Conserved Transcriptomic Profiles Underpin Monogamy across Vertebrates

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Materials and Methods

Sample collection and RNA extraction

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

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

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

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

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

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

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

RNA sequencing and mapping

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

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

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

Orthology inference

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

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

Differential Expression Analysis

To assess concordance of OGG expression in monogamous species across clades we used the differential expression analysis software package DESeq2 (14). For each species, raw read counts for the 1979 OGGs identified across all species were included in the differential expression analysis. Species were labeled as monogamous or nonmonogamous such that species from distinct clades served as biological replicates in the analysis. DESeq2 was performed on different evolutionary sub-groups including mammals, amniotes, tetrapods, and all clades. OGGs with an expression difference of +/- 1 $\log_2$ fold-difference and p-value < 0.1 were characterized as differentially expressed. This fairly liberal cut off captures genes that are generally concordant in expression direction across clades that may otherwise be eliminated.

To extract patterns of gene expression shared among monogamous species across clades, we utilized a hypergeometric approach, the R package Rank-Rank Hypergeometric Overlap (RRHO) (15). In RRHO analysis, rank $\log_2$ fold-differences in monogamous vs. nonmonogamous mRNA levels are binned into steps (we used the default settings which binned our 1979 OGGs into 45 steps). Each step can be set as a threshold making a continuous threshold scale of differential expression unique to each clade. At each pairwise $\log_2$ fold-difference threshold (binned OGG step) enrichment of rank correlations between clades is determined using a hypergeometric distribution. RRHO corrects p-values for multiple comparisons using the B-Y procedure (16). Overall significance of rank correlations for each pairwise comparison of clades was calculated using permutation analysis (17).

Novel candidates and GO analysis

To identify the most robust candidate genes associated with a monogamous mating system across species, we combined the results of our differential gene expression analysis with the RRHO analysis (Fig. 4). Specifically, we identified genes that were concordantly differentially expressed (at +/- 1 $\log_2$ fold-difference) between monogamous and nonmonogamous species in four of the five clades, and equivalently identified in at least five of the ten RRHO comparisons which allows one clade to lack concordant expression (Fig. 5; Dataset S1). To capture genes that are generally concordant in expression direction across clades, which may not be captured by threshold differential expression approaches, we used the more liberal expression cut off of +/- 1 $\log_2$ fold-difference. Notably, these candidate genes have known roles in synaptic transmission, neuroplasticity, and neurological function among other functions possibly reflecting similarities among monogamous species in the mechanisms through which the brain reward circuitry becomes associated with social affiliation (e.g., reproductive and parental behavior). Several candidate genes are critical for neuronal development as well as synaptic function and plasticity, including: Low Density Lipoprotein Receptor-Related Protein 6 (*LRP6*) (18), the Wnt inhibitor Adenomatosis Polyposis Coli Down-Regulated 1 *(APCDD1)* (19) , the Lysophosphatidic Acid Receptor 1 (*LPAR1*) (20), and Notch1 (21). Relatedly, the candidate gene (*Dscam*) remodels microcircuitry through regulation of dendritic arborization (22), and in this way is also thought to enhance learning and memory (27). Two genes on the candidate list have documented roles in synaptic transmission. The excitatory neurotransmitter Metabotropic Glutamate Receptor 6 (*GRM6*) is the only known metabotropic glutamate receptor to directly mediate synaptic transmission in the nervous system (23). Huntingtin Interacting Protein (*Hip1*) regulates AMPA receptor trafficking (24) and, interestingly, also enhances androgen receptor-mediated transcription (25). Finally, candidate genes lysine methyltransferase 2C (*KMT2c*) (26) and solute carrier family 6 member 17 (*SLC6a17*) (27) are involved in cognitive function and cause

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

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

Phylogenetic, life history, and gene expression distances

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

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

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

Accessibility of Data and Data Analyses

RNA sequencing data discussed in this publication have been deposited in NCBI's Gene Expression Omnibus (33) and are accessible through GEO Series accession number GSE123301 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE123301). Additional data and data analyses scripts in R and Python are published and publically available as a dataverse at the Texas Data Repository (https://dataverse.tdl.org/dataverse/monogamy).

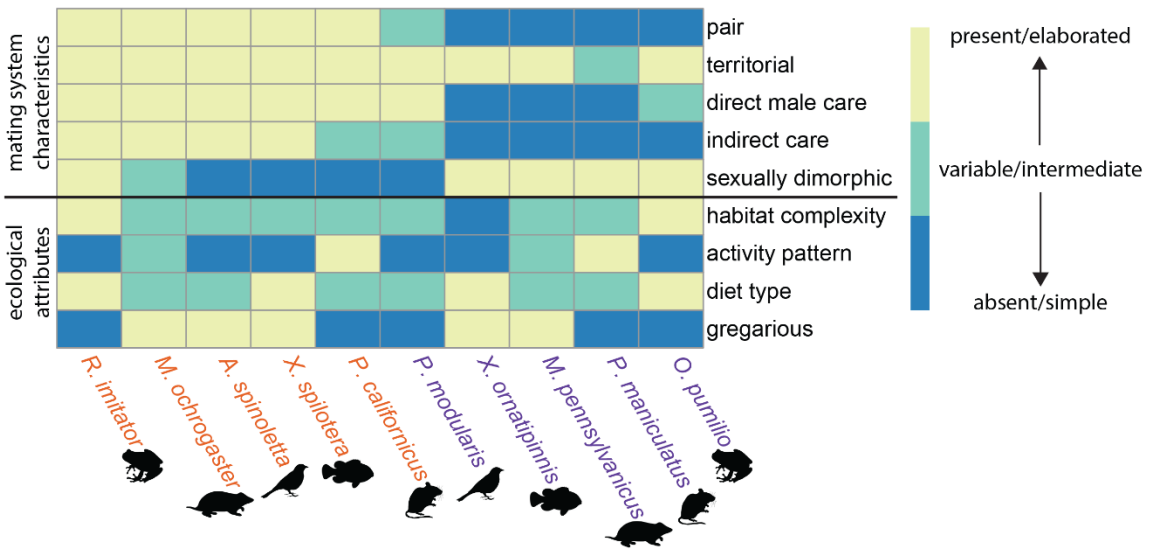


Figure S1. Ecological attributes and mating system characteristics of study species. Species names in orange are the monogamous species, those in purple are the nonmonogamous species for each clade. Color indicates presence or degree of trait elaboration in that species unless otherwise indicated below. Yellow indicates the trait listed on the right is absent or 'simple', green indicates intermediate, and blue indicates present or 'elaborated'. Five mating system characteristics were scored, including: pair bond formation, defense of breeding territories, male engagement in offspring provisioning, cleaning, or transport, males actively defend nests or provision females, and presence of sexual dimorphism (size or color elaboration). Four additional ecological attributes were also scored, including: habitat complexity (simple = yellow, various/intermediate = green, or complex environments = blue), activity pattern (diurnal = yellow, crepuscular/seasonally variable = green, and nocturnal = blue), diet type (primary diet is "herbivorous" = yellow, "omnivorous" = green, and "carnivorous" = blue), and whether communal or gregarious behaviors are observed outside of a reproductive context. Details and references in (SI Appendix, Tables S2 and S3).

