

1 **Conserved Transcriptomic Profiles Underpin Monogamy across**  
2 **Vertebrates**

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4 Rebecca L. Young<sup>a,b,1</sup>, Michael H. Ferkin<sup>c</sup>, Nina F. Ockendon-Powell<sup>d</sup>, Veronica N. Orr<sup>e</sup>,  
5 Steven M. Phelps<sup>a,f</sup>, Ákos Pogány<sup>g</sup>, Corinne L. Richards-Zawacki<sup>h</sup>, Kyle Summers<sup>i</sup>,  
6 Tamás Székely<sup>d,j,k</sup>, Brian C. Trainor<sup>e</sup>, Araxi O. Urrutia<sup>j,l</sup>, Gergely Zachar<sup>m</sup>, Lauren A.  
7 O'Connell<sup>n</sup>, and Hans A. Hofmann<sup>a,b,f,1</sup>

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9 <sup>1</sup>correspondence to: [youngrl@utexas.edu](mailto:youngrl@utexas.edu); [hans@utexas.edu](mailto:hans@utexas.edu)

10

11 **Materials and Methods**

12 **Sample collection and RNA extraction**

13 Reproductive males of each focal species were sacrificed and brains were rapidly  
14 dissected and stored to preserve RNA (species-specific details provided below). All animal  
15 care and use practices were approved by the respective institutions. For each species,  
16 RNA from three individuals was pooled to create an aggregate sample for transcriptome  
17 comparison. The focus of this study is to characterize similarity among species with  
18 independent species-level transitions to a monogamous mating system rather than to  
19 characterize individual-level variation in gene expression. Pooled samples are reflective  
20 of species-level gene expression variation of each species and limit potentially  
21 confounding individual variation for species-level comparisons (1, 2). While exploration of  
22 individual variation is critical to identify mechanisms underlying differences in behavioral  
23 expression, high levels of variation between two pooled samples of conspecifics could  
24 obscure more general species-specific gene expression patterns. Note that two pooled  
25 replicates per species would not be sufficiently large for estimating within species  
26 variance, and the effect of an outlier within a pool of two individuals would be considerable.  
27 Therefore, samples were pooled to minimize the effects of individual variation in mating  
28 behavior within each species. For all samples, Total RNA was extracted from brains using  
29 the TRIzol protocol (Invitrogen) following homogenization of brain tissue. RNA quality and  
30 concentration was determined using the Bioanalyzer (Agilent).

31

32 Voles: We used meadow voles (*M. pennsylvanicus*) that were 3<sup>rd</sup> and 4<sup>th</sup> generation  
33 descendants of those captured near Oshkosh, Wisconsin, USA. In this study, meadow  
34 voles were born and raised under a long photoperiod (14:10 h, L: D, lights on at 0700h  
35 CST). We weaned the voles at 19 days of age. We then housed them with littermates until  
36 34 days of age. When we separated littermates, we housed them individually in clear

37 plastic cages (27 x 16.5 x 12.5 cm, l x w x h) and then maintained the males and females  
38 in different rooms. For both species, on the day of euthanasia, each male vole was  
39 anesthetized with isoflurane and rapidly decapitated. Brains were removed and frozen on  
40 dry ice, then stored at -80° C.

41

42 Mice: Adult male and female *P. maniculatus* and *P. californicus* were obtained from the  
43 Peromyscus Genetic Stock Center (Univ. South Carolina, Columbia, SC). Caging, diet,  
44 and bedding were as previously described (3). For each species we created six male-  
45 female pairs. One week after pairing each mouse was lightly anesthetized with isoflurane  
46 and rapidly decapitated. Brains were removed and frozen on powdered dry ice.

47

48 Birds: Fieldwork was conducted in the Harghita region of Transylvania, Romania, to collect  
49 tissue from wild populations of water pipit, *Anthus spinoletta*, and dunnock, *Prunella*  
50 *modularis*, during their breeding season in May-June 2011 (under permit: Ministerial Order  
51 from the Rumanian government no. 1470/2011). Using song playback, four water pipits  
52 and five dunnocks (adult males) were lured into mist nests. Morphometric data was  
53 collected and collated for each bird. Birds were sacrificed by instantaneous decapitation  
54 within four minutes of capture to prevent stress-induced changes to circulating  
55 testosterone levels and gene expression (Deviche et al., 2010; Van Hout et al., 2010).  
56 Whole brains were dissected out, hindbrains were removed and the remaining material  
57 was finely chopped and placed in Eppendorf tubes free from DNA, DNase and RNase,  
58 and flooded with RNAlater to remove any air bubbles. Samples were stored on ice for  
59 between 8 and 12 hours, to allow the RNAlater to permeate the whole tissue (Applied  
60 Biosciences protocol, Ambion), before being stored at approximately -17°C for up to 10  
61 days before being frozen to -80°C.

62

63 Frogs: We sampled two species of dendrobatid frogs the monogamous *Ranitomeya*  
64 *imitator* and nonmonogamous *Oophaga pumilio*. *R. imitator* individuals were captive-bred  
65 F3s, approximately one year old, from a breeding colony originally collected in Chazuta,  
66 San Martin, Peru. Previous research by one of the authors (K. Summers) and his students  
67 on this population confirms monogamous behavior. Adult *R. imitator* males were  
68 purchased from Understory Enterprises (Chatham, ON, Canada) and were sacrificed upon  
69 arrival. Adult individuals of *O. pumilio* were captured in the field by hand on the island of  
70 Bastimentos, Bocas del Toro, Panama, and transferred to a breeding colony housed at  
71 Tulane University, New Orleans, USA. All individuals used in this study were housed with  
72 a female and successfully reared offspring in the captive colony prior to euthanasia and  
73 brain collection. Animals were euthanized by rapid cervical transection and brains were  
74 rapidly removed and immediately frozen on dry ice.

75

76 Fishes: We chose two sister species from the Ectodine cichlid clade of Lake Tanganyika,  
77 Africa: *Xenotilapia spiloptera*, a monogamous species with the male and female forming  
78 a pair bond and providing parental care, and the closely related *X. ornatipinnis*, a  
79 polygynous species in which only the females provide maternal care (6). Adult and  
80 sexually mature individuals (with large gonads with distinct and mature sperm packages)  
81 were captive-bred F2s, with *X. spiloptera* males engaged in a pair-bond and *X. ornatipinnis*  
82 males actively maintaining a territory. Animals were euthanized by rapid cervical  
83 transection, brains rapidly removed, and immediately frozen on dry ice.

84

### 85 **RNA sequencing and mapping**

86 Following hindbrain removal, RNA was extracted from fore- and midbrain tissue of  
87 reproductively active males using Trizol, according to the manufacturer's instructions. An  
88 aliquot of total RNA was then run on a Bioanalyzer Nano RNA chip (Agilent) to confirm

89 RNA integrity was above 7 for each sample. The three individuals within each species  
90 were then pooled in equal RNA amounts before extraction of polyadenylated RNA with  
91 the Poly A Purist kit (Ambion), according to manufacturer's instructions.

92  
93 RNA library preparation and sequencing was performed by the Genome Sequencing and  
94 Analysis Facility (GSAF) at the University of Texas. Library prep was done using the NEB  
95 small RNA kit (cat #E6160L or #E6160S) and sequencing was performed using Illumina  
96 HiSeq. Trimmomatic (7) was used to filter and trim reads using the following parameters:  
97 -phred33 ILLUMINACLIPadapters.fa:3:30:10 LEADING:3 TRAILING:3  
98 SLIDINGWINDOW:4:15 MINLEN:36, where adapters.fa is a fasta file containing a list of  
99 Illumina barcoded adapters. Transcriptomes were assembled *de novo* with Trinity. To  
100 reduce redundancy in the assembly, we ran cd-hit-est (parameters: -c 0.98) Reads were  
101 mapped to *de novo* assembled transcriptome using the Burrows-Wheeler Alignment tool  
102 (bwa-mem, 8). Raw reads for each gene were calculated as the sum of transcript counts  
103 obtained using SAMtools (9). Raw reads were normalized as reads per million (RPM).

