SI Appendix

SI Materials and Methods

Tissue Samples and Microarray Hybridizations

Total RNA from each tissue sample was extracted, (Absolutely RNA Microprep kit, Stratagene; TRI Reagent, Ambion; RNAqueous Mini, Ambion), amplified (MessageAmp II aRNA Kit, Ambion; Low-input RNA Linear Amplification Kit, Agilent), and Cy3/Cy5 dye-coupled (GE Life Sciences). In some cases two rounds of amplification were used, as indicated (Table S2). RNA from each individual animal was hybridized to a single array (n=6 per group in most cases, Table 1), using the SoNG 20K microarray, which has 20160 addresses including blanks, buffer spots, control cDNAs and replicated cDNAs (21). Arrays were printed in batches of 100 and used within 120 days; where possible, all samples from a single experiment were hybridized to arrays from a single print batch. To enable cross-batch normalizations, each array was hybridized with one experimental sample and a universal reference sample which was a pooled composite of zebra finch (*Taeniopygia guttata*) brain mRNA, also amplified. The Cy3/Cy5 dye coupling was balanced (dye-flipped) between experimental and universal reference samples within each treatment group to control for potential dye incorporation and hybridization biases. The arrays were hybridized overnight at 42°C in individual slide chambers (Corning), washed, scanned using Axon GenePix 4000B slide scanner (Molecular Devices) and visualized with GenePix Pro 6.0 (Molecular Devices). Analyzed slide images were manually edited and aberrant spots were flagged for exclusion in downstream analysis.

Data Preprocessing

Microarray data pre-processing and statistical analyses were done in R 2.13.1 (1), except where noted below. The limma package (2, 3) was used to read in the median foreground and median background fluorescence intensities. Any spots that had been manually flagged (-100) during spot finding were given a weight of zero so they would not be used in any subsequent calculations. Background correction was done using the "half" method, which subtracts the local background estimates from the foreground values, then sets any zero or negative values to 0.5 to avoid losing data (4). Within-array normalization was done using the "printtiploess" method to remove the dyeXintensity biases within each array.

The resulting intensity values are in the form of M-values, which are log2(Cy5 / Cy3). However, all the experiments were done as a "common reference" design, where each sample was hybridized against the same common reference sample to facilitate comparisons. Dye-balancing was also done, so that in half the arrays the reference was in the Cy5 channel in half the reference was in the Cy3 channel. Therefore, instead of M-values, we transformed the intensities so they were all log2(sample / reference). These sample:reference values were used to do an initial comparison between all experiments, before doing any between-array normalizations. Between-array normalization changes depending on which arrays are normalized together and carries the assumption that most genes will not be changing expression. This assumption is almost certainly violated with the many differences species represented by the experiments. The common reference is enough of an internal normalizing factor that the values are reasonable directly comparable without between-array normalization. The initial comparison consisted of principal components analysis as implemented in the affycoretools package (5). Plots of PC1 vs. PC2 were done to see how the arrays clustered, and by coloring the points using various factors we can see which of them are responsible for the most variation in expression data. PC1 separates

and clusters samples according to species (Fig. S2A), PC2 does so according to the number of rounds of amplification during probe preparation (Fig. S2B), and within each cluster sub-groupings by experiment can be seen (Fig. S2C).

The difference in the number of rounds of amplification is potentially problematic if the experiments with two rounds (e01, e07 and e08) resulted in more variation in mRNA abundance between samples, which would lead to lower power to detect expression differences compared with experiments with only one round of amplification. However, two of the experiments with 2X amplification (e01 and e09) involved comparisons of brain regions which showed large numbers of differences. The third experiment with 2X amplification (e07) involved both age and food access comparisons with the age comparison also found a large number of significant differences. We also evaluated the coefficient of variation for each of the 80 treatment groups and found no evidence of greater variation for the 2x amplification groups. Hence we have no reason to think that the additional amplification steps would have significantly interfered with detection of within-experiment treatment differences.

In addition to these effect of species, amplification number and experiment, the Arnold (e04), Ball (e08) and Wingfield (e14) experiments had their arrays cluster into two distinct groups when viewed in a movable 3D plot of PCs 1-3 (not shown), which corresponded to different print batches of the arrays. The two print batches in the Ball (e08) experiment were found in no other group, and the Nordeen (e02) and Dong (e11) experiments all were the only experiments on their respective print-batches, so trying to remove the effect of print batch in a global ANOVA model risked removing real differences in these experiments. Additionally, within each experiment we observed a reference by dye interaction, such that even using the sample:reference values that had been within-array normalized, we could still tell which samples had the reference in Cy5 and which had the reference in Cy3. This refXdye effect was more pronounced in the non-zebra finch samples than in the zebra finch samples, but even within the zebra finch samples the amount of the effect varied. The likely explanation is that all the samples, to a varying degree, were different from the reference sample (mix of adult male and female whole telencephalon), and that these differences were not evenly symmetric around zero. The within-array normalization does force the M-value to be symmetric around zero, so values for a particular gene could be above or below zero depending on which dye the reference was in. This is why the reference dye remains in the expression signature even after within-array normalization and conversion to sample:reference orientation.

ANOVA

The combination of the confounded print-batch effects, the variable refXdye effect, and additional batch effects only found in certain experiments like hybridization date and repeated samples from the same bird, made it impossible to adequately control for all these factors in one global model. Therefore, for our next step we analyzed each experiment separately. The first step in the separate analyses was a between-array scale normalization on the sample:reference values (doing the normalization on the M-values only exacerbated the refXdye effect). Then a statistical model was fit in limma, which uses an empirical Bayes correction to moderate the variance estimates based on all genes together (6). For the experiments with one factor, we conducted a one-way ANOVA F-test and for those experiments with 2 factors, we made contrasts for the main effect of factor 1, the main effect of factor 2 and the interaction term. The Hahn (e03) experiment was a 2x2 factorial design, but it also had one extra treatment group that could only be compared with one of the other 4 groups. Therefore, we made one extra contrasts comparing these two groups. In all cases, the model also accounted for the refXdye effect and any additional batch effects as necessary (Table S3).

Weighted Gene Co-expression Network Analysis (WGCNA)

To look at the patterns of gene expression across all the treatments represented by the 15 experiments, we used the individual ANOVA models to estimate a mean expression value for each treatment group. This collapsed the 488

samples down to 80 treatment groups, and again we used PCA to assess relationships among the treatment groups, as we did for the individual samples in Fig. S2. The patterns (Fig. S3, left column) were remarkably similar to those seen with all 488 samples: groups from zebra finch samples on the right and other species on the left (Figure S3, top left), groups amplified once on the top and twice on the bottom (Figure S3, middle left). Groups from the same experiment clustered more tightly together (Figure S3, bottom left), but this is expected because each experiment was between-array normalized separately.