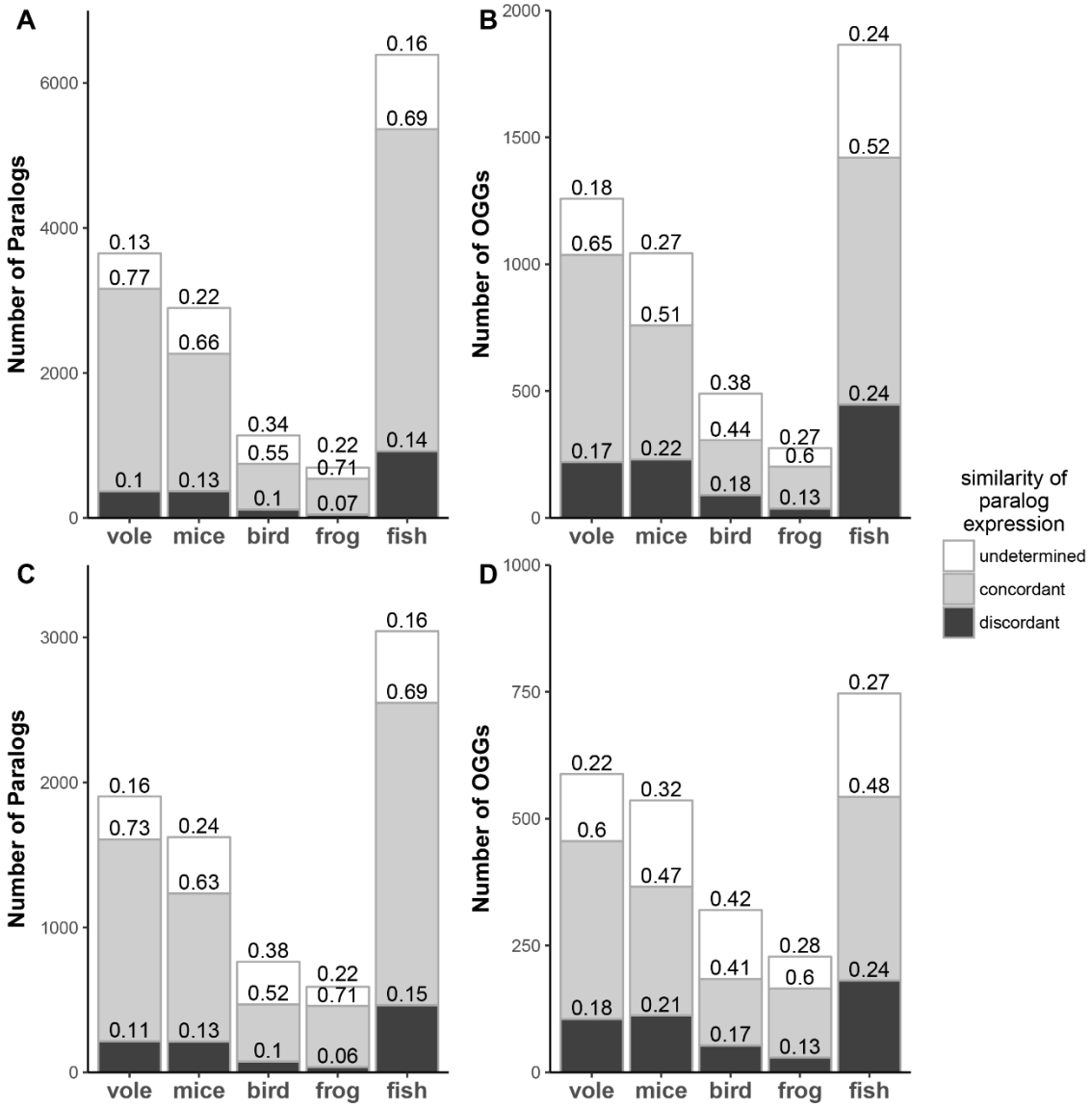


Figure S2. To compare neural gene expression across all clades, genes were grouped into orthologous gene groups (OGGs) using orthoMCL. OrthoMCL identified 6125 OGGs shared across the rat, chicken, frog, and tilapia reference genomes. Of those, 44-76% were identified using RNAseq (Table S3). Due to variation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

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

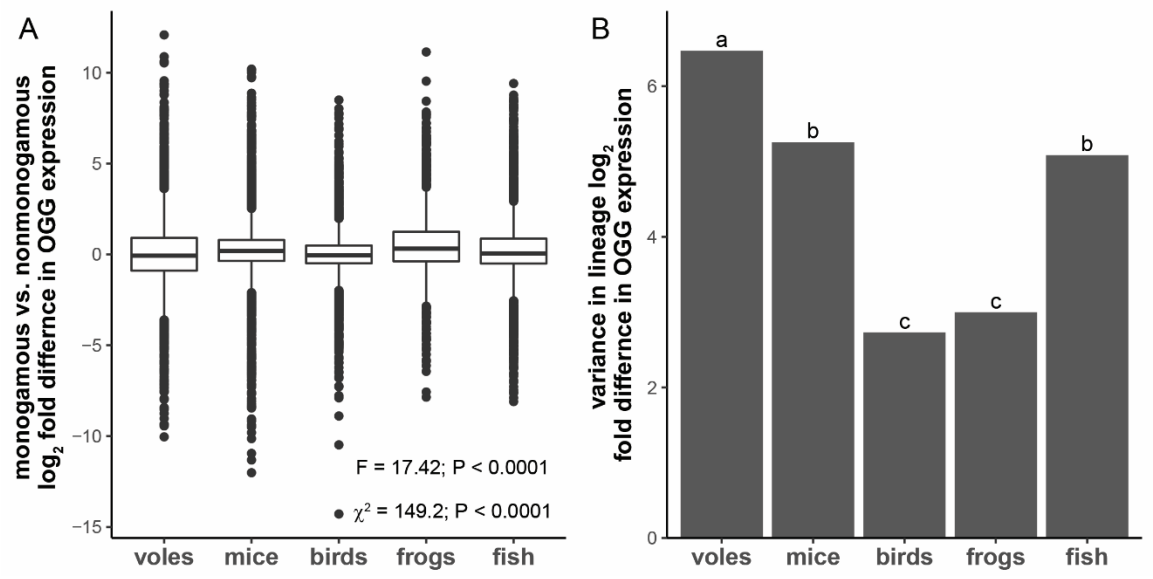


Figure S3. Log_2 fold difference (monogamous vs. nonmonogamous) in orthologous gene group (OGG) expression for all 1979 orthologous gene groups in each clade (A). Variance in log_2 fold-difference (monogamous vs. nonmonogamous) in OGG expression (B). When more than one gene is present in an orthologous gene group the gene with the highest log_2 fold-difference was selected. Log_2 fold-difference in OGG expression in monogamous species is slightly skewed toward increased expression in mice and frogs (median = 0.19 and 0.31, respectively). Birds and frogs exhibit the smallest variance (B) and narrowest interquartile range of expression differences (A) between monogamous and nonmonogamous species. Overall, means (Kruskall-Wallis χ^2) and variances (Levene's test) differ among the clades. F-tests for equality of variances were used to compare variances for all pairwise clades. Clades separated by letters significantly differ after correcting p values for multiple hypothesis testing (p * number of comparisons).