104

### 105 **Orthology inference**

106 Within species pairs, contigs were annotated and gene orthology was defined by aligning  
107 assembled contigs and protein sequences of closely related reference genomes  
108 (mammals: *Rattus norvegicus*; birds: *Gallus gallus*; frogs: *Xenopus tropicalus*; fishes:  
109 *Oreochromis niloticus*) using BLAST (e-value = 1e-5). Genes that reciprocally BLAST to  
110 the clade-specific reference genome in both the monogamous and nonmonogamous  
111 species were called orthologous (number of orthologous genes in each clade: 11,051  
112 voles; 10,519 mice; 6993 birds; 7035 frogs; 13,135 fishes).

113

114 Clade specific gene loss and duplication events obscure the evolutionary history of genes,  
115 such that in many cases gene families will contain distinct numbers of paralogs in different  
116 clades. One solution is to focus on orthologous gene groups. We tested similarity in  
117 transcriptomic profiles of monogamous species across divergent vertebrate clades using  
118 orthologous gene group (OGG) expression patterns. OGGs were identified using the  
119 sequence based ortholog calling software package OrthoMCL (10). Protein sequences of  
120 the reference genomes (listed above) were organized into orthologous gene groups based  
121 on sequence similarity. For each reference genome, genes were grouped into orthologous  
122 gene groups (as paralogs) when sequence similarity was higher among genes within  
123 species than between species. This approach of ortholog calling improves substantially  
124 on reciprocal best BLAST hits, which results in loss of up to 60% of true orthologous  
125 relationships as it eliminates all paralogous genes (11). Alternatively, databases of OGGs  
126 (12, 13) are most useful for traditional model systems with well-sequenced and annotated  
127 genomes. Our method of ortholog calling by sequence similarity of target species using  
128 OrthoMCL (10) identified 1979 OGGs, while only 355 OGGs were identified using a  
129 database approach (eggNOG). While the remaining analysis characterizing monogamy-  
130 related gene-expression patterns focused on OrthoMCL OGGs, similar results were found  
131 when eggNOG OGGs were analyzed. Because our study focused on identifying  
132 monogamy-related expression patterns, and genes in the same OGG were generally  
133 concordant in directionality of expression differences (Fig. S2; Table S3), when an OGG  
134 contained more than one gene (i.e., paralogs: Fig. S2; voles: 573, 29.0%; mice: 521,  
135 28.3%; birds: 320, 16.2%; frogs: 227, 11.5%; fishes: 730, 36.9%) the gene with the highest  
136  $\log_2$  fold-difference between the monogamous and nonmonogamous species pairs was  
137 used for the remainder of the analysis. Similar overall patterns were obtained using the  
138 mean  $\log_2$  fold-differences for the orthologous gene groups.

139

140 **Differential Expression Analysis**

141 To assess concordance of OGG expression in monogamous species across clades we  
142 used the differential expression analysis software package DESeq2 (14). For each  
143 species, raw read counts for the 1979 OGGs identified across all species were included  
144 in the differential expression analysis. Species were labeled as monogamous or  
145 nonmonogamous such that species from distinct clades served as biological replicates in  
146 the analysis. DESeq2 was performed on different evolutionary sub-groups including  
147 mammals, amniotes, tetrapods, and all clades. OGGs with an expression difference of +/-  
148 1 log<sub>2</sub> fold-difference and p-value < 0.1 were characterized as differentially expressed.  
149 This fairly liberal cut off captures genes that are generally concordant in expression  
150 direction across clades that may otherwise be eliminated.

151

152 To extract patterns of gene expression shared among monogamous species across  
153 clades, we utilized a hypergeometric approach, the R package Rank-Rank  
154 Hypergeometric Overlap (RRHO) (15). In RRHO analysis, rank log<sub>2</sub> fold-differences in  
155 monogamous vs. nonmonogamous mRNA levels are binned into steps (we used the  
156 default settings which binned our 1979 OGGs into 45 steps). Each step can be set as a  
157 threshold making a continuous threshold scale of differential expression unique to each  
158 clade. At each pairwise log<sub>2</sub> fold-difference threshold (binned OGG step) enrichment of  
159 rank correlations between clades is determined using a hypergeometric distribution.  
160 RRHO corrects p-values for multiple comparisons using the B-Y procedure (16). Overall  
161 significance of rank correlations for each pairwise comparison of clades was calculated  
162 using permutation analysis (17).

163

164 **Novel candidates and GO analysis**

165 To identify the most robust candidate genes associated with a monogamous mating  
166 system across species, we combined the results of our differential gene expression  
167 analysis with the RRHO analysis (Fig. 4). Specifically, we identified genes that were  
168 concordantly differentially expressed (at +/- 1 log<sub>2</sub> fold-difference) between monogamous  
169 and nonmonogamous species in four of the five clades, and equivalently identified in at  
170 least five of the ten RRHO comparisons which allows one clade to lack concordant  
171 expression (Fig. 5; Dataset S1). To capture genes that are generally concordant in  
172 expression direction across clades, which may not be captured by threshold differential  
173 expression approaches, we used the more liberal expression cut off of +/- 1 log<sub>2</sub> fold-  
174 difference. Notably, these candidate genes have known roles in synaptic transmission,  
175 neuroplasticity, and neurological function among other functions possibly reflecting  
176 similarities among monogamous species in the mechanisms through which the brain  
177 reward circuitry becomes associated with social affiliation (e.g., reproductive and parental  
178 behavior). Several candidate genes are critical for neuronal development as well as  
179 synaptic function and plasticity, including: Low Density Lipoprotein Receptor-Related  
180 Protein 6 (*LRP6*) (18), the Wnt inhibitor Adenomatosis Polyposis Coli Down-Regulated 1  
181 (*APCDD1*) (19), the Lysophosphatidic Acid Receptor 1 (*LPAR1*) (20), and Notch1 (21).  
182 Relatedly, the candidate gene (*Dscam*) remodels microcircuitry through regulation of  
183 dendritic arborization (22), and in this way is also thought to enhance learning and memory  
184 (27). Two genes on the candidate list have documented roles in synaptic transmission.  
185 The excitatory neurotransmitter Metabotropic Glutamate Receptor 6 (*GRM6*) is the only  
186 known metabotropic glutamate receptor to directly mediate synaptic transmission in the  
187 nervous system (23). Huntingtin Interacting Protein (*Hip1*) regulates AMPA receptor  
188 trafficking (24) and, interestingly, also enhances androgen receptor-mediated transcription  
189 (25). Finally, candidate genes lysine methyltransferase 2C (*KMT2c*) (26) and solute carrier  
190 family 6 member 17 (*SLC6a17*) (27) are involved in cognitive function and cause



191 intellectual disability when mutated. Note that in mice, the candidate genes *Tnik*, *Lpar1*,  
192 *Man2a1*, and *Lrp6* predominantly show expression in the forebrain, whereas the remaining  
193 genes are either broadly distributed throughout the brain (28) or their brain expression has  
194 yet to be mapped.

195

196 To characterize similarity of function of monogamy-related OGGs across species we  
197 assessed concordance of over- and under-represented GO annotations using BiNGO  
198 (29). For each clade, enrichment of GO terms was assessed for OGGs up- or down-  
199 regulated at  $\log_2$  fold-difference  $\pm 1$  with the complete list of 1979 OGGs as the reference  
200 set. BiNGO uses a parent-child approach taking into account dependencies among GO  
201 terms. GO terms were identified as over- or under-represented using a hypergeometric  
202 test. p-values were then adjusted for multiple comparisons using Benjamini & Hochberg's  
203 FDR correction.