The PCA shows that the patterns of gene expression are most strongly affected by the species of the sample and the number of rounds of amplification. However, the differences between zebra finch and the other species may not actually represent expression differences, but instead lower hybridization efficiency of the other species due to sequence divergence from the zebra finch cDNAs. Additionally, amplification number is solely a technical artifact that is known to affect expression measurements. Expression patterns due to sequence divergence or amplification number may obscure more subtle patterns due to experimental factors that we would like to assess. Therefore, we removed the batch effect of zebra finch versus "not-zebra finch" (adjusting for each non-zebra finch species separately could have removed experimental treatment effects that were only tested in one species) and the batch effect of amplification number from the group value estimates.

PCA was done again on the batch-corrected group values (Figure S3, right column) to ascertain that the batch effects had been removed and see what the next biggest factors affecting expression might be. As expected, the groups no longer separate by zebra finch versus non-zebra finch or by amplification number, but the within-experiment grouping is still very strong.

The Weighted Gene Co-expression Network Analysis is similar to other clustering methods in that it calculates a distance metric between the expression patterns of all genes, but the difference is the complexity of the distance metric. WGCNA first starts with simple correlation values (usually Pearson) between all pairs of genes. Then the correlations are transformed into an adjacency matrix by raising the correlations to a soft-thresholding power function, β. The parameter β is chosen based on the data set to achieve an approximate scale-free network (7) and favors strong correlations over weak correlations. The adjacencies are next transformed into a topological overlap matrix (8) which as a similarity measure can be subtracted from 1 to give a distance measure. These distance measures are then used in traditional hierarchical clustering to represent the relationships among genes in a familiar dendrogram. The next step in a WGCNA analysis is to break the genes into clusters or "modules". There are many different methods of cutting a dendrogram and WGCNA suggests a computational approach called Dynamic Branch Cut (9).

We performed WGCNA in R using the WGCNA package (10) on the 17,175 probes that had a p-value < 0.001 in at least one of the experimental contrasts. There are many different parameter choices at each step in the process. After assessing a range of soft thresholding values, we chose power $\beta = 8$. We were able calculate Pearson correlation coefficients between all pairs of probes in one block on a laptop computer with 64-bit Windows and 4 GB of RAM. We chose to use a signed adjacency and signed topological overlap matrix to preserve the differences between positive correlation and negative correlations. Average linkage hierarchical clustering was used and modules were determined using the Dynamic Hybrid method with deepSplit = 2 and a minimum module size = 30. A second Partitioning Around Mediods-like stage of module detection was done with pamRespectsDendro = TRUE. At the end, modules with similar expression patterns were merged at mergeCutHeight = 0.2. Otherwise, the default values of the blockwiseModules() function were used.

Once modules have been defined, an average expression profile of all the genes in the model can be determined by calculating an eigengene value for each treatment group in the model by taking the first principal component of the

expression values of the genes in the module. Relatively higher expression values are represented by positive eigengene values and relatively lower expression values are represented by negative eigengene values, such that the set of eigengene values for a module can be taken as a proxy for the average expression pattern of all the genes in that module (Figure S6). Our original 17,175 probes X 80 treatment groups data matrix has now been reduced down to 95 modules X 80 groups. We then used a series of one-way ANOVA models to assess the effect of experiment (15 levels), species (5 levels), tissue (12 levels), age (8 levels) or sex (2 levels), photoperiod after sacrifice (6 levels) and song exposure prior to sampling (2 levels) (Table S4). Experiment was the most significant factor for 74 modules; this dominant effect of experiment was not unexpected because 1) the within-experiment normalizations, while necessary, artificially increased the expression similarity of treatment groups within each experiment, and 2) many of the experiments were partially or completely confounded with the other factors so that any effects of those factors would also show up as an experimental effect. At the other end of the spectrum, neither photoperiod at sacrifice nor song exposure had any appreciable effect on any of the modules. Therefore, we ignored experiment, photoperiod at sacrifice and song exposure and looked to see which other factor had the most significant effect for each module as shown in Figure 2 and Table S4.

SI APPENDIX REFERENCES

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Figure S1: phylogeny of species under study. Dates as in: Cracraft, J., and F.K. Barker. 2009. Passeriformes. Pp. 423-431 in S.B. Hedges and S. Kumar, eds., *The Timetree of Life*. Oxford University Press, New York.
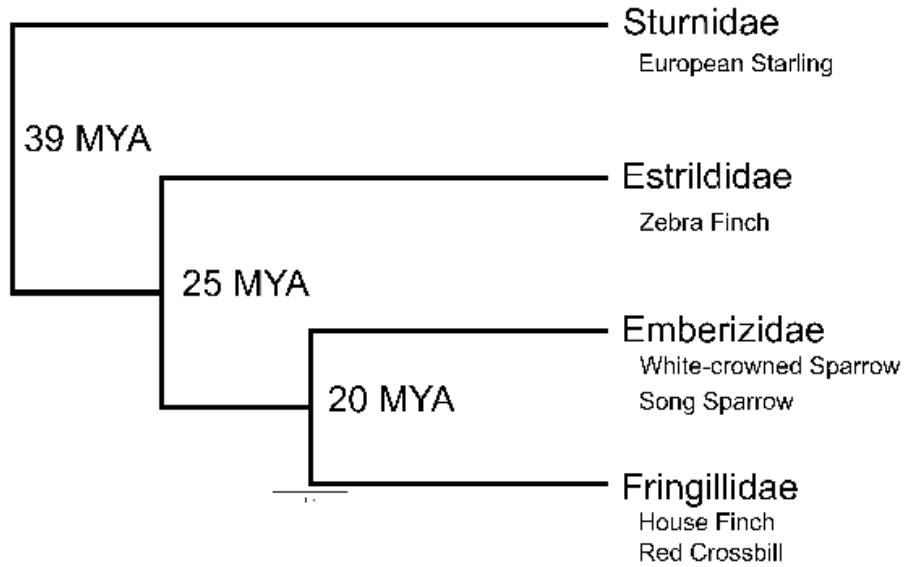
Figure S2.

