social variation, and this convergence is not specific to sex or level of social variation (i.e., inter-individual versus inter-species). Future studies should now examine whether these systems have also converged with rodents functionally, testing the hypothesis that social variation emerges from differential nonapeptide tuning of social recognition, mu-opioid tuning of positive hedonics, and dopaminergic tuning the learned association between the two.

Can similar mechanistic modifications more *generally* explain repeated instances of social variation within and between species? In butterflyfishes, we found that neural patterning of social variation in oval butterflyfish did not translate across *Chaetodon* species, consistent with reports of prairie voles and their *Microtus* congeners (male *Microtus* NAcc OTR notwithstanding [4,7,29,30]). Consistently, certain neuromodulatory patterns of social variation found in a few rodent species do not translate more broadly across their genus [9,38,39]. Recent studies have compared multiple independent evolutionary social transitions across several species, in Lake Tanganyikan cichlids [40], and recently across vertebrates [41], and suggest that on a whole-brain level, repeated evolutionary transitions from promiscuous to monogamous living were accompanied by convergent changes in gene expression patterns. On a brain region-specific level, neuromodulation of social evolution across several species can be robust, as exemplified in Estrildid finches [42] and butterflyfishes [15]. Yet, it can also be labile, even across relatively short divergence times and few transitional events. For example, two independent transitions from promiscuity to monogamy were accompanied by nonconcordant V1aR patterning throughout the brain in *Peromyscus* mice [39]. Similarly, a single transition from monogamy to promiscuity was accompanied by nonconcordant MOR patterning in *Microtus* voles [9]. In our study, we found that the transition from pair bonding to solitary living in six species of *Chaetodon* butterflyfishes was associated with nonconcordant *MOR*, *D1R*, *D2R*, and *V1aR* receptor expression patterning. Importantly, however, these negative results should be considered tentative, since the small sample size among solitary males likely resulted in limited experimental power. The only neuromodulatory pattern we found to be linked to species divergence in sociality is *OTR* expression in the Vs (teleost homologue of the mammalian medial amygdala/bed nucleus of the stria terminalis, meAMY/BNST), which is higher in pair bonding species than solitary counterparts, specifically in males. This result mirrors that of *Microtus* voles (although in *Microtus* voles, this exists for both sexes) [34]. In male rodents, amygdala activity is necessary for pair bond affiliation [43], and OT signaling within the meAMY is critical for social recognition [44,45]. Whether amygdalar OTR signaling functions to gate species differences in sociality via tuning social recognition is an interesting line of inquiry for both butterflyfishes and rodents alike. Taken together, these studies suggest that neuromodulatory patterning of social evolution can be labile, highlighting the importance of comparing several species that ideally represent multiple independent social transitions when seeking generalizable mechanistic principles.

The differences in gene expression associated with social variation between sexes, and within versus among species found in this study might be attributed to several factors. One may be differences in pre-existing neural phenotypes (e.g., level and location of ligand synthesis, projection pathways, receptor patterning) available for recruitment during the development and or evolution of sociality. For example, sexual dimorphism in nonapeptide expression across brain nuclei has been reported in teleosts [46,47], and we found that the trend for female-specific Vd *OTR* modulation of individual social variation coincides with higher base-line expression of this receptor within this brain regions relative to males (table S3). An additional potential source of variation is incomplete parallelism in behavioral details of social variation. For example, in *C. lunulatus*, while female pair bonders display lower territory defense than solitary counterparts; this is not observed in males [19]. Likewise, while some solitary individuals of *C. lunulatus* might represent “divorcees” or “widows” that were previously pair bonded; this is highly unlikely for solitary species. Regardless of these inconsistencies, butterflyfishes represent an excellent research clade for understanding the evolution of neural mechanisms underlying sociality.