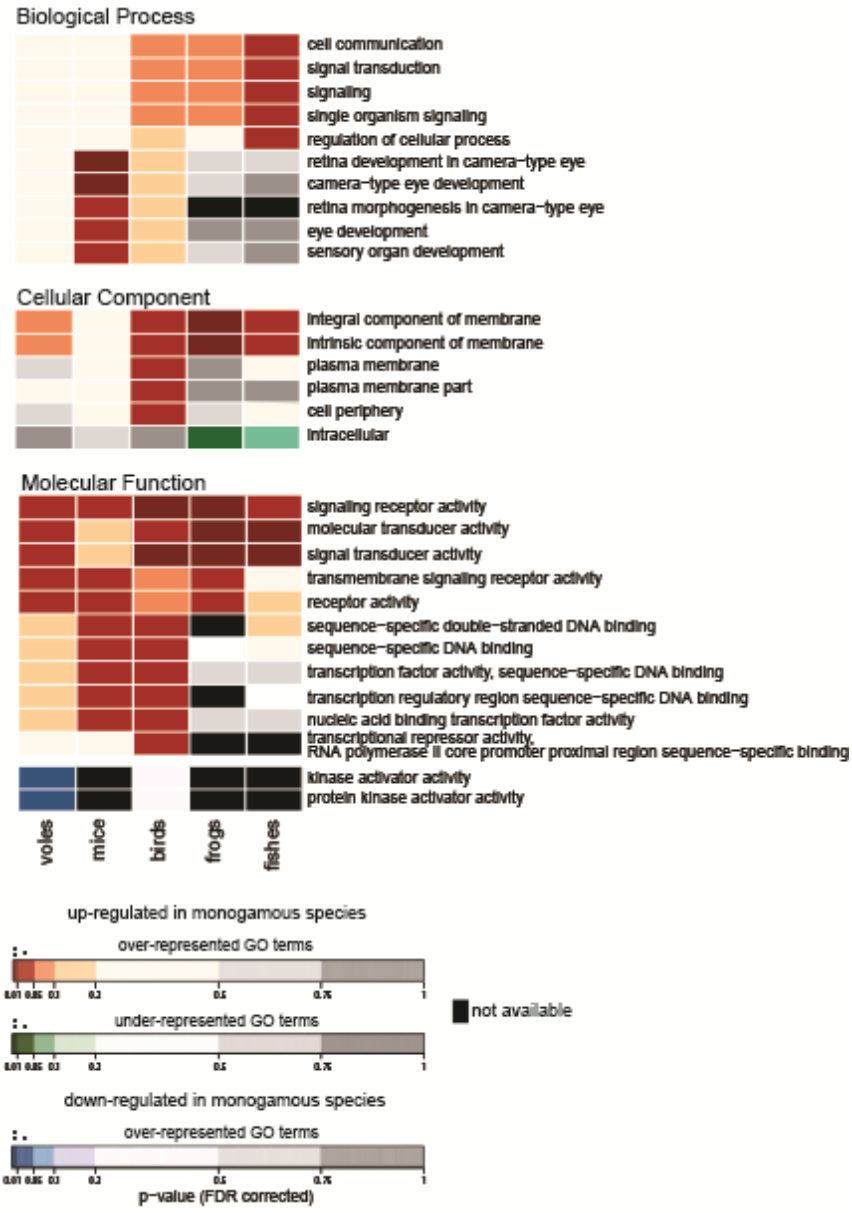
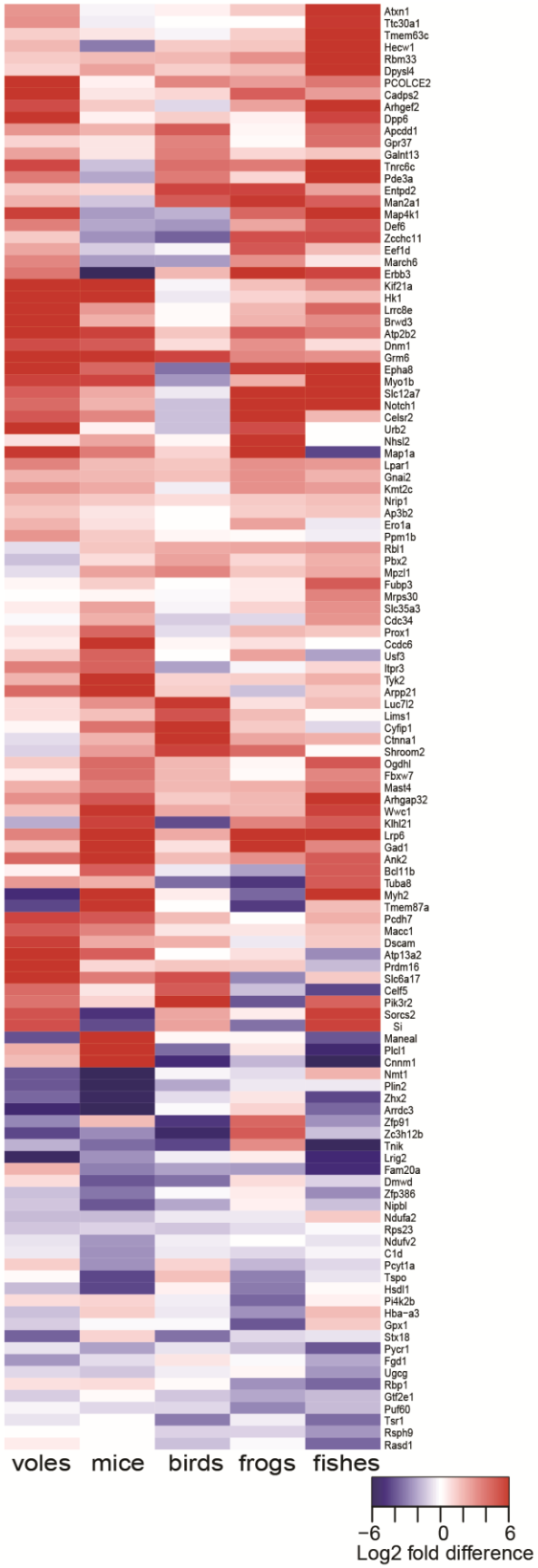
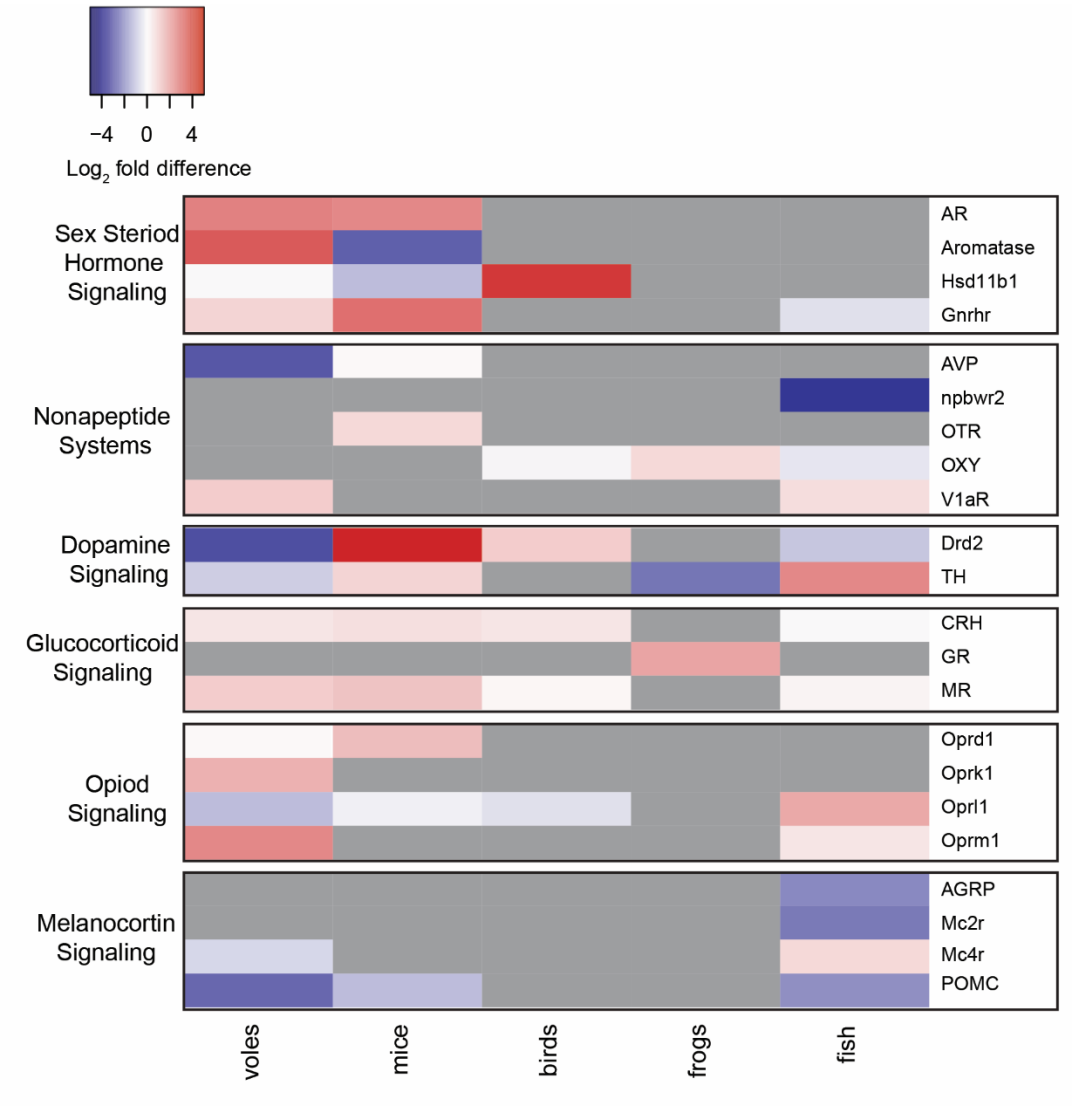