PCA plots (first two principal components) of all samples labeled for species, amplification and experiment. The same plot is repeated three times, with samples colored according to species (A), rounds of amplification during cDNA labeling (B) or Experiment (C). The samples cluster into four distinct groups, with the zebra finch samples separated to the right from the other species mainly along the PC1 axis (A), whereas PC2 appears to reflect the effect of different rounds of amplification during probe preparation (B). Within the four main clusters, sub-grouping by experiment can also be seen (C). GWCS is Gambel's White-crowned sparrow.
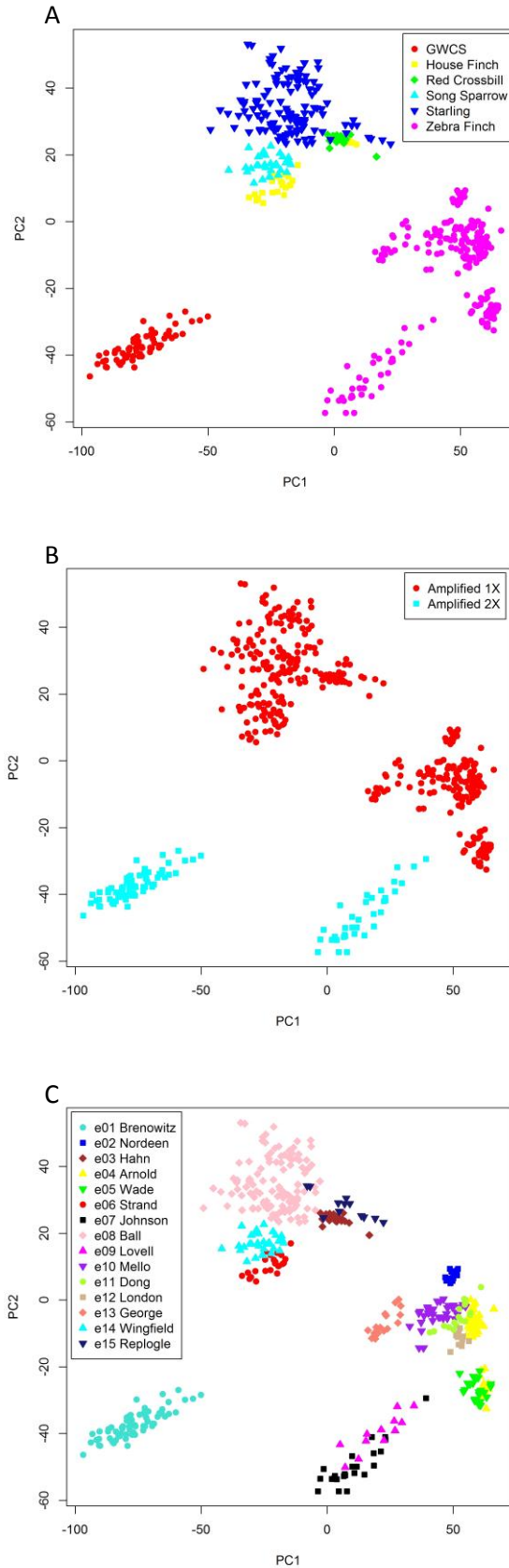
Figure S3. PCA plots (first two principal components) of the 80 treatment groups showing the clustering before (left column) and after (right column) batch corrections for zebra finch vs. non-zebra finch and amplification number. In each column the same plot is repeated 3 times with groups colored according to species (top), rounds of amplification during cDNA labeling (middle) or Experiment (bottom). The before correction plots (left column) show almost identical patterns to the PCA plots of all 488 samples (Figure S2).
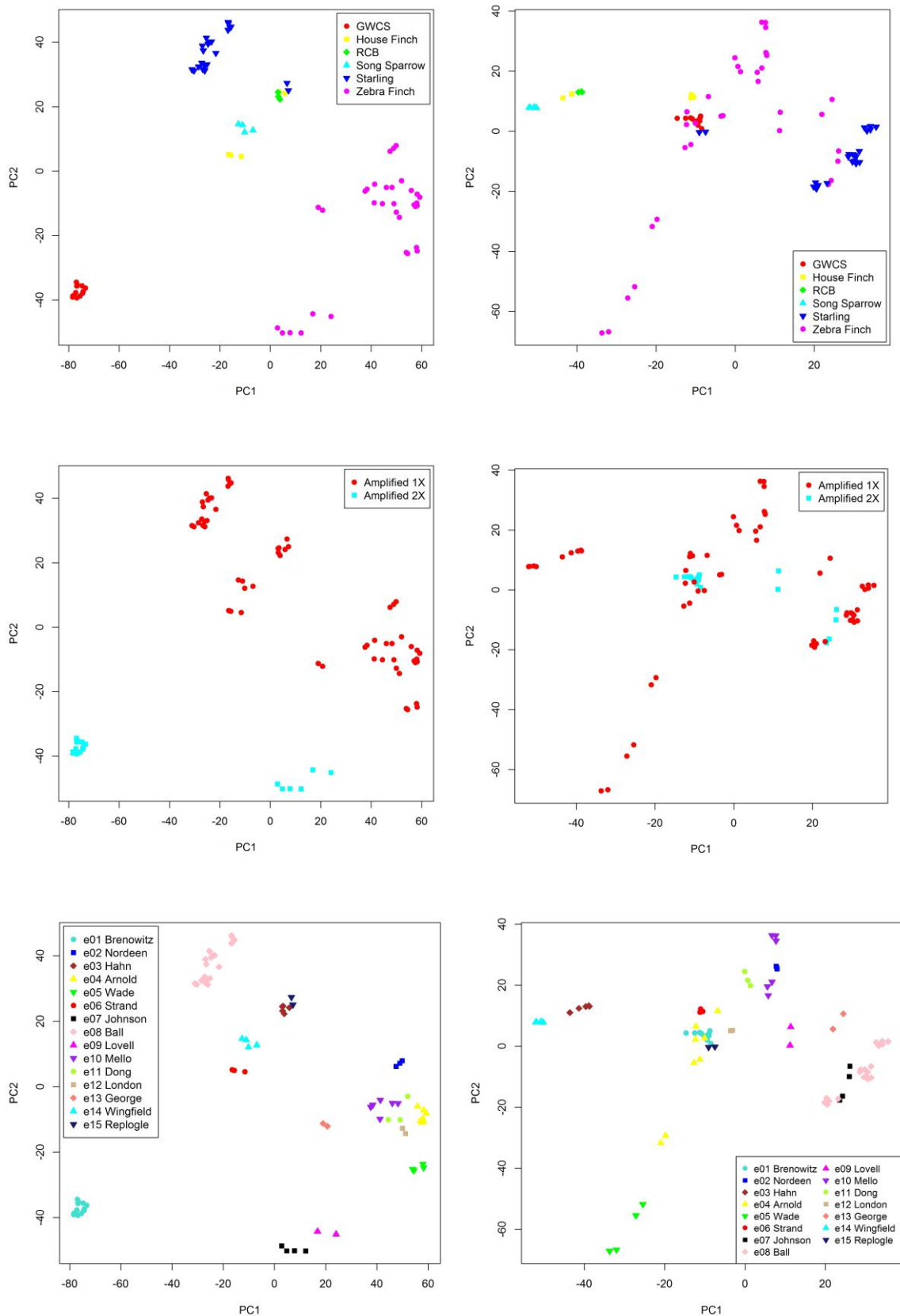
Figure S4   **Histogram of the number of contrasts for which each cDNA had p-values < 0.001.** The twelve genes significant for 9 or more contrasts are identified in Figure S5.
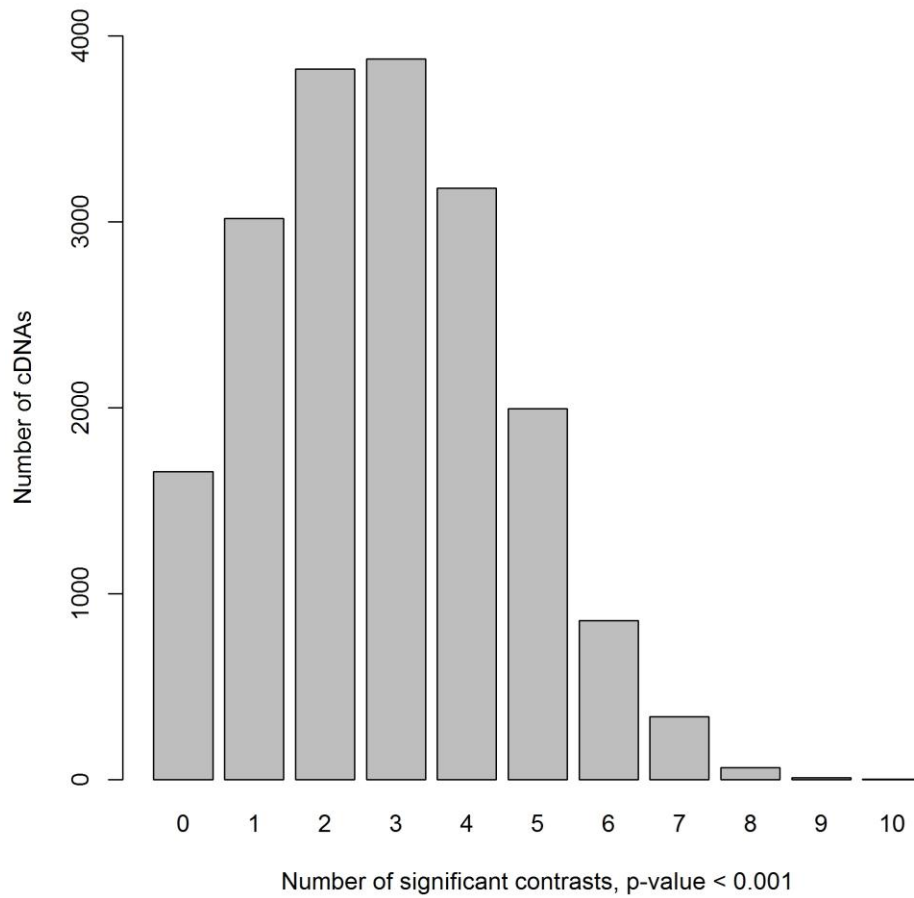
Figure S5   **Genes significant in the most contrasts.** In each column, contrasts for that gene that are significant at p<0.001 are shaded, with darker shading indicating lower p-value.