204

### 205 **Phylogenetic, life history, and gene expression distances**

206 As described above, we chose species pairs with similar ecological attributes (except for  
207 mating system characteristics) for each clade to control for the potential confounding  
208 effects of a species' natural history. For each species, we consulted the literature to score  
209 characteristics of mating system (i.e., presence of a pair bond, territoriality, and direct and  
210 indirect paternal care) and ecology (i.e., habitat complexity, activity patterns, diet, and  
211 gregariousness) (SI Appendix, Tables S1 and S2).

212

213 To assess the contribution of evolutionary history and mating system on neural gene  
214 expression, we compared evolutionary distance, and similarities in mating system  
215 characteristics to gene expression distance between all pairs (Fig. 6A and B). A mating  
216 system score was calculated for each species as the sum of the mating system

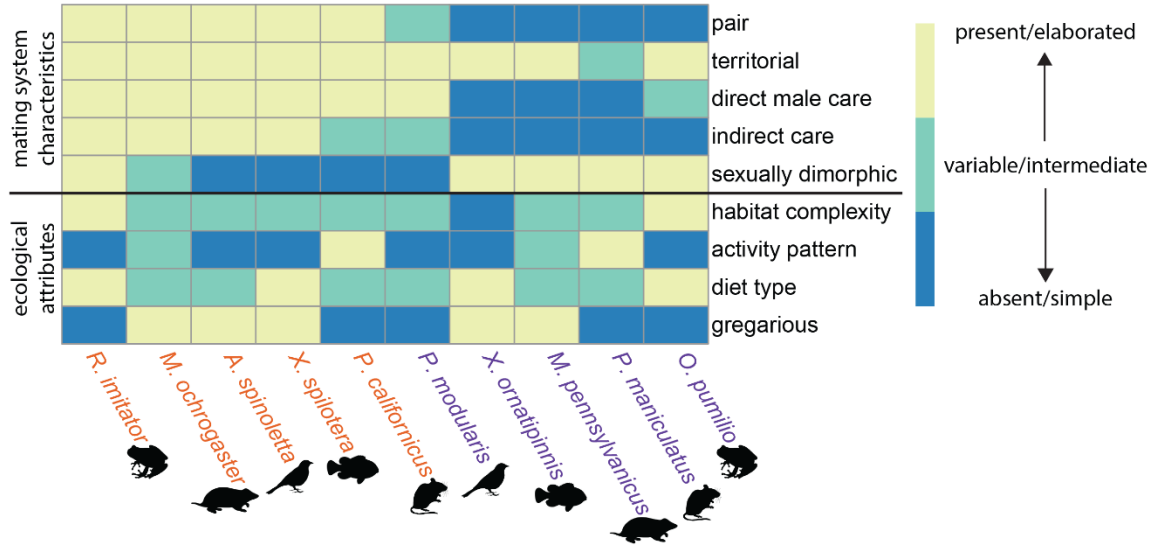
217 characteristics, where higher values indicate more elaborated monogamy (i.e., males  
218 consistently form pair bonds, provide both direct and indirect parental care, exhibit high  
219 levels of territoriality, and are less sexually dimorphic). We estimated evolutionary  
220 divergence between clades using TimeTree (30). For each clade, expression distance  
221 was estimated as the Euclidean distance in  $\log_2$  fold-difference of expression between the  
222 monogamous and nonmonogamous species (31). To remove the variation in gene  
223 expression and mating system characteristics due to phylogeny, we calculated  
224 phylogenetic independent contrast on the first principal component (PC) of gene  
225 expression and mating system score using the R package ape (32). In both the Euclidean  
226 distance comparisons (Fig. 6A and B) and the principal components analysis (PCA) (Fig.  
227 6C), we limited the OGGs to include only those that were differentially expressed ( $\pm 1 \log_2$   
228 fold difference in at least one clade) and variable (upper quartile of variance across all  
229 species). This subset included 401 OGGs that overlap significantly with candidate OGGs  
230 discovered using RRHO (overlap = 35, hypergeometric distribution  $p = 7.6e-07$ ) and  
231 differential expression analysis (overlap = 83, hypergeometric distribution  $p = 3.7e-13$ )  
232 (Fig. S7). A PCA including  $\log_2$ -transformed RPM for this subset of OGGs in all 10 species  
233 was performed using the prcomp function in R. PC1 described 22.8% of the variation in  
234 expression.

235

### 236 **Accessibility of Data and Data Analyses**

237 RNA sequencing data discussed in this publication have been deposited in NCBI's Gene  
238 Expression Omnibus (33) and are accessible through GEO Series accession number  
239 GSE123301 (<https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE123301>).

240 Additional data and data analyses scripts in R and Python are published and publically  
241 available as a dataverse at the Texas Data Repository  
242 (<https://dataverse.tdl.org/dataverse/monogamy>).



244

245 **Figure S1.** Ecological attributes and mating system characteristics of study species.

246 Species names in orange are the monogamous species, those in purple are the

247 nonmonogamous species for each clade. Color indicates presence or degree of trait

248 elaboration in that species unless otherwise indicated below. Yellow indicates the trait

249 listed on the right is absent or 'simple', green indicates intermediate, and blue indicates

250 present or 'elaborated'. Five mating system characteristics were scored, including: pair

251 bond formation, defense of breeding territories, male engagement in offspring

252 provisioning, cleaning, or transport, males actively defend nests or provision females, and

253 presence of sexual dimorphism (size or color elaboration). Four additional ecological

254 attributes were also scored, including: habitat complexity (simple = yellow,

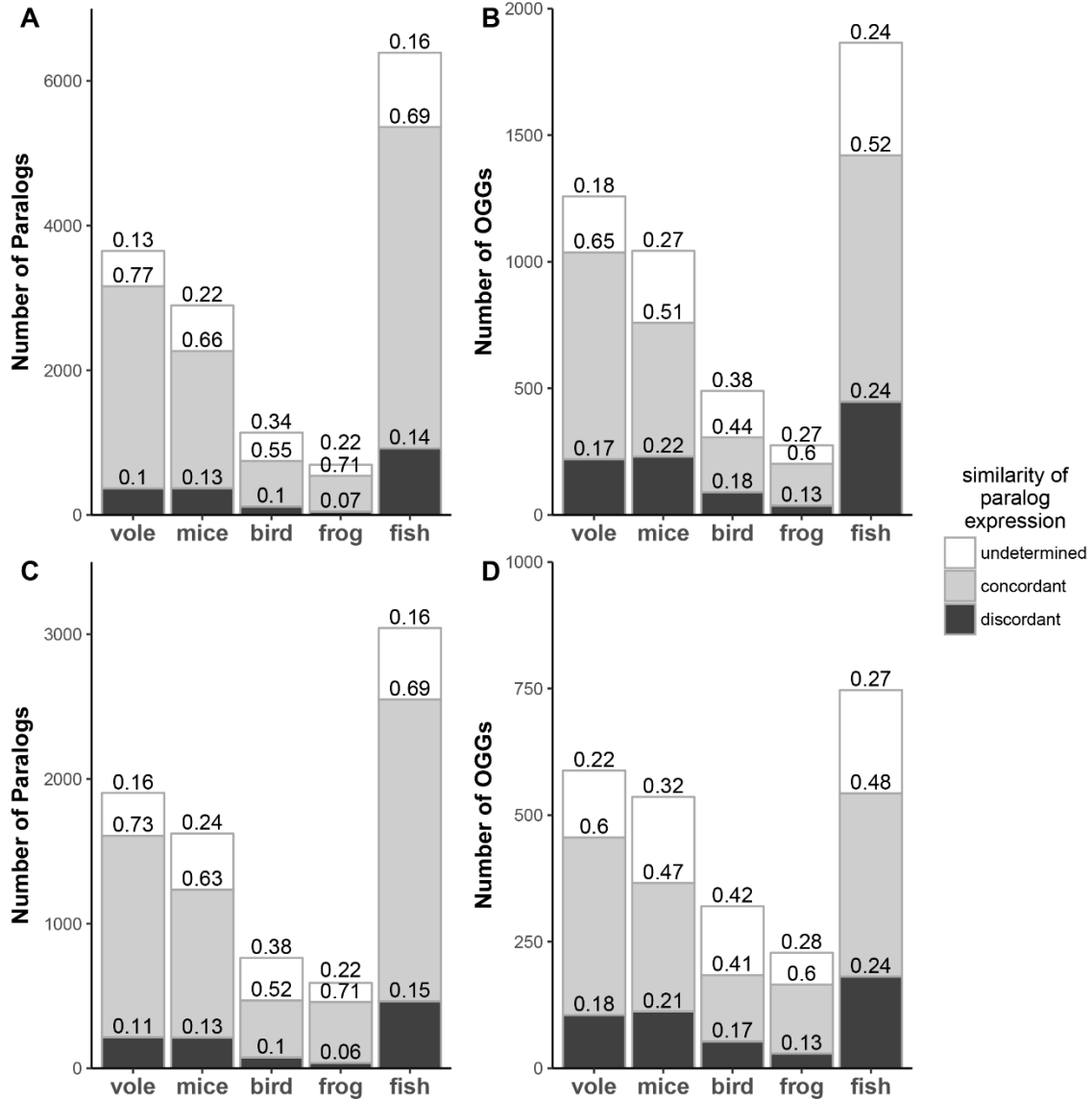
255 various/intermediate = green, or complex environments = blue), activity pattern (diurnal =