Conclusions

Social variation (i.e., pair bonded vs. solitary living) has repeatedly and independently evolved across vertebrates, the neural basis of which has been mostly resolved in mammals. We present one of the most comprehensive single studies on the neural basis of social variation outside of mammals, spanning several levels of mechanistic (i.e., from brain regions to receptor gene expression) and organismic (i.e., from individuals to species, in both sexes) organization in a phylogenetically distant lineage, teleost fishes. Our results suggest that nonapeptide, dopamine, and mu-opioid receptor signaling within the forebrain mediates social variation among *Chaetodon* butterflyfishes, although the brain region-specific sites of action are context-specific. Taken together with previous studies in mammals, our results support the emerging idea that ancient nonapeptide, dopamine, and mu-opioid systems have been repeatedly recruited to modulate social variation across both short and vast evolutionary distances in both sexes, albeit using different "solutions" across different contexts [14]. A comprehensive assessment of neuromodulatory patterning throughout the brain across many taxonomically diverse species is now needed to further resolve major mechanistic themes of animal social variation.

Acknowledgements

We thank Manuela Giammusso for field assistance; Makhail Matz, Eva Fischer, and Rayna Harris for statistical guidance; Adam Dewan for generously sharing pre-published drafts of a *Chaetodon* brain atlas; and the O'Connell lab for comments on earlier versions of the manuscript. We acknowledge the fishes that were used to conduct the present study. We thank two anonymous reviewers for feedback that improved the manuscript during the review process.

Ethics

Animal collections followed Great Barrier Reef Marine Park Authority permit approvals: G10/33239.1, G13/35909.1, G14/37213.1; and James Cook University General Fisheries permit 170251. Animal handling and sacrifice procedures were approved by James Cook University Animal Ethics committee (approval: A1874).

Author contributions

JPN conceived of the study; JPN, LAO, MSP and SPW designed and coordinated the study; JPN and DJC collected samples; JPN and LAO performed molecular work, JPN analyzed data; JPN wrote the manuscript with input and editing from all authors; All authors gave final approval for publication and agree to be held accountable for the work performed therein.

Funding

This work was supported by the ARC Centre of Excellence for Coral Reef Studies (grant no. CEO561435) through funds awarded to MSP and SPWW, an NSF EDEN Research Travel Award to JPN (grant no. 0955517), and a Harvard Bauer Fellowship and L'Oreal For Women in Science Award won by LAO. LAO is a New York Stem Cell Foundation – Robertson Investigator.

References

1. Goodson JL. 2013 Deconstructing sociality, social evolution and relevant nonapeptide functions. *Psychoneuroendocrinology* **38**, 465–478.

2. McGraw L, Székely T, Young LJ. 2010 Pair bonds and parental behaviour. *Social behaviour: Genes*

3. Nowicki JP, O'Connell LA, Cowman PF, Walker SPW, Coker DJ, Pratchett MS. 2018 Variation in social systems within Chaetodon butterflyfishes, with special reference to pair bonding. *PLoS One* **13**, e0194465.

4. Zheng D-J, Larsson B, Phelps SM, Ophir AG. 2013 Female alternative mating tactics, reproductive success and nonapeptide receptor expression in the social decision-making network. *Behavioural Brain Research*. **246**, 139–147. (doi:10.1016/j.bbr.2013.02.024)

5. Firth JA, Cole EF, Ioannou CC, Quinn JL, Aplin LM, Culina A, McMahon K, Sheldon BC. 2018 Personality shapes pair bonding in a wild bird social system. *Nat Ecol Evol* **2**, 1696–1699.

6. Walum H *et al.* 2008 Genetic variation in the vasopressin receptor 1a gene (AVPR1A) associates with pair-bonding behavior in humans. *Proc. Natl. Acad. Sci. U. S. A.* **105**, 14153–14156.

7. Ophir AG, Gessel A, Zheng D-J, Phelps SM. 2012 Oxytocin receptor density is associated with male mating tactics and social monogamy. *Horm. Behav.* **61**, 445–453.

8. Freeman SM, Young L. 2013 Oxytocin, vasopressin, and the evolution of mating systems in mammals. *Oxytocin, Vasopressin and Related Peptides in the Regulation of Behavior* , 128–144.

9. Shapiro LE, Insel TR. 1992 Oxytocin receptor distribution reflects social organization in monogamous and polygamous voles. *Ann. N. Y. Acad. Sci.* **652**, 448–451.