Contrast		ADAMTS1	APOH	CRHBP	CTSB	EGR1	GPR98	JUND	MTMR2	MURC	NR4A3	PCSK1	RCAN2
e01.A	photoperiod + T												
e01.B	region (HVC, RA)												
e01.C	photoperiod X region												
e03.A	species												
e03.C	species X diet												
e04.A	age (p1 to adult)												
e04.B	sex												
e04.C	age X sex												
e05.B	age (p1, p7)												
e06.A	photoperiod + T												
e07.A	age (p55, Adult)												
e07.B	food (singing)												
e07.C	age X food												
e08.A	region (4)												
e08.B	photoperiod + T												
e08.C	region X photoperiod												
e09.A	region (HVC, shelf)												
e10.A	song playback												
e10.B	region (NCM, L2a)												
e10.C	playback X region												
e11.A	song playback (adult)												
e12.A	song playback (p20)												
e13.A	age (p25, p45)												
e15.A	song playback (starling)												
Module		26	26	73	20	17	12	12	80	16	42	1	0	26	78	78

Figure S6. Correlation between expression heatmap for the 829 cDNAs in module 1 and the calculated eigengene values for each treatment group in module 1. For each module, the eigengenes are standardized to a mean of 0 and a standard deviation of 0.1125. The eigengene values can be used as a proxy for the overall expression pattern of the cDNAs in down-stream analyses, such as ANOVAs.
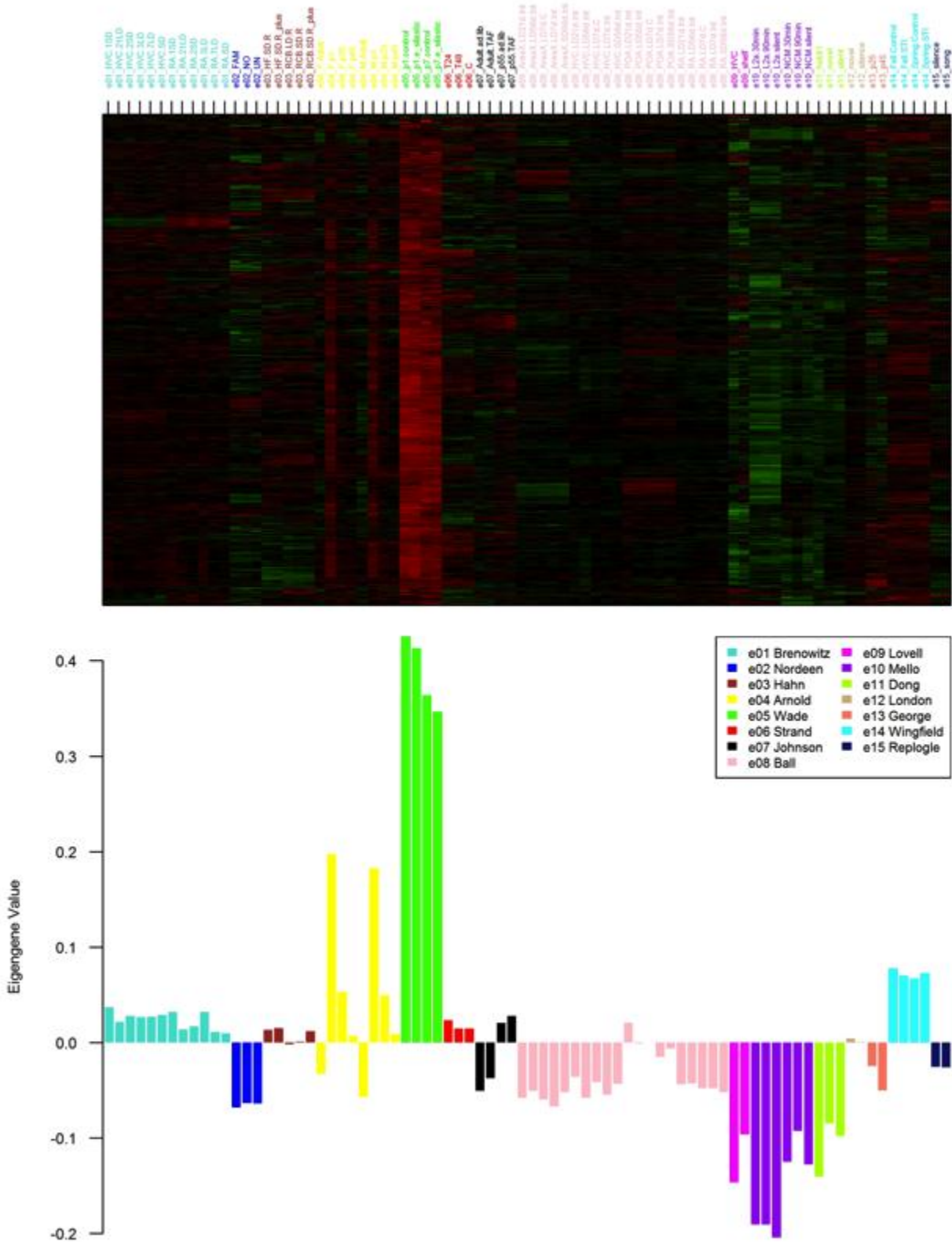
Figure S7. Plots of the eigengene values for modules 1 (top two), and 13 (bottom two); in each module set the first graph is colored by brain region and the second graph by age. Each bar represents one of the 80 treatment groups (see Figure S6 for treatment names). Both modules had significantly over-represented GO terms relating to ribosomes and highly significant ANOVA p-values for both brain region and age.
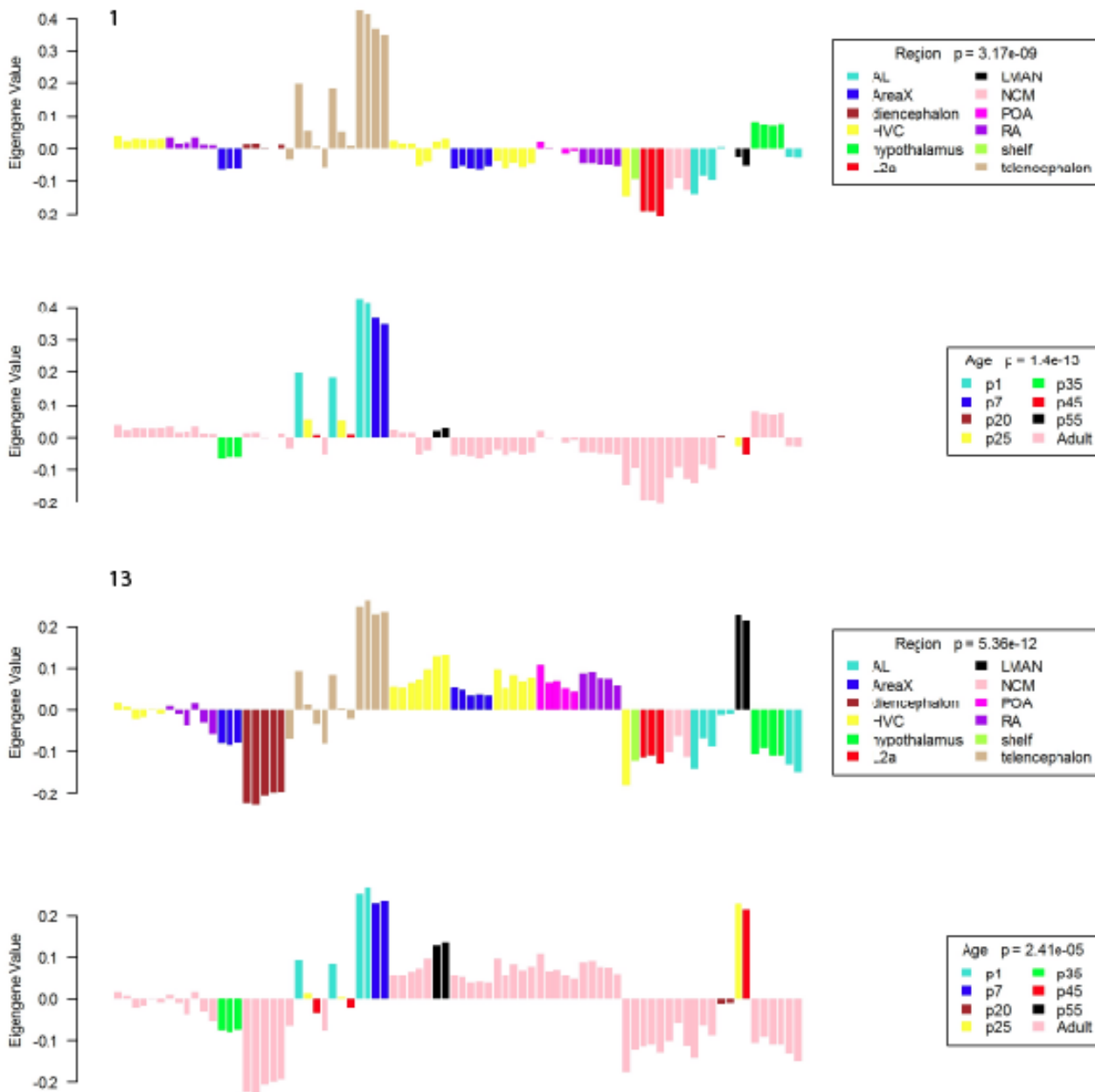
Figure S8. Plots of the eigengene values for modules 82 (top) and 95 (bottom), colored by sex. Each bar represents one of the 80 treatment groups (see Figure S6 for treatment names). Both modules had significantly over-represented GO terms and highly significant ANOVA p-values for sex.
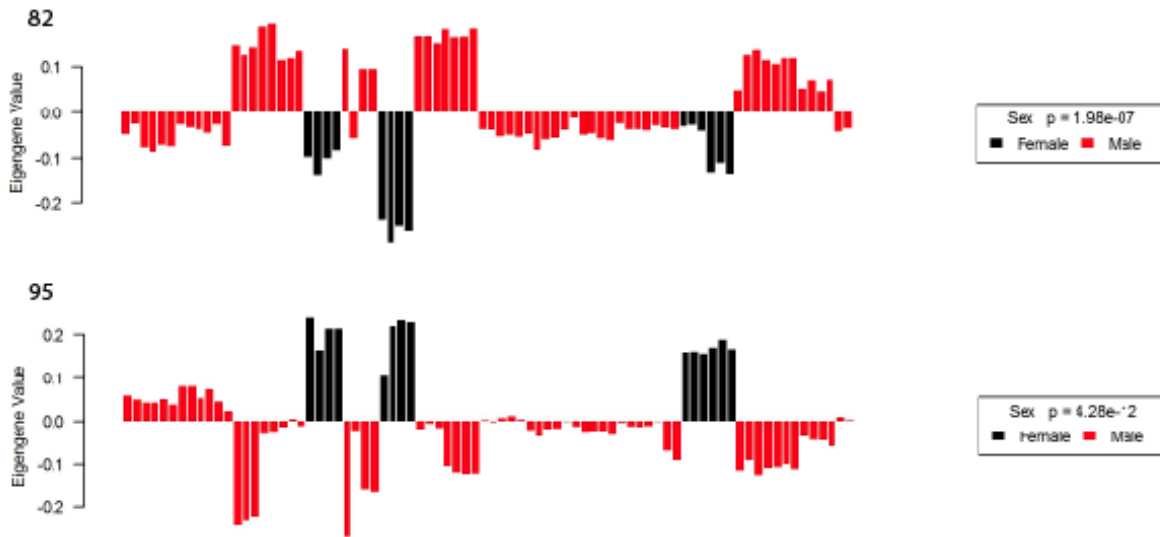
Table S1. Summary of microarray experimental designs for the 15 experiments compared in the ANOVA and WGCNA analyses.