256 yellow, crepuscular/seasonally variable = green, and nocturnal = blue), diet type (primary

257 diet is "herbivorous" = yellow, "omnivorous" = green, and "carnivorous" = blue), and

258 whether communal or gregarious behaviors are observed outside of a reproductive

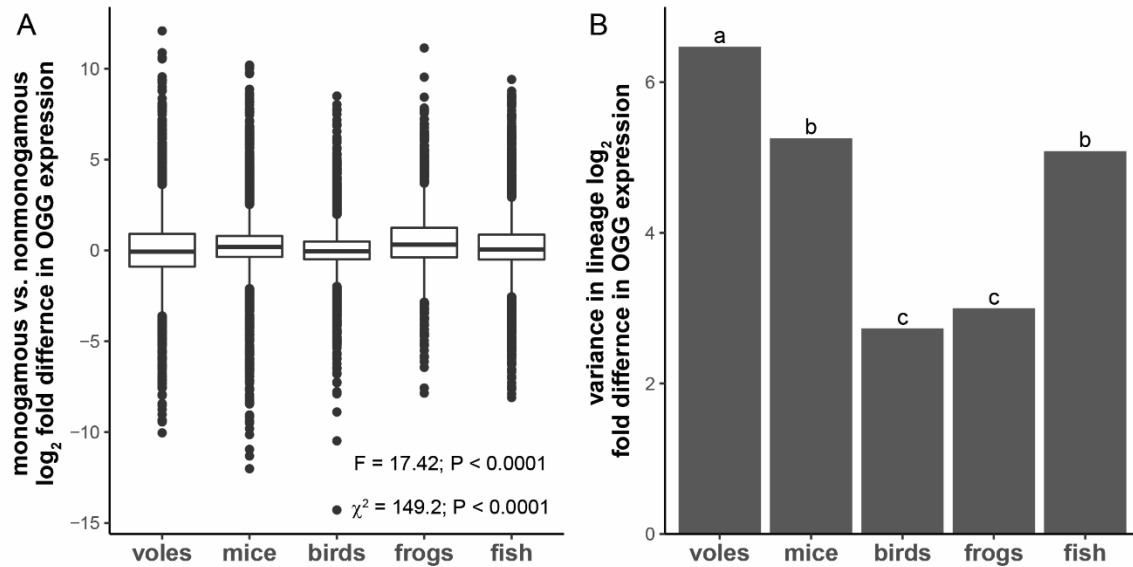
259 context. Details and references in (SI Appendix, Tables S2 and S3).



260

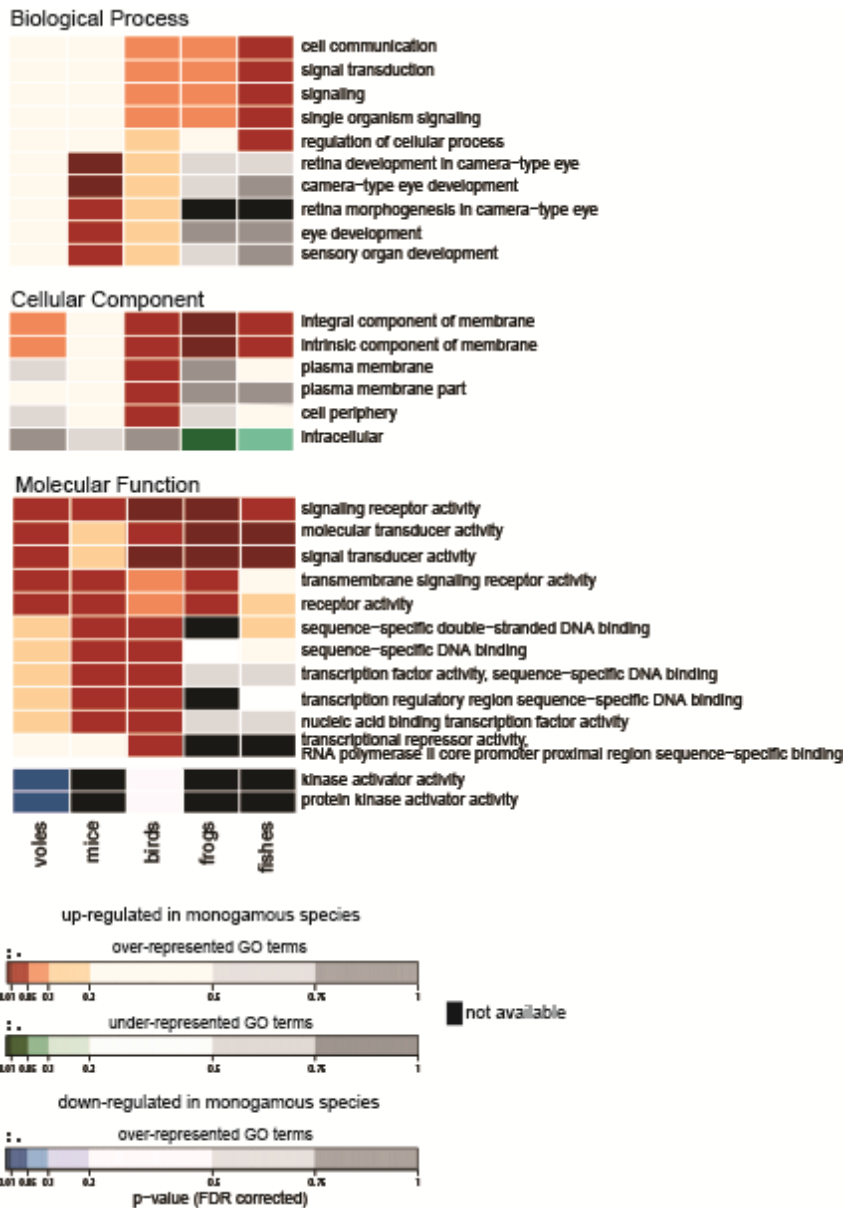
261 **Figure S2.** To compare neural gene expression across all clades, genes were grouped  
 262 into orthologous gene groups (OGGs) using orthoMCL. OrthoMCL identified 6125 OGGs  
 263 shared across the rat, chicken, frog, and tilapia reference genomes. Of those, 44-76%  
 264 were identified using RNAseq (Table S3). Due to variation in paralog identification,  
 265 different numbers of genes were included for each clade (voles: 7540, mice: 7046, birds:  
 266 4914, frogs: 3703, fish: 10154) (Table S3). 1979 OGGs were shared across all clades  
 267 with different numbers of paralogs in each clade (Table S4). We assess the concordance  
 268 in paralog expression at the gene (A and C) and OGG (B and D) levels for all clade-specific