10. O'Connell LA, Hofmann HA. 2012 Evolution of a vertebrate social decision-making network. *Science* **336**, 1154–1157.

11. O'Connell LA, Hofmann HA. 2011 The vertebrate mesolimbic reward system and social behavior network: a comparative synthesis. *J. Comp. Neurol.* **519**, 3599–3639.

12. Carter CS, Cushing BS. 2004 Proximate mechanisms regulating sociality and social monogamy, in the context of evolution. *The origins and nature of sociality* , 99–121.

13. Ondrasek NR. 2016 Emerging Frontiers in social neuroendocrinology and the study of nonapeptides. *Ethology* **122**, 443–455.

14. Fischer EK, Nowicki J, O'Connell LA. 2019 Evolution of affiliation: patterns of convergence from genomes to behavior. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* (doi:10.1098/rstb.2018.0242)

15. Dewan AK, Ramey ML, Tricas TC. 2011 Arginine vasotocin neuronal phenotypes, telencephalic fiber varicosities, and social behavior in butterflyfishes (Chaetodontidae): potential similarities to birds and mammals. *Horm. Behav.* **59**, 56–66.

16. Dewan AK, Tricas TC. 2011 Arginine vasotocin neuronal phenotypes and their relationship to aggressive behavior in the territorial monogamous multiband butterflyfish, Chaetodon multicinctus. *Brain Res.* **1401**, 74–84.

17. Cowman PF, Bellwood DR. 2011 Coral reefs as drivers of cladogenesis: expanding coral reefs, cryptic extinction events, and the development of biodiversity hotspots. *J. Evol. Biol.* **24**, 2543–2562.

18. Bellwood DR, Pratchett MS. 2013 The origins and diversification of coral reef butterflyfishes. In *Biology of Butterflyfishes* (eds MS Pratchett, ML Berumen, BG Kapoor), pp. 1–18. Florida: CRC Press.

19. Nowicki JP, Walker SPW, Coker DJ, Hoey AS, Nicolet KJ, Pratchett MS. 2018 Pair bond endurance promotes cooperative food defense and inhibits conflict in coral reef butterflyfish. *Sci. Rep.* **8**, 6295.

20. Fricke HW. 1986 Pair swimming and mutual partner guarding in monogamous butterflyfish (pisces, chaetodontidae) - a joint advertisement for territory. *Ethology* **73**, 307–333.

21. Pratchett MS, Pradjakusuma OA, Jones GP. 2006 Is there a reproductive basis to solitary living versus pair-formation in coral reef fishes? *Coral Reefs* **25**, 85–92.

22. Reese ES. 1991 How behavior influences community structure of butterflyfishes (family Chaetodontidae) on Pacific coral reefs. *Ecol Int Bull* **19**, 29–41.

23. Pratchett MS, Berumen ML, Kapoor BG. 2013 *Biology of Butterflyfishes*. CRC Press.

24. Roberts CM, Ormond FG. 1992 Butterflyfish social behaviour, with special reference to the incidence of territoriality: a review. *Environ. Biol. Fishes* **34**, 79–93.

25. Bauchot R, Ridet JM, Bauchot ML. 1989 The brain organization of butterflyfishes. *Environ. Biol. Fishes* **25**, 204–219.

26. Dewan AK, Tricas TC. 2014 Cytoarchitecture of the telencephalon in the coral reef multiband butterflyfish (*Chaetodon multicinctus*: Perciformes). *Brain Behav. Evol.* **84**, 31–50.

27. Matz MV, Wright RM, Scott JG. 2013 No control genes required: Bayesian analysis of qRT-PCR data. *PLoS One* **8**, e71448.

28. Benjamini Y, Hochberg Y. 1995 Controlling the False Discovery Rate: A Practical and Powerful Approach to Multiple Testing. *J. R. Stat. Soc. Series B Stat. Methodol.* **57**, 289–300.

29. Ophir AG, Wolff JO, Phelps SM. 2008 Variation in neural V1aR predicts sexual fidelity and space use among male prairie voles in semi-natural settings. *Proc. Natl. Acad. Sci. U. S. A.* **105**, 1249–1254.