Figure S4. To characterize similarity of function of monogamy-related orthologous gene groups (OGGs) across species we assessed concordance of over- and under-represented GO annotations using BiNGO (29). For each clade, enrichment of GO terms was assessed for OGGs up- or down-regulated at $\log_2$ fold-difference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


Atxn1
Ttc30a1
Tmem63c
Hecw1
Rbm33
Dpysl4
PCOLCE2
Cadps2
Arhgef2
Dpp6
Apodd1
Gpr37
Galnt13
Tnrc6c
Pde3a
Entpd2
Man2a1
Map4k1
Def6
Zcchc11
Eef1d
March6
Erbb3
Kif21a
Hk1
Lrrc8e
Brwd3
Atp2b2
Dnm1
Grm6
Epha8
Myo1b
Slc12a7
Notch1
Celsr2
Urb2
Nhsl2
Map1a
Lpar1
Gnai2
Kmt2c
Nrip1
Ap3b2
Ero1a
Ppm1b
Rbl1
Pbx2
Mpzl1
Fubp3
Mrps30
Slc35a3
Cdc34
Prox1
Ccdc6
Usf3
Itpr3
Tyk2
Arpp21
Luc7l2
Lims1
Cyfip1
Ctnna1
Shroom2
Ogdhl
Fbxw7
Mast4
Arhgap32
Wwc1
Klhl21
Lrp6
Gad1
Ank2
Bcl11b
Tuba8
Myh2
Tmem87a
Pcdh7
Macc1
Dscam
Atp13a2
Prdm16
Slc6a17
Celf5
Pik3r2
Sorcs2
Si
Maneal
Plcl1
Cnnm1
Nmt1
Plin2
Zhx2
Arrdc3
Zfp91
Zc3h12b
Tnik
Lrig2
Fam20a
Dmwd
Zfp386
Nipbl
Ndufa2
Rps23
Ndufv2
C1d
Pcyt1a
Tspo
Hsd11
Pi4k2b
Hba-a3
Gpx1
Stx18
Pycr1
Fgd1
Ugcg
Rbp1
Gtf2e1
Puf60
Tsr1
Rsph9
Rasd1
voles
mice
birds
frogs
fishes
−6
0
6
Log2 fold difference

301

Figure S5. Genes concordantly expressed in monogamous brains across vertebrates. 121 genes were identified with DESeq2 $\log_2$ fold-difference +/- 1 and p-value < 0.1. DESeq2 provides a base mean and a $\log_2$ fold-difference. As with any other analysis with replicates, not all replicates are expected to show the same directional difference in gene expression. However, on average, these 121 OGGs are differentially expressed between monogamous and nonmonogamous species across all clades. Gene symbols are provided.



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310 **Figure S6.** Relative expression (log_2 fold-difference) in monogamous versus
311 nonmonogamous species of each clade for 22 candidate genes previously implicated in
312 regulating complex social behavior across vertebrates. These often studied candidate
313 genes represent six neuroendocrine and neuro-modulatory systems that have previously
314 implicated in the regulation of (aspects of) monogamous behavior or, more generally,
315 complex social behavior. Reds (from light to dark) indicate increased expression in the
316 monogamous species; blues (from light to dark) indicate decreased expression in the
317 monogamous species. Grey indicates that expression data was not available for one or
318 both species of that clade. Note that in several clades many of these candidate genes

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

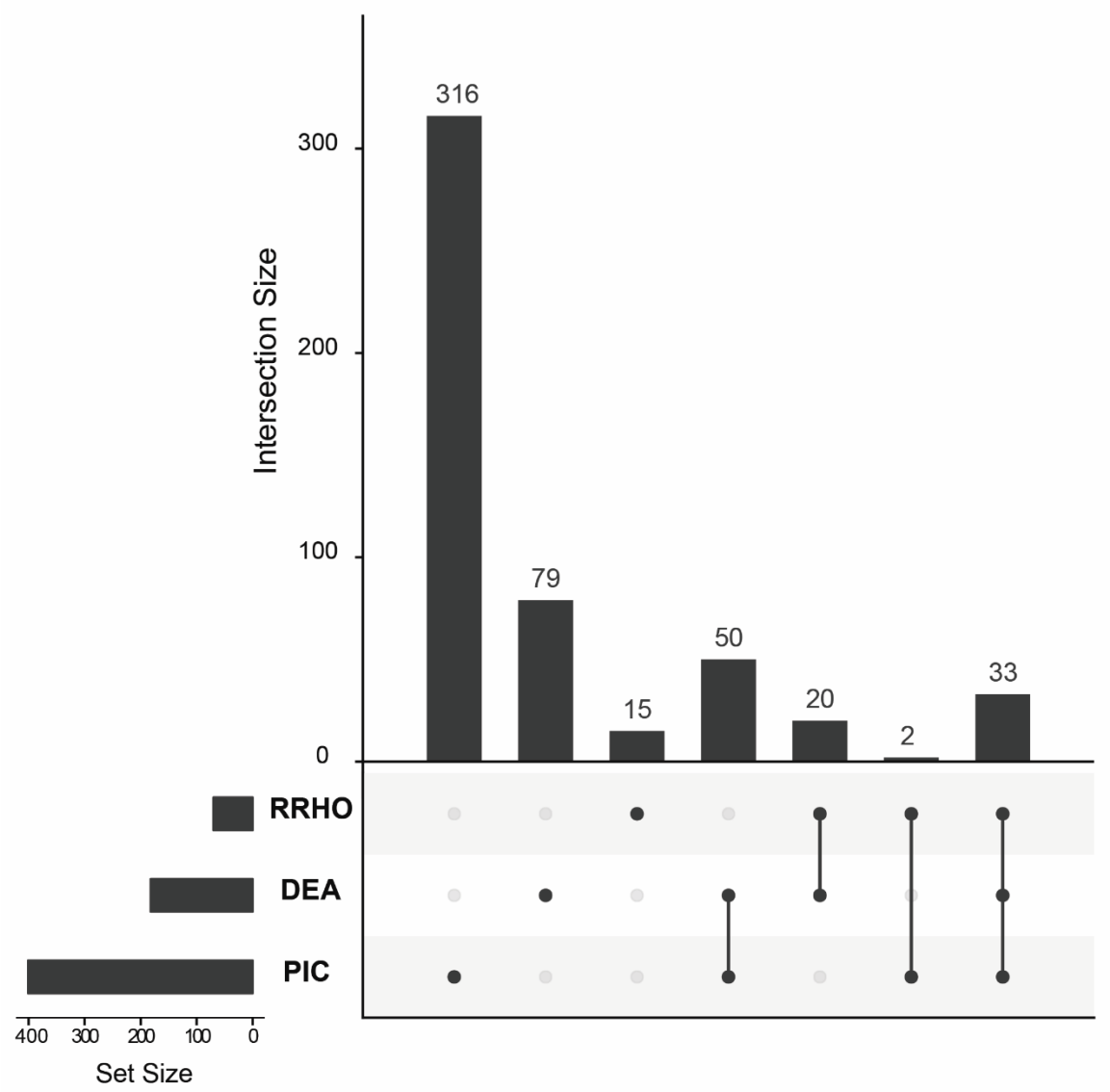

Figure S7. 401 OGGs exhibit both differential expression between the monogamous and nonmonogamous species in at least one clade (at +/- 1 log2 fold difference) and high expression (RPM) variance across all species (variance in the upper quartile). This subset included 401 OGGs used in the phylogenetic independent contrast (PIC) overlap significantly with 70 OGGs identified by RRHO (overlap = 35, hypergeometric distribution p = 7.6e-07) and 182 OGGs identified using differential expression analysis (overlap = 83, hypergeometric distribution p = 3.7e-13). Intersections were identified and plotted using the R package UpSetR (34). P-values are corrected for multiple hypothesis

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

Table S1. Mating system characteristics of each species used.