269 OGGs (A and B) and for OGGs shared across clades (C and D). Paralogs are generally  
270 concordant in expression direction between the monogamous and nonmonogamous  
271 species (i.e. higher or lower expression). The paralog with the largest fold-difference in  
272 expression between the monogamous and nonmonogamous species pairs was selected  
273 as the representative gene for each OGG. If this value was  $< \pm 1 \log_2$  fold-difference the  
274 OGG and its containing paralogs were labeled at “undetermined” (white; all plots). The  
275 number of paralogs directionally discordant from the representative gene are shown for  
276 each clades (black; A and C). Any OGG containing a discordant paralog is considered  
277 discordant (black; B and D). The number of concordant paralogs and OGGs are shown in  
278 grey. Values at the boundaries indicate the proportion of paralogs (A and C) or OGGs  
279 containing paralogs (B and D).



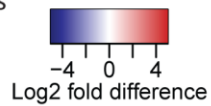
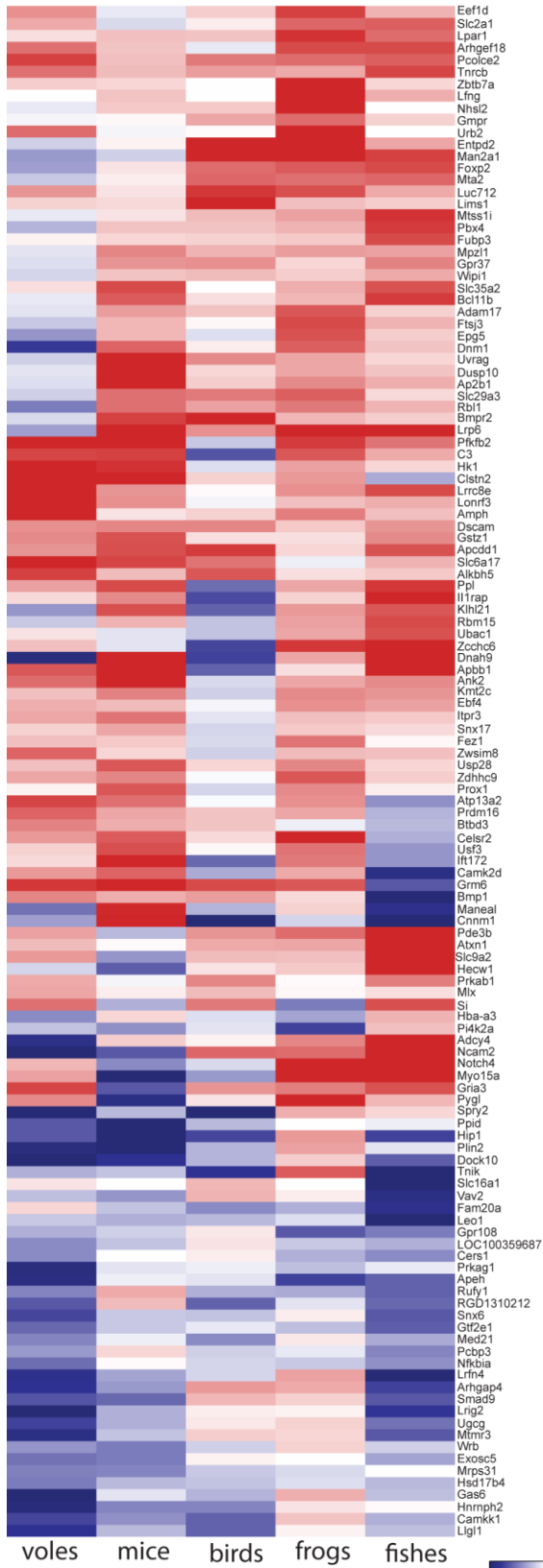
280

281 **Figure S3.** Log<sub>2</sub> fold difference (monogamous vs. nonmonogamous) in orthologous gene  
 282 group (OGG) expression for all 1979 orthologous gene groups in each clade (A). Variance  
 283 in log<sub>2</sub> fold-difference (monogamous vs. nonmonogamous) in OGG expression (B). When  
 284 more than one gene is present in an orthologous gene group the gene with the highest  
 285 log<sub>2</sub> fold-difference was selected. Log<sub>2</sub> fold-difference in OGG expression in monogamous  
 286 species is slightly skewed toward increased expression in mice and frogs (median = 0.19  
 287 and 0.31, respectively). Birds and frogs exhibit the smallest variance (B) and narrowest  
 288 interquartile range of expression differences (A) between monogamous and  
 289 nonmonogamous species. Overall, means (Kruskall-Wallis  $\chi^2$ ) and variances (Levene's  
 290 test) differ among the clades. F-tests for equality of variances were used to compare  
 291 variances for all pairwise clades. Clades separated by letters significantly differ after  
 292 correcting p values for multiple hypothesis testing (p \* number of comparisons).