| Exp.	Author	Species	Description	Pub.
e01	Brenowitz	Gambel's White-Crowned Sparrow (Family Emberizidae)	Castrated, short-day (8:16 light:dark) synchronized birds were implanted with testosterone (T) capsules and shifted to long days (20:4 light:dark). HVC and RA song nuclei were collected from birds after 3, 7, and 21 days, remaining birds shifted back to short days with HVC and RA collected after 1 and 2 days. Short day controls were collected at the time other birds were implanted with T.	(11)
e02	Nordeen	Zebra Finch (Family Estrildidae)	Area X of p35 males who had been individually acoustically isolated at p30, then at p35 placed next to a stimulus female and exposed to no tutor, father tutor, or unfamiliar tutor for 2 hours then sacrificed.	
e03	Hahn	Red Crossbill, House Finch (both Family Fringillidae)	Diencephalons were collected under short days (8:16 light:dark) from birds on either standard or enriched diets, in 2 species that differ in effects of diet on reproductive behavior (responsive red crossbills, non-responsive house finches). Red crossbills on a standard diet and long days (20:4 light:dark) were also compared to the short day group to assess effects of photostimulation in this species.	
e04	Arnold	Zebra Finch	Whole telencephalon of p1, p25, p45 and adult birds, both males and females.	(12, 13)
e05	Wade	Zebra Finch	Whole telencephalon of p1 and p7 females, implanted on hatch day with estrogen-containing silastic capsules or blank controls.	
e06	Strand	House Finch	HVC of adult males on short days (8:16 light:dark) at time zero, 24, and 48 hours after implantation with testosterone-containing silastic capsules.	
e07	Johnson	Zebra Finch	HVC of p55 and adult males after 20 days of food *ad libitum* or Timed Access to Food (TAF) to assess singing-driven differences in gene expression.	
e08	Ball	Starling (Family Sturnidae)	Area X, HVC, POA and RA from adult males that were gonadally-intact and photostimulated (7 and 21 days on long days, 16:8 light: dark), castrated and photostimulated (7 days on long days), and photorefractory (56 days on long days), with short-day control (56 days on short days, 11:13 light:dark).	(14)
e09	Lovell/Mello	Zebra Finch	HVC and shelf regions from adult males.	(15)
e10	Mello/Lovell	Zebra Finch	NCM and L2a from adult females, 30 or 90 minutes after onset of novel song playback, or silence control.	
e11	Dong/Clayton	Zebra Finch	Auditory lobule (AL) from adult males after 30 minutes of novel or familiar song playback, or silence control.	(16)
e12	London/Clayton	Zebra Finch	AL from p20 males after 30 minutes of novel song playback or silence control.	(17)
e13	George/Clayton	Zebra Finch	LMAN from p25 and p45 males.	
e14	Wingfield	Song Sparrow (Family Emberizidae)	Hypothalamus from adult males 30 minutes after Simulated Territorial Intrusion (STI) or control, in spring and fall.	(18)
e15	Replogle/Ball/ Clayton	Starling	AL from adult males after 30 minutes of novel song playback or silence control.	

Table S2. Summary of the sample variables, collection and preparation practices for the 15 experiments.

SoNG-	# sam ples	Author	Species	Age	Sex	Tissue	Treat-ment1	Treatment2	tiss collec	Extrac.	Amplific.	site	bred	Sac mo.	Sac year
e01	71	Brenowitz	GWCS	Adult	M	HVC RA	HVC RA	SD 3LD+T 7LD+T 21LD+T 1SD-T 2SD-T	punches	RNaqueo	2x MessAmp	lab	wild	Nov-Dec	2005
e02	16	Nordeen	zf	p35	M	Area X	No tutor Fam Unfam	N/A	punches	RNaqueo	1x Agilent	lab	Rochester	Aug to Jan	2005 to
e03	24	Hahn	Red House	Adult	M	dienceph	LD SD	Roudybush Roudybush+	dissectio	Trizol	1x Agilent	lab	wild	Mar	2006
e04	48	Arnold	zf	p1 p25 p45 Adult	M F	telenceph	p1 p25 p45 Adult	M F	dissectio	Trizol	1x Agilent	lab	UCLA	Jun-Oct	2005
e05	24	Wade	zf	p1 p7	F	telenceph	p1 p7	Control E-silastic	dissectio	Trizol	1x Agilent	lab	MSU	Jul-Sep	2005
e06	24	Strand	House	Adult	M	HVC	0T 24hT 48hT	N/A	punches	RNaqueo	1x Agilent	lab	wild	A. Sep B. Mar	2005 2006
e07	24	Johnson	zf	p55 Adult	M	HVC	p55 Adult	Ad lib TAF	punches	Stratagen	2x MessAmp	lab	Acadiana	Oct to Feb	2005 to
e08	120	Ball	Starling	Adult	M	Area X HVC POA RA	Area X HVC POA RA	SD56d LD7dC LD7dl LD21dl LD56dl	punches	Stratagen	1x MessAmp	lab	wild	Jan-Jun	2008
e09	12	Lovell	zf	Adult	M	HVC / shelf	HVC HVC	N/A	LCM/Lo	Stratagen	2x MessAmp	lab	OHSU	Feb-Mar	2006
e10	36	Mello/ Lovell	zf	Adult	F	NCM L2a	NCM L2a	Silence 30min 90min	punches	Stratagen	1x MessAmp	lab	OHSU	Feb	2006
e11	18	Dong	zf	Adult	M	AL	Silence Novel Habituat	N/A	dissectio	RNaqueo	1x Agilent	lab	UIUC Magnolia	Feb-Mar	2005
e12	12	London	zf	p20	M	AL	Silence Novel	N/A	dissectio	RNaqueo	1x Agilent	lab	UIUC bred & MBF	Jun to Jan	2005 to
e13	16	George	zf	p25 p45	M	LMAN	p25 p45	N/A	punches	Stratagen	1x Agilent	lab	UIUC bred & MBF	Jun-Aug	2008
e14	31	Wingfield	Song	Adult	M	hypothala	Spring Fall	Control STI	dissectio	Trizol	1x Agilent	field	wild	A. Jun 05 B. Nov to	2005 2005 to
e15	12	Replogle	Starling	Adult	M	AL	Silence 30min	N/A	dissectio	Trizol	1x Agilent	lab	wild	Dec	2005