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

Table S2. Ecological attributes of the study species.

species	habitat complexity	activity	diet	communal/gregarious outside of breeding
M. ochrogaster	Intermediate: un-grazed grassland/savanna (37)	Various, seasonal (83)	Omnivorous: forbs, foliage, fruits, tubers, insects (84, 85)	Yes: communal group living (86, 87)
M. pennsylvanicus	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)
P. californicus	Intermediate: dense chaparral/broad-sclerophyll woodland (48)	Nocturnal (93)	Omnivorous: acorns, flowers seeds, fungi and arthropods (94)	No: non-overlapping territories (51)
P. maniculatus	Various (95)	Nocturnal (96)	Omnivorous: mostly insects & arthropods, but also seeds, flowers, and leaves (63, 97)	No (62, 63)
A. spinoletta	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)
P. modularis	Intermediate: woodlands, hedgerows, gardens	Diurnal	Omnivorous: mostly insects and seeds (102)	No: solitary in winter (103)
R. imitator	Complex: leaf litter, secondary to old growth forest (73)	Diurnal	Carnivorous: ants, mites, beetles (A. Stuckert, pers. obs.)	No (K. Summers pers. obs.)
O. pumilio	Complex: premontane forest leaf litter	Diurnal (104)	Carnivorous: ants, mites, beetles, springtails(105, 106)	No; (76–78)
X. spilotera	Intermediate (82, 107)	Diurnal (HAH pers. obs.)	Carnivorous (108)	Yes (82, 108)
X. ornatipinnis	Simple, sand (82)	Diurnal (HAH pers. obs.)	Carnivorous (108)	Yes (82, 108)

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

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

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

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)

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

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

Table S6. Maximum, mean, and median $-Log_{10}$ p-values for each quadrant of the Rank Rank Hypergeometic Overlap analysis (Fig. 4).

clade A	clade B	Concordant		Discordant	
		Downs	Ups	Down,Up	Up,Down
		Max *P*values			
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 *P*values			
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 *P*values			
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

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

Symbol	Name	Protein ID	voles	mice	birds	frogs	fishes
Ank2	Ankyrin 2	ENSRNOP00000015386	4.087	10.012	1.705	2.900	4.523
Apcdd1	Adenomatosis Polyposis Coli Down-Regulated 1	ENSRNOP00000059242	2.816	2.005	4.526	0.280	3.987
Arhgap32	neuron-associated GTPase-activating protein	ENSRNOP00000011589	2.884	4.567	1.352	1.857	6.610
Arpp21	cAMP-regulated phosphoprotein 21	ENSRNOP00000039268	4.021	6.166	1.201	-1.366	1.422
Atp2b2	ATPase Plasma Membrane Ca2+ Transporting 2	ENSRNOP00000060489	6.414	5.284	1.473	4.378	3.593
Brwd3	Bromodomain And WD Repeat Domain Containing 3	ENSRNOP00000050434	7.942	2.055	0.219	1.974	3.062
Ctnna1	Catenin Alpha 1	ENSRNOP00000008041	-0.745	1.964	7.495	2.321	2.102
Dpysl4	Dihydropyrimidinase Like 4	ENSRNOP00000029334	1.187	2.449	1.260	1.791	6.443
Dscam	Down Syndrome Cell Adhesion Molecule (cell adhesion, PAK pathway)	ENSRNOP00000022476	5.447	2.189	2.064	-0.492	1.439
Entpd2	Ectonucleoside Triphosphate Diphosphohydrolase 2	ENSRNOP00000018560	1.372	1.009	5.261	5.266	2.429
Epha8	Ephrin Receptor A8	ENSRNOP00000017559	7.482	4.169	-3.140	5.547	8.402
Erbb3	Erb-B2 Receptor Tyrosine Kinase 3	ENSRNOP00000006796	3.671	-6.491	1.806	6.163	5.232
Fam20a	Golgi associated secretory pathway pseudokinase	ENSRNOP00000005367	1.991	-2.827	-2.156	-2.273	-4.851
Gad1	Glutamate Decarboxylase 1	ENSRNOP00000000008	1.527	5.965	0.826	6.420	3.232
Galnt13	Polypeptide N-Acetylgalactosaminyltransferase 13	ENSRNOP00000042772	2.470	0.680	3.471	1.022	1.471
Gnai2	G Protein Subunit Alpha I2	ENSRNOP00000022550	1.970	1.918	1.588	2.919	1.963
Grm6	Glutamate Receptor, Metabotropic 6	ENSRNOP00000000249	7.102	5.860	5.261	3.171	2.965
Gtf2e1	General Transcription Factor IIE Subunit 1	ENSRNOP00000039601	-1.125	0.230	-1.319	-1.967	-1.547
Hecw1	HECT, C2 and WW domain containing E3 ubiquitin protein ligase 1	ENSRNOP00000021703	1.867	-2.903	1.384	1.530	7.697
Kif21a	member of the KIF4 subfamily of kinesin-like motor proteins	ENSRNOP00000044677	9.344	6.399	-0.358	1.606	3.096
Kmt2c	lysine methyltransferase 2C	ENSRNOP00000063937	2.764	2.171	-0.394	2.958	2.552
Lpar1	Lysophosphatidic Acid Receptor 1	ENSRNOP00000043652	3.300	1.765	1.453	2.904	2.723
Lrp6	Low Density Lipoprotein Receptor-Related Protein 6	ENSRNOP00000063261	3.250	6.296	2.258	7.213	5.889

Table S7 cont.

Symbol	Name	Protein ID	voles	mice	birds	frogs	fishes
Lrrc8e	Leucine-Rich Repeat Containing 8 Family Member E	ENSRNOP00000037487	10.538	2.713	0.229	1.823	4.147
Man2a1	Mannosidase Alpha Class 2A Member 1	ENSRNOP00000020767	1.935	-1.249	4.456	5.717	4.381
Mast4	Microtubule Associated Serine/Threonine Kinase Family Member 4	ENSRNOP00000067460	2.129	3.461	1.890	2.117	3.578
Mpzl1	Myelin Protein Zero Like 1	ENSRNOP00000004376	-0.798	2.453	3.194	1.492	2.185
Myo1b	Myosin IB	ENSRNOP00000059455	5.353	5.197	-2.363	2.059	6.865
Notch1	Notch 1	ENSRNOP00000026212	4.015	2.024	-1.424	6.514	7.403
Nrip1	Nuclear Receptor Interacting Protein 1	ENSRNOP00000002152	1.877	1.387	0.907	1.376	1.594
Ogdhl	Oxoglutarate Dehydrogenase Like	ENSRNOP00000027054	1.426	4.064	1.805	0.325	4.619
Pcdh7	Protocadherin 7	ENSRNOP00000042179	5.270	4.670	1.758	0.104	2.097
PCOLCE2	procollagen C-endopeptidase enhancer 2	ENSRNOP00000067519	6.402	0.408	3.162	2.589	3.690
Pik3r2	Phosphoinositide-3-Kinase Regulatory Subunit 2	ENSRNOP00000026210	3.727	1.128	8.494	-3.772	4.228
Rbl1	RB Transcriptional Corepressor Like 1	ENSRNOP00000063017	-0.752	1.504	2.227	2.291	2.525
Rbm33	RNA Binding Motif Protein 33	ENSRNOP00000030808	1.344	1.766	1.825	2.909	8.013
Slc12a7	Solute Carrier Family 12 Member 7	ENSRNOP00000022635	4.439	2.250	-0.550	5.992	8.764
Slc6a17	Solute Carrier Family 6 Member 17	ENSRNOP00000065179	9.553	3.581	4.832	-2.679	1.371
Tnik	TRAF2 and NCK interacting kinase	ENSRNOP00000016799	-1.729	-3.351	-4.096	3.004	-5.800
Tnrc6c	Trinucleotide Repeat Containing 6B	ENSRNOP00000049430	5.153	-1.430	3.906	3.512	5.937
Tyk2	Tyrosine Kinase 2	ENSRNOP00000048018	2.024	7.602	1.188	1.245	2.103
Wwc1	cytoplasmic phosphoprotein (possible memory enhancer)	ENSRNOP00000011061	1.670	5.841	2.191	1.866	5.397

376

377

Dataset S1. Novel candidate genes associated with monogamous mating system across species. Gene functions are provided by Gene Cards (www.genecards.org) unless otherwise noted. Localizations are provided by the Allen Mouse Brain Atlas (28).