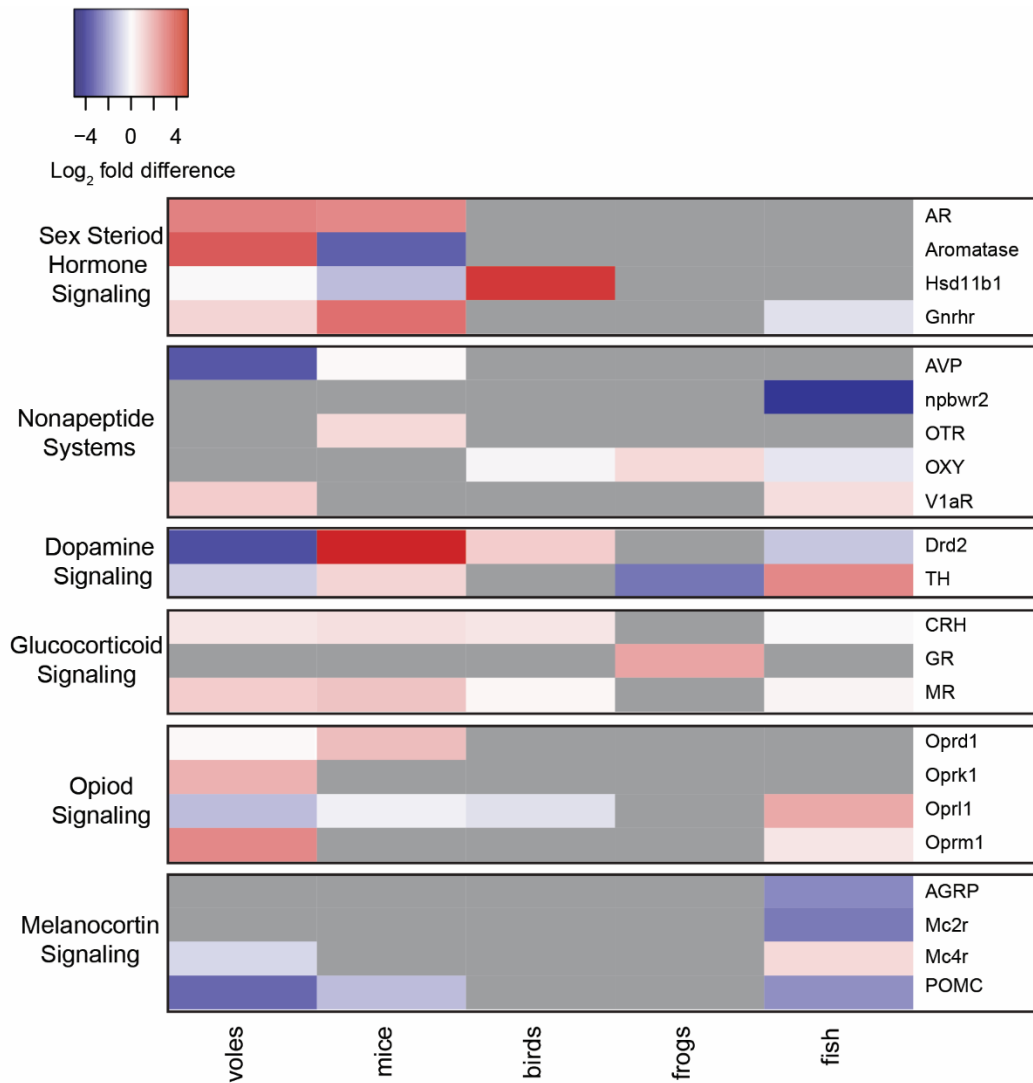
293

294 **Figure S4.** To characterize similarity of function of monogamy-related orthologous gene  
 295 groups (OGGs) across species we assessed concordance of over- and under-represented  
 296 GO annotations using BiNGO (29). For each clade, enrichment of GO terms was assessed  
 297 for OGGs up- or down-regulated at  $\log_2$  fold-difference  $\pm 1$  with the complete list of 1979  
 298 OGGs as the reference set.





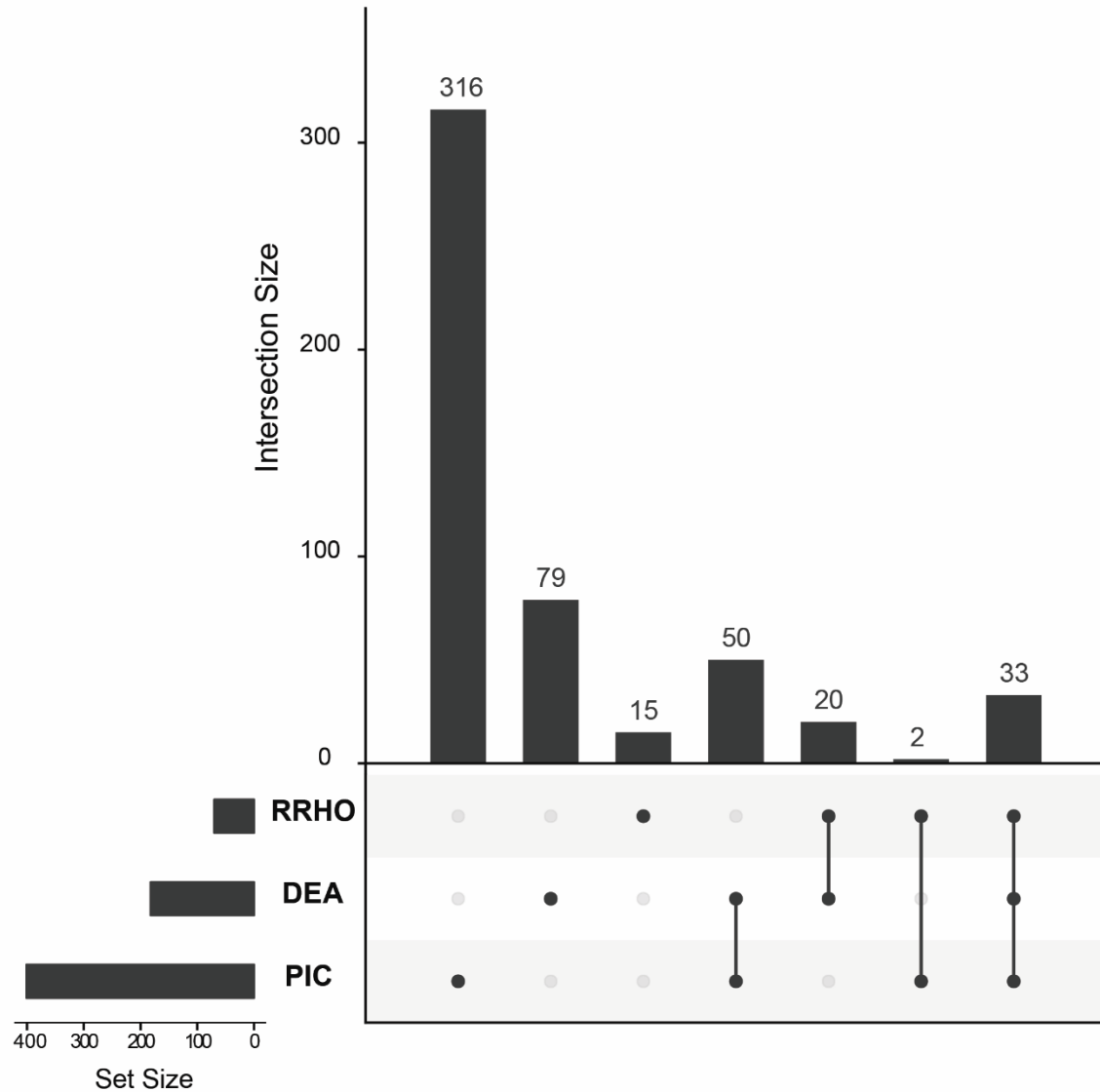
300 **Figure S5.** Genes concordantly expressed in monogamous brains across vertebrates.  
301 123 genes were identified with DESeq2  $\log_2$  fold-difference  $\pm 1$  and p-value  $< 0.1$ .  
302 DESeq2 provides a base mean and a  $\log_2$  fold-difference. As with any other analysis with  
303 replicates, not all replicates are expected to show the same directional difference in gene  
304 expression. However, on average, these 123 OGGs are differentially expressed between  
305 monogamous and nonmonogamous species across all clades. Gene symbols are  
306 provided where available, when gene symbols are not available the Ensembl rat protein  
307 id is provided.



308

309 **Figure S6.** Relative expression (log<sub>2</sub> fold-difference) in monogamous versus  
 310 nonmonogamous species of each clade for 22 candidate genes previously implicated in  
 311 regulating complex social behavior across vertebrates. These often studied candidate  
 312 genes represent six neuroendocrine and neuro-modulatory systems that have previously  
 313 implicated in the regulation of (aspects of) monogamous behavior or, more generally,  
 314 complex social behavior. Reds (from light to dark) indicate increased expression in the  
 315 monogamous species; blues (from light to dark) indicate decreased expression in the  
 316 monogamous species. Grey indicates that expression data was not available for one or  
 317 both species of that clade. Note that in several clades many of these candidate genes

318 were not detected in either the RNAseq analysis or during the orthology inference step,  
319 therefore limiting the interpretability of these data.  
320



321

322 **Figure S7.** 401 OGGs exhibit both differential expression between the monogamous  
 323 and nonmonogamous species in at least one clade (at +/- 1 log<sub>2</sub> fold difference) and  
 324 high expression (RPM) variance across all species (variance in the upper quartile). This  
 325 subset included 401 OGGs used in the phylogenetic independent contrast (PIC) overlap  
 326 significantly with 70 OGGs identified by RRHO (overlap = 35, hypergeometric distribution  
 327  $p = 7.6e-07$ ) and 182 OGGs identified using differential expression analysis (overlap =  
 328 83, hypergeometric distribution  $p = 3.7e-13$ ). Intersections were identified and plotted  
 329 using the R package UpSetR (34). P-values are corrected for multiple hypothesis