30. King LB, Walum H, Inoue K, Eyrich NW, Young LJ. 2016 Variation in the Oxytocin Receptor Gene Predicts Brain Region–Specific Expression and Social Attachment. *Biol. Psychiatry* **80**, 160–169.

31. Donaldson ZR, Young LJ. 2008 Oxytocin, vasopressin, and the neurogenetics of sociality.

Science **322**, 900–904.

32. Cushing BS, Martin JO, Young LJ, Carter CS. 2001 The effects of peptides on partner preference formation are predicted by habitat in prairie voles. *Horm. Behav.* **39**, 48–58.

33. Insel TR, Wang ZX, Ferris CF. 1994 Patterns of brain vasopressin receptor distribution associated with social-organization in microtine rodents. *Journal of Neuroscience* **14**, 5381–5392.

34. Insel TR, Shapiro LE. 1992 Oxytocin receptor distribution reflects social organization in monogamous and polygamous voles. *Proceedings of the National Academy of Sciences* **89**, 5981–5985.

35. Aragona BJ, Liu Y, Yu YJ, Curtis JT, Detwiler JM, Insel TR, Wang ZX. 2006 Nucleus accumbens dopamine differentially mediates the formation and maintenance of monogamous pair bonds. *Nat. Neurosci.* **9**, 133–139.

36. Inoue K, Burkett JP, Young LJ. 2013 Neuroanatomical distribution of μ-opioid receptor mRNA and binding in monogamous prairie voles (Microtus ochrogaster) and non-monogamous meadow voles (Microtus pennsylvanicus). *Neuroscience* **244**, 122–133.

37. Young LJ, Murphy Young AZ, Hammock EAD. 2005 Anatomy and neurochemistry of the pair bond. *J. Comp. Neurol.* **493**, 51–57.

38. Bester-Meredith JK, Young LJ, Marler CA. 1999 Species differences in paternal behavior and aggression in peromyscus and their associations with vasopressin immunoreactivity and receptors. *Horm. Behav.* **36**, 25–38.

39. Turner LM, Young AR, Rompler H, Schoneberg T, Phelps SM, Hoekstra HE. 2010 Monogamy evolves through multiple mechanisms: Evidence from V1aR in Deer mice. *Mol. Biol. Evol.* **27**, 1269–1278.

40. Renn SCP, Machado HE, Duftner N, Sessa AK, Harris RM, Hofmann HA. 2018 Gene expression signatures of mating system evolution. *Genome* **61**, 287–297.

41. Young RL *et al.* 2019 Conserved transcriptomic profiles underpin monogamy across vertebrates. *Proceedings of the National Academy of Sciences* **January 7, 2019**.

42. Goodson JL, Evans AK, Wang Y. 2006 Neuropeptide binding reflects convergent and divergent evolution in species-typical group sizes. *Horm. Behav.* **50**, 223–236.

43. Kirkpatrick B, Carter CS, Newman SW, Insel TR. 1994 Axon-sparing lesions of the medial nucleus of the amygdala decrease affiliative behaviors in the prairie vole (Microtus ochrogaster): behavioral and anatomical specificity. *Behav. Neurosci.* **108**, 501.

44. Ferguson JN, Young LJ, Hearn EF, Matzuk MM, Insel TR, Winslow JT. 2000 Social amnesia in mice lacking the oxytocin gene. *Nat. Genet.* **25**, 284–288.

45. Ferguson JN, Aldag JM, Insel TR, Young LJ. 2001 Oxytocin in the medial amygdala is essential for social recognition in the mouse. *J. Neurosci.* **21**, 8278–8285.

46. Ohya T, Hayashi S. 2006 Vasotocin/isotocin-immunoreactive neurons in the medaka fish brain are sexually dimorphic and their numbers decrease after spawning in the female.

Zoolog. Sci. **23**, 23–29.

47. Kawabata Y, Hiraki T, Takeuchi A, Okubo K. 2012 Sex differences in the expression of vasotocin/isotocin, gonadotropin-releasing hormone, and tyrosine and tryptophan hydroxylase family genes in the medaka brain. *Neuroscience* **218**, 65–77.