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

References:

1. Nugent BM, Stiver KA, Alonzo SH, Hofmann HA (2016) Neuroendocrine profiles associated with discrete behavioral variation in Symphodus ocellatus, a species with male alternative reproductive tactics. *Mol Ecol*.
2. Stiver KA, Harris RM, Townsend JP, Hofmann HA, Alonzo SH (2015) Neural Gene Expression Profiles and Androgen Levels Underlie Alternative Reproductive Tactics in the Ocellated Wrasse, Symphodus ocellatus. *Ethology* 121(2):152–167.
3. Duque-Wilckens N, et al. (2016) Effects of V1a receptor in bed nucleus of stria terminalis on social behavior and anxiety in males and females. *Neuropharmacology* 110:406–414.
4. Deviche PJ, et al. (2010) Acute stress rapidly decreases plasma testosterone in a free-ranging male songbird: potential site of action and mechanism. *Gen Comp Endocrinol* 169(1):82–90.
5. Van Hout AJ-M, Eens M, Darras VM, Pinxten R (2010) Acute stress induces a rapid increase of testosterone in a songbird: implications for plasma testosterone sampling. *Gen Comp Endocrinol* 168(3):505–10.
6. Kidd MR, Duftner N, Koblmüller S, Sturmbauer C, Hofmann HA (2012) Repeated Parallel Evolution of Parental Care Strategies within Xenotilapia, a Genus of Cichlid Fishes from Lake Tanganyika. *PLoS One* 7(2):e31236.
7. Bolger AM, Lohse M, Usadel B (2014) Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics* 30(15):2114–2120.
8. Li H, Durbin R (2009) Fast and accurate short read alignment with Burrows–Wheeler transform. *Bioinforma* 25(14):1754–1760.
9. Li H, et al. (2009) The Sequence Alignment/Map (SAM) Format and SAMtools. *Bioinformatics* 25(16):2078–9.
10. Li L, Stoeckert CJ, Roos DS (2003) OrthoMCL: Identification of Ortholog Groups for Eukaryotic Genomes. *Genome Res* 13(9):2178–2189.
11. Dalquen DA, Dessimoz C (2013) Bidirectional best hits miss many orthologs in duplication-rich clades such as plants and animals. *Genome Biol Evol* 5(10):1800–6.
12. Jensen LJ, et al. (2008) eggNOG: automated construction and annotation of orthologous groups of genes. *Nucleic Acids Res* 36(Database issue):D250-4.
13. Waterhouse RM, Tegenfeldt F, Li J, Zdobnov EM, Kriventseva E V (2013) OrthoDB: a hierarchical catalog of animal, fungal and bacterial orthologs. *Nucleic Acids Res* 41(Database issue):D358-65.
14. Love M, Huber W, Anders S (2014) Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biol* 15(12):550.
15. Rosenblatt J, Stein J (2014) RRHO: Test overlap using the Rank-Rank Hypergeometric test.
16. Benjamini Y, Yekutieli D (2001) The Control of the False Discovery Rate in Multiple Testing under Dependency. *Ann Stat* 29(4):1165–1188.
17. Plaisier SB, Taschereau R, Wong JA, Graeber TG (2010) Rank–rank hypergeometric overlap: identification of statistically significant overlap between gene-expression signatures. *Nucleic Acids Res* 38(17):e169–e169.
18. Liu C-C, et al. (2014) Deficiency in LRP6-Mediated Wnt Signaling Contributes to Synaptic Abnormalities and Amyloid Pathology in Alzheimer's Disease. *Neuron* 84(1):63–77.
19. Cruciat C-M, Niehrs C (2013) Secreted and Transmembrane Wnt Inhibitors and Activators. *Cold Spring Harb Perspect Biol* 5(3). doi:10.1101/cshperspect.a015081.
20. Garcia-Diaz B, et al. (2015) Loss of lysophosphatidic acid receptor LPA1 alters

oligodendrocyte differentiation and myelination in the mouse cerebral cortex. *Brain Struct Funct* 220(6):3701–3720.
21. Lasky JL, Wu H (2005) Notch signaling, brain development, and human disease. *Pediatr Res* 57(5 Pt 2):104R–109R.
22. Maynard KR, Stein E (2012) DSCAM Contributes to Dendrite Arborization and Spine Formation in the Developing Cerebral Cortex. *J Neurosci* 32(47):16637–16650.
23. Huang Y-Y, Haug MF, Gesemann M, Neuhauss SCF (2012) Novel Expression Patterns of Metabotropic Glutamate Receptor 6 in the Zebrafish Nervous System. *PLoS One* 7(4):e35256.
24. Metzler M, et al. (2003) Disruption of the endocytic protein HIP1 results in neurological deficits and decreased AMPA receptor trafficking. *EMBO J* 22(13):3254–3266.
25. Mills IG, et al. (2005) Huntingtin interacting protein 1 modulates the transcriptional activity of nuclear hormone receptors. *J Cell Biol* 170(2):191–200.
26. Kleefstra T, et al. (2012) Disruption of an EHMT1-associated chromatin-modification module causes intellectual disability. *Am J Hum Genet* 91(1):73–82.
27. Waltl S (2015) Mutations in SLC6A17 cause autosomal-recessive intellectual disability. *Clin Genet* 88(2):136–137.
28. Allen Mouse Brain Atlas *2004*. Available at: http://mouse.brain-map.org/.
29. Maere S, Heymans K, Kuiper M (2005) BiNGO: a Cytoscape plugin to assess overrepresentation of Gene Ontology categories in Biological Networks. *Bioinforma* 21(16):3448–3449.
30. Hedges SB, Dudley J, Kumar S (2006) TimeTree: a public knowledge-base of divergence times among organisms. *Bioinforma* 22(23):2971–2972.
31. Gibbons FD, Roth FP (2002) Judging the Quality of Gene Expression-Based Clustering Methods Using Gene Annotation. *Genome Res* 12(10):1574–1581.
32. Paradis E, Claude J, Strimmer K (2004) APE: Analyses of phylogenetics and evolution in R language. *Bioinformatics* 20. doi:10.1093/bioinformatics/btg412.
33. Edgar R, Domrachev M, Lash AE (2002) Gene Expression Omnibus: NCBI gene expression and hybridization array data repository. *Nucleic Acids Res* 30(1):207–210.
34. Conway JR, Lex A, Gehlenborg N (2017) UpSetR: an R package for the visualization of intersecting sets and their properties. *Bioinformatics* 33(18):2938–2940.
35. Getz LL, Carter CS (1980) Social organization in Microtus ochrogaster populations. *Biologist* 62(1–4):56–69.
36. Getz LL, Carter CS, Gavish L (1981) The Mating System of the Prairie Vole, Microtus ochrogaster: Field and Laboratory Evidence for Pair-Bonding. *Behav Ecol Sociobiol* 8(3):189–194.
37. Stalling DT (1990) Microtus ochrogaster. *Mamm Species*:1–9.
38. Solomon NG (1993) Comparison of parental behavior in male and female prairie voles (Microtus ochrogaster). *Can J Zool* 71(2):434–437.
39. Lonstein JS, De Vries GJ (1999) Comparison of the parental behavior of pair-bonded female and male prairie voles (Microtus ochrogaster). *Physiol Behav* 66(1):33–40.
40. Thomas JA, Birney EC (1979) Parental Care and Mating System of the Prairie Vole, Microtus ochrogaster. *Behav Ecol Sociobiol* 5(2):171–186.
41. Oliveras D, Novak M (1986) A comparison of paternal behaviour in the meadow vole Microtus pennsylvanicus, the pine vole M. pinetorum and the prairie vole M. cchrogaster. *Anim Behav* 34(2):519–526.