330 testing. RRHO candidate OGGs are among the most up- or the most- down-regulated in  
331 6 of the 10 comparisons (as in Fig. 5). DEA candidate OGGs include those identified  
332 with DESeq2 across all vertebrates ( $\log_2$  fold-difference  $\pm 1$  and p-value  $< 0.1$ ) or those  
333 that exhibit a  $\pm 1 \log_2$  fold expression difference between the monogamous and  
334 nonmonogamous species in at least four clades (as in Fig. S5 and Fig. 5, respectively).  
335

**Table S1.** Mating system characteristics of each species used.

<b>species</b>	<b>pair bond</b>	<b>territorial</b>	<b>paternal offspring care</b>	<b>indirect paternal care</b>	<b>sexually dimorphic</b>
<i>M. ochrogaster</i>	Yes (35, 36)	Yes (36, 37)	Yes (38)(39)	Yes (40, 41)	Various (3, No: 8, Yes: in the lab: 9)
<i>M. pennsylvanicus</i>	No (44)	Yes (45, 46)	No (41)	No (41)	Yes (47)
<i>P. californicus</i>	Yes (48, 49)	Yes: <i>highly</i> aggressive and territorial (50, overlapping territories: , 51)	Yes (52–54)	Yes (55–58)	No (most sources say no;but, brain regions are sexually dimorphic 59)
<i>P. maniculatus</i>	No (60, 61)	Yes: much less so than <i>P. californicus</i> (62, 63)	No (64, 65) (note: pup licking/nest sitting reported in one study)	No (no reports)	Yes (43)
<i>A. spinoletta</i>	Variable (86%: 66)	Yes (66)	Yes (67)	Yes: alarm calling, feeding & incubating females (66)	No (66)
<i>P. modularis</i>	Variable depending on space use (68, 69)	Yes: mate guarding (70)	Yes: provisioning (70)	Sometimes (71)	No (72)
<i>R. imitator</i>	Yes (73, 74)	Yes (73)	Yes: egg attendance; tadpole feeding and transport (75)	Yes (73)	Yes: (females are slightly, but significantly larger 73)
<i>O. pumilio</i>	No (76, 77)	Yes (76, 78)	Yes: intermediate: periodic water shedding (79)	No (80)	Yes: (females larger than males, C. Richards-Zawacki pers. obs.)
<i>X. spilotera</i>	Yes (81)	Yes (81)	Yes (81, 82)	Yes (81)	No (HAH pers. obs.)
<i>X. ornatipinnis</i>	No (82)	Yes: 'roving territories' (HAH pers. obs.)	No (82)	No (82)	Yes: chin pigmentation in males (HAH pers. obs.)

**Table S2.** Ecological attributes of the study species.

<b>species</b>	<b>habitat complexity</b>	<b>activity</b>	<b>diet</b>	<b>communal/gregarious outside of breeding</b>
<i>M. ochrogaster</i>	Intermediate: un-grazed grassland/savanna (37)	Various, seasonal (83)	Omnivorous: forbs, foliage, fruits, tubers, insects (84, 85)	Yes: communal group living (86, 87)
<i>M. pennsylvanicus</i>	Intermediate: grasslands, woodlands, riparian (88)	Various depending on landscape and season (89)	Omnivorous: grasses, foliage, fruits, tubers, insects (89, 90)	Yes: communal nesting in winter populations (91, 92)
<i>P. californicus</i>	Intermediate: dense chaparral/broad-sclerophyll woodland (48)	Nocturnal (93)	Omnivorous: acorns, flowers seeds, fungi and arthropods (94)	No: non-overlapping territories (51)
<i>P. maniculatus</i>	Various (95)	Nocturnal (96)	Omnivorous: mostly insects & arthropods, but also seeds, flowers, and leaves (63, 97)	No (62, 63)
<i>A. spinoletta</i>	Intermediate: open, heterogeneous habitats: shrub lands, medium woodlands, wet inlands (98, 99)	Diurnal	Omnivorous: mostly insects and seeds (100)	Yes: feeds in large groups outside of the breeding season (101)
<i>P. modularis</i>	Intermediate: woodlands, hedgerows, gardens	Diurnal	Omnivorous: mostly insects and seeds (102)	No: solitary in winter (103)
<i>R. imitator</i>	Complex: leaf litter, secondary to old growth forest (73)	Diurnal	Carnivorous: ants, mites, beetles (A. Stuckert, pers. obs.)	No (K. Summers pers. obs.)
<i>O. pumilio</i>	Complex: premontane forest leaf litter	Diurnal (104)	Carnivorous: ants, mites, beetles, springtails (105, 106)	No; (76–78)
<i>X. spilotera</i>	Intermediate (82, 107)	Diurnal (HAH pers. obs.)	Carnivorous (108)	Yes (82, 108)
<i>X. ornatipinnis</i>	Simple, sand (82)	Diurnal (HAH pers. obs.)	Carnivorous (108)	Yes (82, 108)

342 **Table S3.** For each species, the number of genes and orthologous gene groups (OGGs)  
 343 are shown. For each clade the number of shared genes and OGGs are shown. For each  
 344 evolutionary group (i.e., mammals, amniotes, tetrapods, and vertebrates) the number of  
 345 shared OGGs is shown. The starting set is limited to 6125 orthologous OGGs identified  
 346 by orthoMCL as containing genes from each of references genome used in this study (i.e.,  
 347 rat, chicken, *Xenopus*, and tilapia).

348

Species	Genes	OGGs	clade genes	clade OGGs	mammal OGGs	amniote OGGs	tetrapod OGGs	vertebrate OGGs
<i>M. ochrogaster</i>	9733	6007	7840	5449 (0.890)	4746 (0.775)	3508 (0.573)	2081 (0.340)	1979 (0.323)
<i>M. pennsylvanicus</i>	8088	5555						
<i>P. californicus</i>	7936	5578	7046	5193 (0.839)	4746 (0.775)	3508 (0.573)	2081 (0.340)	1979 (0.323)
<i>P. manipulatus</i>	8298	5679						
<i>A. spinoletta</i>	5378	4914	4914	4267 (0.697)	4746 (0.775)	3508 (0.573)	2081 (0.340)	1979 (0.323)
<i>P. modularis</i>	6441	5378						
<i>R. imitator</i>	6170	5035	3703	3284 (0.536)	4746 (0.775)	3508 (0.573)	2081 (0.340)	1979 (0.323)
<i>O. pumilio</i>	4895	4098						
<i>X. spiloptera</i>	11296	5889	10154	5628 (0.919)	4746 (0.775)	3508 (0.573)	2081 (0.340)	1979 (0.323)
<i>X. ornatipinnis</i>	11401	5882						

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352 **Table S4.** For the 1979 orthologous gene groups (OGGs) shared among the clades  
 353 compared, we show the total numbers and proportions of genes, genes with paralogs, and  
 354 paralogs with directionally concordant, discordant, and undetermined expression for each  
 355 clade. In addition, we show the number and proportion of OGGs containing paralogs and  
 356 OGGs containing directionally concordant, discordant, undetermined paralogs. The  
 357 paralog with the largest fold-difference in expression between the monogamous and  
 358 nonmonogamous species pairs was selected as the representative gene for each OGG.  
 359 If this value was  $< \pm 1 \log_2$  fold-difference the OGG and its containing paralogs were  
 360 labeled as “undetermined.”