Table S3. number of treatment groups per experiment, plus the extra batch effects included in the ANOVA models.

Experiment	# treatment groups	Extra batch effects
e01 Brenowitz	12	refXdye, bird
e02 Nordeen	3	refXdye
e03 Hahn	5	refXdye
e04 Arnold	8	refXdye, print batch
e05 Wade	4	refXdye
e06 Strand	3	refXdye, hyb date
e07 Johnson	4	refXdye + 3 outliers removed
e08 Ball	20	refXdye, print batch, bird
e09 Lovell	2	refXdye, bird
e10 Mello	6	refXdye, bird
e11 Dong	3	refXdye
e12 London	2	refXdye
e13 George	2	refXdye
e14 Wingfield	4	refXdye, print batch
e15 Replogle	2	refXdye

Table S4. P-values for the ANOVAs associating the eigengene values for each module with each factor. See Figure 1 for a heat map of the p-values for region, species, age and sex.

	Expt	species	region	age	sex	Low Factor	Low Factor No Expt
ME1	1.83E-28	4.4E-01	3.2E-09	1.4E-13	2.6E-02	Expt	age
ME2	1.20E-58	3.7E-29	7.7E-09	8.2E-01	2.7E-01	Expt	species
ME3	6.06E-59	1.0E-22	3.1E-09	5.6E-01	1.3E-01	Expt	species
ME4	1.75E-57	4.2E-10	1.9E-14	1.3E-01	1.8E-01	Expt	region
ME5	3.14E-44	8.5E-07	5.7E-23	2.5E-02	7.2E-01	Expt	region
ME6	7.29E-51	2.1E-08	9.2E-09	5.4E-06	2.0E-03	Expt	region
ME7	1.15E-48	4.0E-03	3.5E-21	5.9E-01	5.4E-01	Expt	region
ME8	6.41E-47	1.6E-17	1.1E-21	8.4E-01	4.7E-01	Expt	region
ME9	1.75E-30	1.4E-07	1.5E-17	1.6E-04	1.2E-01	Expt	region
ME10	1.01E-60	1.7E-07	1.6E-02	1.1E-03	8.7E-03	Expt	species
ME11	1.70E-46	4.0E-25	1.6E-17	8.8E-01	5.5E-01	Expt	species
ME12	2.00E-06	3.3E-01	4.4E-16	1.1E-07	3.8E-03	region	region
ME13	8.96E-37	7.7E-04	5.4E-12	2.4E-05	3.5E-01	Expt	region
ME14	1.50E-59	2.6E-40	1.8E-13	1.0E+00	7.8E-01	Expt	species
ME15	3.02E-05	7.1E-01	7.4E-32	1.3E-04	3.2E-02	region	region
ME16	2.01E-48	1.0E-05	1.0E-05	2.7E-03	2.8E-03	Expt	region
ME17	6.39E-21	5.0E-01	1.5E-12	1.0E-06	4.8E-01	Expt	region
ME18	2.84E-28	8.0E-03	1.7E-08	9.0E-09	3.2E-01	Expt	age
ME19	5.40E-36	9.6E-13	2.1E-09	1.1E-03	5.0E-03	Expt	species
ME20	1.73E-07	4.6E-01	1.0E-18	2.6E-05	5.5E-01	region	region
ME21	1.88E-50	4.6E-07	5.8E-07	1.9E-02	5.3E-03	Expt	species
ME22	2.24E-13	1.6E-02	2.7E-27	1.3E-06	2.9E-02	region	region
ME23	1.03E-33	4.9E-05	1.6E-12	1.1E-02	8.7E-02	Expt	region
ME24	5.44E-19	2.3E-04	4.5E-12	5.8E-07	1.0E-01	Expt	region
ME25	3.13E-39	4.2E-04	2.6E-10	1.9E-03	8.4E-01	Expt	region
ME26	5.05E-25	2.3E-06	1.2E-04	1.1E-05	8.9E-03	Expt	species