42. Heske EJ, Ostfeld RS (1990) Sexual Dimorphism in Size, Relative Size of Testes, and Mating Systems in North American Voles. *J Mammal* 71(4):510–519.
43. Dewsbury DA, Baumgardner DJ, Evans RL, Webster DG (1980) Sexual Dimorphism for Body Mass in 13 Taxa of Muroid Rodents under Laboratory Conditions. *J Mammal* 61(1):146–149.
44. Gruder-Adams S, Getz LL (1985) Comparison of the Mating System and Paternal Behavior in Microtus ochrogaster and M. pennsylvanicus. *J Mammal* 66(1):165 LP-167.
45. Getz LL (1961) Home Ranges, Territoriality, and Movement of the Meadow Vole. *J Mammal* 42(1):24–36.
46. Sadleir RMFS (1965) The Relationship between Agonistic Behaviour and Population Changes in the Deermouse, Peromyscus maniculatus (Wagner). *J Anim Ecol* 34(2):331–352.
47. Boonstra R, Xia X, Pavone L (1993) Mating system of the meadow vole, Microtus pennsylvanicus. *Behav Ecol* 4(1):83–89.
48. McCabe T, Blanchard B (1950) *Three species of Peromyscus* (Rood Association, Santa Barbara, CA).
49. Ribble DO (1992) Lifetime Reproductive Success and its Correlates in the Monogamous Rodent, Peromyscus californicus. *J Anim Ecol* 61(2):457–468.
50. Merritt J (1999) California Mouse. *The Smithsonian Book of North American Mammals*, eds Wilson D, Ruff S (Smithsonian Institute Press, Washington, D.C.), pp 565–566.
51. Ribble D, Salvioni M (1990) Social organization and nest co-occupancy in Peromyscus californicus, a monogamous rodent. *Behav Ecol Sociobiol* 26(1):9–15.
52. Gubernick DJ, Alberts JR (1987) The biparental care system of the California mouse, Peromyscus californicus. *J Comp Psychol* 101(2):169–177.
53. 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(1):25–38.
54. Dudley D (1974) Contributions of paternal care to the growth and development of the young in Peromyscus californicus. *Behav Biol* 11(2):155–166.
55. Gubernick DJ, Teferi T (2000) Adaptive Significance of Male Parental Care in a Monogamous Mammal. *Proc Biol Sci* 267(1439):147–150.
56. Bredy TW, Brown RE, Meaney MJ (2007) Effect of resource availability on biparental care, and offspring neural and behavioral development in the California mouse (Peromyscus californicus). *Eur J Neurosci* 25(2):567–575.
57. Trainor BC, Finy MS, Nelson RJ (2008) Paternal aggression in a biparental mouse: Parallels with maternal aggression. *Horm Behav* 53(1):200–207.
58. Trainor BC, Marler CA (2001) Testosterone, Paternal Behavior, and Aggression in the Monogamous California Mouse (Peromyscus californicus). *Horm Behav* 40(1):32–42.
59. Campi KL, Jameson CE, Trainor BC (2013) Sexual Dimorphism in the Brain of the Monogamous California Mouse (Peromyscus californicus). *Brain Behav Evol* 81(4):236–249.
60. Kirkland GL, Layne NJ (1989) *Advances in the Study of Peromyscus (Rodentia)* (Texas Tech University Press, Lubbock, Texas).
61. Ribble DO, Millar JS (1996) The mating system of northern populations of Peromyscus maniculatus as revealed by radiotelemetry and DNA fingerprinting. *Écoscience* 3(4):423–428.
62. Wolff JO, Freeberg MH, Dueser RD (1983) Interspecific Territoriality in Two

Sympatric Species of Peromyscus (Rodentia: Cricetidae). *Behav Ecol Sociobiol* 12(3):237–242.
63. Wolff JO, Dueser RD, Berry KS (1985) Food Habits of Sympatric Peromyscus leucopus and Peromyscus maniculatus. *J Mammal* 66(4):795–798.
64. Hartung TG, Dewsbury DA (1979) Paternal behavior in six species of muroid rodents. *Behav Neural Biol* 26(4):466–478.
65. Jašarević E, et al. (2013) Evolution of monogamy, paternal investment, and female life history in Peromyscus. *J Comp Psychol* 127(1):91–102.
66. Bollmann K, Reyer H-U (1999) Why does monogamy prevai l in the Alpine Water Pipit? *Proceedings of the 22nd International Ornithological Congress*, eds Adams NJ, Slotow RH (BirdLife South Africa, Johannesburg), pp 2666–2688.
67. Rauter C, Reyer H-U (1997) Incubation pattern and foraging effort in the female Water Pipit Anthus spinoletta. *Ibis (Lond 1859)* 139(3):441–446.
68. Santos ESA, Nakagawa S (2013) Breeding Biology and Variable Mating System of a Population of Introduced Dunnocks Prunella modularis in New Zealand. *PLoS One* 8(7):e69329.
69. Davies N (1992) *Dunnock behaviour and social evolution* (Oxford University Press, Oxford).
70. Burke T, Daviest NB, Bruford MW, Hatchwell BJ (1989) Parental care and mating behaviour of polyandrous dunnocks Prunella modularis related to paternity by DNA fingerprinting. *Nature* 338(6212):249–251.
71. Davies NB (1985) Cooperation and conflict among dunnocks, Prunella modularis, in a variable mating system. *Anim Behav* 33:628–648.
72. Møller AP, Birkhead TR, Moller AP, Birkhead TR (1994) The Evolution of Plumage Brightness in Birds is Related to Extrapair Paternity. *Evolution (N Y)* 48(4):1089–1100.
73. Brown JL, Twomey E, Morales V, Summers K (2008) Phytotelm size in relation to parental care and mating strategies in two species of Peruvian poison frogs. *Behaviour* 145(9):1139–1165.
74. Brown JL, Morales V, Summers K (2010) A key ecological trait drove the evolution of biparental care and monogamy in an amphibian. *Am Nat* 175(4):436–446.
75. Tumulty J, Morales V, Summers K (2014) The biparental care hypothesis for the evolution of monogamy: experimental evidence in an amphibian. *Behav Ecol* 25(2):262–270.
76. Pröhl H, Hödl W (1999) Parental investment, potential reproductive rates, and mating system in the strawberry dart-poison frog, Dendrobates pumilio. *Behav Ecol Sociobiol* 46(4):215–220.
77. Meuche I, Linsenmair KE, Pröhl H (2012) Intrasexual competition, territoriality and acoustic communication in male strawberry poison frogs (Oophaga pumilio). *Behav Ecol Sociobiol* 66(4):613–621.
78. Bunnell P (1973) Vocalizations in the territorial behavior of the frog Dendrobates pumilio. *Copeia*:277–284.
79. Weygoldt P (1987) Evolution of parental care in dart poison frogs (Amphibia: Anura: Dendrobatidae). *J Zool Syst Evol Res* 25(1):51–67.
80. Pröhl H, Berke O (2001) Spatial Distributions of Male and Female Strawberry Poison Frogs and Their Relation to Female Reproductive Resources. *Oecologia* 129(4):534–542.
81. Konings A, Dieckhoff HW (1992) *Tanganyika Secrets* (Cichlid Press, Miami, Florida).
82. Konings A (1996) *Guide to Tanganyika Cichlids* (Back to Nature, Jonsered, Sweden).