361

Clade	genes	total paralogs (proportion of genes)	Paralogs (proportion of paralogs)			OGGs with paralogs (proportion of OGGs)	OGGs (proportion of OGGs with paralogs)		
			concordant	discordant	undetermined		concordant	discordant	undetermined
voles	3294	1903 (0.58)	1392 (0.73)	215 (0.11)	296 (0.16)	588 (0.30)	351 (0.60)	105 (0.18)	132 (0.22)
mice	3066	1623 (0.53)	1023 (0.63)	212 (0.13)	388 (0.24)	536 (0.27)	254 (0.47)	112 (0.21)	170 (0.32)
birds	2421	762 (0.31)	395 (0.52)	74 (0.10)	293 (0.38)	320 (0.16)	131 (0.41)	53 (0.17)	136 (0.43)
frogs	2341	590 (0.25)	421 (0.71)	37 (0.06)	132 (0.22)	228 (0.12)	136 (0.60)	29 (0.13)	63 (0.28)
fish	4275	3043 (0.71)	2087 (0.69)	463 (0.15)	493 (0.16)	747 (0.38)	362 (0.48)	181 (0.24)	204 (0.27)

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363

364 **Table S5.** For each clade, taxon median divergence time estimates and references are  
 365 reported from the TimeTree Database (109). No data is available in the TimeTree  
 366 Database for *Xenotilapia spiloptera*. References used to estimate divergence time between  
 367 the *Xenotilapia* species are provided.

368

<b>Taxon A</b>	<b>Taxon B</b>	<b>divergence time median (MYA)</b>	<b>References:</b>
<i>M. pennsylvanicus</i>	<i>M. ochrogaster</i>	10.5	(110–113)
<i>P. maniculatus</i>	<i>P. californicus</i>	11.5	(110, 111, 113, 114)
<i>Peromyscus</i>	<i>Microtus</i>	18.9	(110, 111, 113, 115–120)
<i>P. modularis</i>	<i>A. spinoletta</i>	29	(121–127)
<i>O. pumilio</i>	<i>R. imitator</i>	34.2	(128, 129)
<i>X. ornatipinnis</i>	<i>X. spiloptera</i>	2.5	(6, 130)

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370 **Table S6.** Maximum, mean, and median  $-\text{Log}_{10}$  p-values for each quadrant of the Rank  
 371 Rank Hypergeometric Overlap analysis (Fig. 4).

clade A	clade B	Concordant		Discordant	
		Downs	Ups	Down,Up	Up,Down
		Max Pvalues			
voles	mice	35.41	6.21	15.70	6.61
voles	birds	1.71	0.00	0.00	0.00
voles	frogs	22.33	18.05	20.06	12.35
voles	fish	22.54	3.40	8.33	6.07
mice	birds	7.52	10.02	0.27	5.23
mice	frogs	14.84	14.17	14.84	13.36
mice	fish	16.95	4.98	9.93	9.55
birds	frogs	7.91	2.22	8.11	0.00
birds	fish	5.81	5.43	0.21	0.00
frogs	fish	16.72	6.42	8.07	14.46

**Mean Pvalues**

voles	mice	12.10	0.18	1.52	0.35
voles	birds	0.17	0.00	0.00	0.00
voles	frogs	10.48	2.24	6.51	0.94
voles	fish	8.36	0.16	0.39	0.35
mice	birds	1.88	0.51	0.00	0.22
mice	frogs	5.27	0.84	3.18	0.83
mice	fish	6.52	0.26	0.45	0.86
birds	frogs	2.54	0.09	2.38	0.00
birds	fish	1.10	0.59	0.00	0.00
frogs	fish	7.14	0.28	0.26	3.82

**Median Pvalues**

voles	mice	11.51	0.00	0.00	0.00
voles	birds	0.00	0.00	0.00	0.00
voles	frogs	11.27	0.00	4.89	0.00
voles	fish	7.67	0.00	0.00	0.00
mice	birds	0.93	0.00	0.00	0.00
mice	frogs	5.38	0.00	0.00	0.00
mice	fish	6.02	0.00	0.00	0.00
birds	frogs	2.29	0.00	1.28	0.00
birds	fish	0.01	0.00	0.00	0.00
frogs	fish	7.29	0.00	0.00	3.31

372

373 **Table S7.** Expression of novel candidate genes in monogamous vs. nonmonogamous  
 374 species pairs.

Symbol	Name	Protein ID	log <sub>2</sub> fold-difference in expression				
			voles	mice	birds	frogs	fish
Ank2	Ankyrin 2	ENSRNOP00000015386	4.09	10.01	1.70	2.90	4.52
Apcdd1	Adenomatosis Polyposis Coli Down-Regulated 1	ENSRNOP00000059242	2.82	2.01	4.53	0.28	3.99
Dscam	Down Syndrome Cell Adhesion Molecule (cell adhesion, PAK pathway)	ENSRNOP00000022476	5.45	2.19	2.06	-0.49	1.44
NA	ENSRNOP00000013363	ENSRNOP00000013363	1.48	8.34	1.22	1.35	1.64
ENTPD2	Ectonucleoside triphosphate diphosphohydrolase 2	ENSRNOP00000018560	1.37	1.01	5.26	5.27	2.43
Fam20a	Golgi associated secretory pathway pseudokinase	ENSRNOP00000005367	1.99	-2.83	-2.16	-2.27	-4.85
Grm6	Glutamate Receptor, Metabotropic 6	ENSRNOP00000000249	7.10	5.86	5.26	3.17	2.96
Gtf2e1	General Transcription Factor IIE Subunit 1	ENSRNOP00000039601	-1.12	0.23	-1.32	-1.97	-1.55
Hecw1	HECT, C2 and WW domain containing E3 ubiquitin protein ligase 1	ENSRNOP00000021703	1.87	-2.90	1.38	1.53	7.70
Hip1	Huntingtin interacting protein 1	ENSRNOP00000031153	-2.71	-7.67	-3.54	1.64	-4.70
Kmt2c	Lysine methyltransferase 2C	ENSRNOP00000063937	2.76	2.17	-0.39	2.96	2.55
Lpar1	Lysophosphatidic Acid Receptor 1	ENSRNOP00000043652	3.30	1.77	1.45	2.90	2.72
Lrp6	Low Density Lipoprotein Receptor-Related Protein 6	ENSRNOP00000063261	3.25	6.30	2.26	7.21	5.89
Lrrc8e	Leucine rich repeat containing 8 VRAC subunit E	ENSRNOP00000037487	10.54	2.71	0.23	1.82	4.15
Man2a1	Mannosidase, alpha, class 2A, member 1	ENSRNOP00000020767	1.93	-1.25	4.46	5.72	4.38
Mpzl1	Myelin protein zero-like 1	ENSRNOP00000004376	-0.80	2.45	3.19	1.49	2.18
Notch1	Notch 1	ENSRNOP00000026212	4.01	2.02	-1.42	6.51	7.40
PCOLCE2	Procollagen C-endopeptidase enhancer 2	ENSRNOP00000067519	6.40	0.41	3.16	2.59	3.69
Rbl1	RB transcriptional corepressor like 1	ENSRNOP00000063017	-0.75	1.50	2.23	2.29	2.53
Slc29a3	Solute carrier family 29 member 3	ENSRNOP00000000689	1.88	1.95	3.11	2.91	0.30
Slc6a17	Solute carrier family 6 member 17	ENSRNOP00000065179	9.55	3.58	4.83	-2.68	1.37
SMAD9	Mothers against decapentaplegic homolog 9	ENSRNOP00000000102	-2.70	-2.04	-2.01	0.30	-3.20
Tnik	TRAF2 and NCK interacting kinase	ENSRNOP00000016799	-1.73	-3.35	-4.10	3.00	-5.80
Tnrc6b	Trinucleotide Repeat Containing 6B	ENSRNOP00000049430	5.15	-1.43	3.90	3.51	5.94

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377 **Dataset S1.** Novel candidate genes associated with monogamous mating system across  
378 species. Gene functions are provided by Gene Cards ([www.genecards.org](http://www.genecards.org)) unless  
379 otherwise noted. Localizations are provided by the Allen Mouse Brain Atlas (28).

380

381 **Dataset S2.** For each orthologous gene group (OGG) and each clade the gene with the  
382 largest expression difference between the monogamous and nonmonogamous species is  
383 selected as the representative gene. Ensembl IDs are provided for each OGG and clade  
384 in monogamous to nonmonogamous  $\log_2$  fold-difference.

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