ME27	3.61E-23	3.2E-02	2.3E-06	6.7E-12	1.5E-02	Expt	age
ME28	2.72E-17	2.0E-06	5.0E-07	7.1E-05	1.2E-01	Expt	region
ME29	2.32E-23	2.4E-11	7.7E-15	9.5E-02	1.3E-01	Expt	region
ME30	1.06E-26	2.9E-14	5.0E-14	6.7E-03	1.7E-01	Expt	species
ME31	1.03E-47	6.4E-16	1.2E-17	1.7E-01	5.5E-02	Expt	region
ME32	2.86E-28	3.1E-03	2.5E-09	4.1E-07	7.5E-01	Expt	region
ME33	1.15E-12	7.5E-02	5.0E-22	3.6E-02	1.8E-01	region	region
ME34	3.22E-15	1.8E-03	5.8E-18	1.2E-04	3.2E-01	region	region
ME35	3.69E-32	1.0E-02	1.7E-10	3.0E-08	2.7E-01	Expt	region
ME36	8.87E-33	3.3E-04	2.1E-04	2.5E-05	6.3E-01	Expt	age
ME37	6.90E-10	5.9E-03	2.9E-31	4.6E-06	1.0E-01	region	region
ME38	7.35E-10	7.6E-02	2.5E-15	3.5E-05	5.7E-01	region	region
ME39	5.39E-29	1.7E-03	9.7E-08	2.8E-06	1.3E-02	Expt	region
ME40	1.37E-28	7.7E-07	3.4E-12	6.9E-03	6.5E-01	Expt	region
ME41	6.40E-14	2.1E-04	4.2E-14	4.7E-05	7.8E-02	region	region
ME42	6.25E-50	9.6E-21	1.1E-13	9.7E-01	1.6E-01	Expt	species
ME43	1.13E-45	1.5E-01	2.2E-11	2.9E-03	7.6E-02	Expt	region
ME44	2.03E-37	9.7E-18	3.2E-17	1.5E-02	7.7E-02	Expt	species
ME45	1.78E-41	5.6E-33	3.9E-35	9.3E-01	7.0E-01	Expt	region
ME46	1.53E-45	8.3E-11	4.8E-09	1.7E-03	2.1E-03	Expt	species
ME47	1.19E-45	6.6E-21	1.8E-06	5.3E-01	2.0E-01	Expt	species
ME48	5.67E-15	7.6E-03	3.3E-16	1.9E-08	1.1E-01	region	region
ME49	1.15E-40	1.7E-06	1.9E-08	6.0E-04	6.9E-01	Expt	region
ME50	2.57E-24	7.9E-14	4.4E-27	6.5E-03	3.8E-01	region	region
ME51	3.63E-29	1.9E-03	2.0E-04	9.6E-09	1.8E-01	Expt	age
ME52	1.06E-39	4.8E-07	1.8E-06	2.4E-03	8.6E-02	Expt	species
ME53	1.54E-43	3.7E-09	2.8E-09	2.2E-02	9.9E-02	Expt	region
ME54	1.18E-27	2.7E-03	5.1E-17	2.3E-02	6.3E-01	Expt	region
ME55	3.68E-23	7.6E-07	2.2E-12	3.4E-01	5.6E-07	Expt	region
ME56	4.24E-06	2.6E-03	1.1E-22	2.1E-01	7.5E-01	region	region

ME57	5.58E-12	7.7E-02	5.3E-11	1.8E-06	4.7E-01	Expt	region
ME58	4.23E-30	1.5E-04	4.9E-05	4.5E-07	3.1E-01	Expt	age
ME59	3.08E-46	2.9E-29	1.3E-29	6.4E-01	6.0E-01	Expt	region
ME60	1.86E-44	1.8E-18	4.5E-24	1.4E-01	3.6E-01	Expt	region
ME61	1.88E-23	4.6E-11	3.4E-20	7.5E-03	2.5E-01	Expt	region
ME62	6.13E-19	1.4E-01	2.1E-09	5.9E-09	2.5E-01	Expt	region
ME63	5.98E-18	2.1E-03	1.8E-17	4.9E-04	1.1E-01	Expt	region
ME64	9.19E-29	1.6E-05	1.7E-15	3.3E-05	4.8E-02	Expt	region
ME65	6.06E-42	2.4E-09	3.5E-04	1.4E-02	7.1E-02	Expt	species
ME66	6.91E-30	1.7E-09	3.0E-11	5.9E-03	9.0E-02	Expt	region
ME67	6.33E-42	4.9E-11	9.0E-10	3.4E-02	1.0E-01	Expt	species
ME68	1.78E-30	9.9E-02	8.4E-15	2.6E-07	9.3E-02	Expt	region
ME69	7.43E-20	9.3E-06	1.2E-11	2.7E-02	9.3E-01	Expt	region
ME70	1.27E-34	1.2E-07	1.4E-08	4.0E-01	8.1E-05	Expt	region
ME71	2.95E-39	2.1E-05	6.2E-13	4.5E-08	4.4E-02	Expt	region
ME72	0.052673709	2.8E-01	2.6E-26	9.8E-02	9.3E-02	region	region
ME73	1.45E-32	1.5E-02	2.5E-09	7.8E-04	1.1E-03	Expt	region
ME74	6.32E-05	3.3E-01	2.2E-26	2.9E-05	5.4E-02	region	region
ME75	1.76E-05	1.2E-03	2.5E-29	7.6E-02	4.0E-02	region	region
ME76	2.13E-25	1.9E-04	3.5E-12	4.6E-08	1.4E-01	Expt	region
ME77	7.49E-35	9.2E-08	1.9E-05	4.5E-03	4.5E-01	Expt	species
ME78	7.74E-18	7.0E-01	1.5E-15	2.6E-09	4.2E-01	Expt	region
ME79	1.76E-17	3.0E-17	9.0E-24	9.1E-01	4.2E-01	region	region
ME80	2.61E-21	1.2E-03	1.6E-05	1.4E-02	3.8E-02	Expt	region
ME81	6.44E-42	1.7E-08	2.8E-11	3.1E-04	8.0E-01	Expt	region
ME82	6.02E-25	4.9E-05	3.7E-05	7.1E-07	2.0E-07	Expt	sex
ME83	3.65E-20	3.4E-04	6.9E-07	6.1E-02	6.1E-10	Expt	sex
ME84	1.17E-35	5.2E-21	1.7E-19	4.7E-01	3.8E-01	Expt	species
ME85	3.89E-36	1.9E-18	1.5E-10	3.1E-01	5.7E-01	Expt	species
ME86	8.52E-18	6.7E-05	8.4E-13	1.9E-08	6.4E-03	Expt	region

ME87	1.03E-30	5.8E-08	2.7E-13	3.0E-04	2.1E-01	Expt	region
ME88	3.17E-14	1.8E-10	2.3E-30	4.1E-02	3.7E-01	region	region
ME89	2.01E-25	9.8E-03	2.9E-22	2.6E-05	9.5E-03	Expt	region
ME90	6.31E-38	6.0E-05	7.2E-09	6.6E-03	1.6E-01	Expt	region
ME91	3.63E-09	2.5E-05	1.6E-26	4.0E-02	3.4E-02	region	region
ME92	0.000725016	5.0E-01	4.7E-22	1.5E-03	4.9E-01	region	region
ME93	6.66E-11	1.1E-04	1.9E-26	1.7E-01	2.5E-01	region	region
ME94	7.69E-21	4.4E-03	1.2E-17	2.1E-09	2.0E-02	Expt	region
ME95	6.85E-12	5.8E-01	3.0E-05	6.1E-06	4.3E-12	sex	sex
ME0	2.08E-24	6.4E-04	8.7E-08	1.8E-02	6.0E-01	Expt	region

SI Datasets Available As Separate Files

SI Dataset 1: "ANOVAplusModules_08feb12.txt". Master file giving p-values from ANOVA expression analysis, all spots, all 32 contrasts (also lists each spot's primary module membership assignment).

SI Dataset 2: "KMEvalues_09feb12.xslx". Module membership scores for all spots in all modules.

SI Dataset 3: "moduleBarplots_pValues.zip". Compressed archive of 96 jpeg files showing eigengene expression profiles and results of ANOVA factor analyses for each module.

SI Dataset 4: "Modules-GO.xslx". Results of GO term enrichment analysis for each module.