83. Madison DM (1985) Activity rhythms and spacing. *Biology of New World Microtus*, ed Tamarin RH (American Society of Mammalogists, Stillwater, Oklahoma), pp 373–419.
84. Cole FR, Batzli GO (1979) Nutrition and Population Dynamics of the Prairie Vole, Microtus ochrogaster, in Central Illinois. *J Anim Ecol* 48(2):455–470.
85. Zimmerman EG (1965) A Comparison of Habitat And Food of Two Species of Microtus. *J Mammal* 46(4):605–612.
86. Getz LL, McGuire B (1997) Communal nesting in prairie voles (Microtus ochrogaster): formation, composition, and persistence of communal groups. *Can J Zool* 75(4):525–534.
87. Getz L, Hofmann J (1986) Social organization in free-living prairie voles, Microtus ochrogaster. *Behav Ecol Sociobiol* 18(4):275–282.
88. Burt WH, Grossenheider RP (1976) *A field guide to the mammals* (Houghton Mifflin, Boston).
89. Reich LM (1981) Microtus pennsylvanicus. *Mamm Species Arch* 159:1 LP-8.
90. Lindroth RL, Batzli GO (1984) Food habits of the meadow vole (Microtus pennsylvanicus) in bluegrass and prairie habitats. *J Mammal* 65(4):600–606.
91. Webster AB, Brooks RJ (1981) Social Behavior of Microtus pennsylvanicus in Relation to Seasonal Changes in Demography. *J Mammal* 62(4):738–751.
92. Madison DM, FitzGerald RW, McShea WJ (1984) Dynamics of Social Nesting in Overwintering Meadow Voles (Microtus pennsylvanicus): Possible Consequences for Population Cycling. *Behav Ecol Sociobiol* 15(1):9–17.
93. Hudson JW (1967) Variations inthe patterns of torpidity of small homoiotherms. *Proceedings of the Third International Symposium of Mammalian Hibernation*, eds Fisher KC, Lyman CP, Schonbaum E, South FE (Elsevier, New York), pp 30–46.
94. Merritt JF (1974) Factors Influencing the Local Distribution of Peromyscus californicus in Northern California. *J Mammal* 55(1):102–114.
95. King JA (1968) *Biology of Peromyscus (Rodentia)* (The American Society of Mammalogists, Stillwater, Oklahoma).
96. O'Farrell MJ (1974) Seasonal Activity Patterns of Rodents in a Sagebrush Community. *J Mammal* 55(4):809–823.
97. Whitaker Jr. JO, Cudmore WW (1987) Food and ectoparasites of shrews of south cental Indiana with emphasis on Sorex fumeus and Sorex hoyi. *Proc Indiana Acad Sci* 96(543–552).
98. Melendez L, Laiolo P (2014) The role of climate in constraining the elevational range of the Water Pipit Anthus spinoletta in an alpine environment. *Ibis (Lond 1859)* 156(2):276–287.
99. Alström P, Mild K (1996) The identification of Rock, Water and Buff-bellied Pipits. *Alula* 2(4):161–175.
100. Frey-Roos F, Brodmann PA, Reyer H-U (1995) Relationships between food resources, foraging patterns, and reproductive success in the water pipit, Anthus sp. spinoletta. *Behav Ecol* 6(3):287–295.
101. Bollmann K, Brodmann PA, Reyer HU (1997) Territory quality and reproductive success: Can Water Pipits Anthus spinoletta assess the relationship reliably? *Ardea-Wageningen* 85:83–98.
102. Bishton G (1986) The diet and foraging behaviour of the Dunnock Prunella modularis in a hedgerow habitat. *Ibis (Lond 1859)* 128(4):526–539.
103. Lack P (1986) *The Atlas of Wintering Birds in Britain and Ireland* (A&C Black Publisher, London).
104. Limerick S (1980) Courtship Behavior and Oviposition of the Poison-Arrow Frog Dendrobates pumilio. *Herpetologica* 36(1):69–71.

105. Caldwell JP, Summers KD (2003) Imitating poison frog, Dendrobates imitator. *Grzimek's Animal Life Encyclopedia, Volume 6, Amphibians*, eds Hutchins M, Duellman WE, Schlager N (Gale Group, Farmington Hills, Michigan). 2nd Ed.
106. Donnelly MA (1991) Feeding Patterns of the Strawberry Poison Frog, Dendrobates pumilio (Anura: Dendrobatidae). *Copeia* 1991(3):723–730.
107. Pollen AA, et al. (2007) Environmental Complexity and Social Organization Sculpt the Brain in Lake Tanganyikan Cichlid Fish. *Brain Behav Evol* 70(1):21–39.
108. Herrmann H-J (1996) *Agualex-catalog: Cichlids from Tanganyika* (Dahne Verlag, Ettlingen, Germany).
109. Kumar S, Stecher G, Suleski M, Hedges SB (2017) TimeTree: A Resource for Timelines, Timetrees, and Divergence Times. *Mol Biol Evol* 34(7):1812–1819.
110. Julie P, et al. (2015) Out of Himalaya: the impact of past Asian environmental changes on the evolutionary and biogeographical history of Dipodoidea (Rodentia). *J Biogeogr* 42(5):856–870.
111. Fabre P-H, Hautier L, Dimitrov D, P Douzery EJ (2012) A glimpse on the pattern of rodent diversification: a phylogenetic approach. *BMC Evol Biol* 12(1):88.
112. Babb PL, Fernandez-Duque E, Schurr TG (2010) AVPR1A Sequence Variation in Monogamous Owl Monkeys (Aotus azarai) and Its Implications for the Evolution of Platyrrhine Social Behavior. *J Mol Evol* 71(4):279–297.
113. Fritz SA, Bininda-Emonds OR, Purvis A (2009) Geographical variation in predictors of mammalian extinction risk: big is bad, but only in the tropics. *Ecol Lett* 12(6):538–549.
114. León-Paniagua L, Navarro-Sigüenza AG, Hernández-Baños BE, Morales JC (2007) Diversification of the arboreal mice of the genus Habromys (Rodentia: Cricetidae: Neotominae) in the Mesoamerican highlands. *Mol Phylogenet Evol* 42(3):653–664.
115. Davies KTJ, Bates PJJ, Maryanto I, Cotton JA, Rossiter SJ (2013) The Evolution of Bat Vestibular Systems in the Face of Potential Antagonistic Selection Pressures for Flight and Echolocation. *PLoS One* 8(4):e61998.
116. Tapaltsyan V, et al. (2015) Continuously Growing Rodent Molars Result from a Predictable Quantitative Evolutionary Change over 50 Million Years. *Cell Rep* 11(5):673–680.
117. Psouni E, Janke A, Garwicz M (2012) Impact of Carnivory on Human Development and Evolution Revealed by a New Unifying Model of Weaning in Mammals. *PLoS One* 7(4):e32452.
118. Soria-Carrasco V, Castresana J (2012) Diversification rates and the latitudinal gradient of diversity in mammals. *Proc R Soc B Biol Sci* 279(1745):4148 LP-4155.
119. Abramson NI, Lebedev VS, Tesakov AS, Bannikova AA (2009) Supraspecies relationships in the subfamily Arvicolinae (Rodentia, Cricetidae): An unexpected result of nuclear gene analysis. *Mol Biol* 43(5):834.
120. Steppan SJ, Adkins RM, Anderson J (2004) Phylogeny and Divergence-Date Estimates of Rapid Radiations in Muroid Rodents Based on Multiple Nuclear Genes. *Syst Biol* 53(4):533–553.
121. Selvatti AP, Gonzaga LP, Russo CA de M (2015) A Paleogene origin for crown passerines and the diversification of the Oscines in the New World. *Mol Phylogenet Evol* 88:1–15.
122. Claramunt S, Cracraft J (2015) A new time tree reveals Earth history's imprint on the evolution of modern birds. *Sci Adv* 1(11). Available at: http://advances.sciencemag.org/content/1/11/e1501005.abstract.
123. Roquet C, Lavergne S, Thuiller W (2014) One Tree to Link Them All: A Phylogenetic Dataset for the European Tetrapoda. *PLoS Curr*

6:ecurrents.tol.5102670fff8aa5c918e78f5592790e48.
124. Drovetski S V., et al. (2013) Geographic mode of speciation in a mountain specialist Avian family endemic to the Palearctic. *Ecol Evol* 3(6):1518–1528.
125. Jetz W, Thomas GH, Joy JB, Hartmann K, Mooers AO (2012) The global diversity of birds in space and time. *Nature* 491:444.
126. Braun EL, et al. (2011) Homoplastic microinversions and the avian tree of life. *BMC Evol Biol* 11(1):141.
127. Fjeldså J, Irestedt M, Ericson Pgp, Zuccon D (2010) The Cinnamon Ibon Hypocryptadius cinnamomeus is a forest canopy sparrow. *Ibis (Lond 1859)* 152(4):747–760.
128. Pyron RA (2014) Biogeographic Analysis Reveals Ancient Continental Vicariance and Recent Oceanic Dispersal in Amphibians. *Syst Biol* 63(5):779–797.
129. Gomez-Mestre I, Pyron RA, Wiens JJ (2012) Phylogenetic Analyses Reveal Unexpected Patterns In The Evolution Of Reproductive Modes In Frogs. *Evolution (N Y)* 66(12):3687–3700.
130. Koblmüller S, Salzburger W, Sturmbauer C (2004) Evolutionary Relationships in the Sand-Dwelling Cichlid Lineage of Lake Tanganyika Suggest Multiple Colonization of Rocky Habitats and Convergent Origin of Biparental Mouthbrooding. *J Mol Evol* 58(1):79–96.