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RELATING QUANTITATIVE VARIATION WITHIN A BEHAVIOR TO VARIATION IN TRANSCRIPTION

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Many studies have shown that variation in transcription is associated with changes in behavioral state, or with variation within a state, but little has been done to address if the same genes are involved in both. Here we investigate the transcriptional basis of variation in parental provisioning using two species of burying beetle, *Nicrophorus orbicollis* and *Nicrophorus vespilloides*. We used

RNA-seq to compare transcription in parents that provided high amounts of provisioning behavior versus low amounts in males and females of each species. We found no overarching transcriptional patterns distinguishing high from low caring parents, and no informative transcripts that displayed particularly large expression differences in either sex. However, we did find subtler gene expression differences between high and low provisioning parents that are consistent across both sexes and species. Furthermore, we show that transcripts previously implicated in transitioning into parental care in *N. vespilloides* had high variance in the levels of transcription and were unusually likely to display differential expression between high and low provisioning parents. Thus, quantitative behavioral variation appears to reflect many transcriptional differences of small effect. Furthermore, the same transcripts required for the transition between behavioral states are also related to variation within a behavioral state.

Intermediate between a variant allele and the quantitative effect it produces is some variable mechanistic process (Maynard Smith et al. 1985). One such mechanism is variation in gene expression (Albert and Kruglyak 2015), the study of which has been enhanced by the development of transcriptomic methods (Todd et al. 2016) leading to a plethora of evolutionary studies using this technique. However, most evolutionary transcriptomic studies examine the changes in transcription associated with transitions in phenotypic state: transitions from one state to another. In evolution, however, it is variation within a phenotypic state – typically quantitative variation (Lynch and Walsh 1998) – that is exposed to selection and evolves. If variation in transcription is in fact a mediator of phenotypic effects, then two things must be true: allelic variation must influence transcriptional variation, and transcriptional variation must influence phenotypic variation. Through research mapping QTLs for gene expression phenotypes (eQTL), evidence for the first of these is rapidly

mounting (Albert and Kruglyak 2015). However, empirical work addressing the latter is less common. Research searching for quantitative trait transcripts (QTT; Passador-Gurgel et al. 2007) in *Drosophila* (Passador-Gurgel et al. 2007, Ayroles et al. 2009), human disease (Petretto et al. 2008, Lodder et al. 2012), and crops (Yang et al. 2012) has suggested that many genes are transcriptionally linked to phenotype (Mackay et al. 2009). Furthermore, work in *Drosophila* has suggested that predicting candidate transcripts with phenotypic associations may be relatively difficult (Nuzhdin et al. 2009). However, to date, QTT-like studies have only been performed in a handful of organisms, and none in natural populations, limiting the evolutionary conclusions that can be drawn. Here we begin to address this void by examining how transcriptional variation is related to behavioral variation in a complex quantitative behavior, parental provisioning of food by regurgitation to begging offspring.

Behavioral phenotypes can be among the most complex traits found in animals. Behavior is typically highly quantitative in nature, and is produced variably but reliably in response to environmental conditions (Székely et al. 2010). As such, behaviors are expected to be underpinned by an especially complicated set of genetic influences (Boake et al. 2002). Furthermore, the genetic basis of behavior can be logically split into two processes: first, a process that produces a qualitative change in behavioral state; and second, a process that produces a specific quantitative value of the new behavior. Many studies of transcription and behavior have focused on how transcription changes with changes in behavioral state (reviewed in Harris and Hofmann 2014). For example, in honeybees, differential expression is associated with the transition between nursing behavior and foraging behavior (Ben-Shahar et al. 2002, Whitfield et al. 2003, Grozinger et al. 2007, Ament et al. 2008). Other studies investigate how transcription relates to quantitative variation in behavior (QTT) or behavioral type in *Drosophila* (Dierick and Greenspan 2006, Edwards et al. 2006), fish (Cummings et al. 2008, Sanogo et al. 2012, Rey et al. 2013, Fraser et al. 2014 Wong et al. 2015, Bell et al. 2016) and

honey bees (Alaux et al. 2009), and have ascribed a strong and multigenic transcriptional basis to behavioral variation.

Information linking transcriptional associations with behavioral transitions to transcriptional associations with within-behavior variation is lacking. This requires data on levels of transcription in the same behavior using both types of comparisons. If there is overlap between these two transcriptional mechanisms, it suggests that the production of specific behavioral phenotypes is a single process; transcripts are adjusted to produce behavioral change and the specific level of that adjustment determines the quantitative phenotype. In this scenario, the transcriptional changes required to produce behavioral transitions are loose, only needing to follow a broad pattern. If, on the other hand, there is little or no overlap between these mechanisms, it instead suggests that two distinct processes are needed to produce a specific behavioral phenotype; one tightly controlled process to produce a transition, and a separately regulated process producing the quantitative phenotype. It has been predicted that regulatory mechanisms for different types of behavioral change will be different (Cardoso et al. 2015), though one study has found transcriptional overlap between behavioral transitions and behavioral variation in sticklebacks (Bell et al. 2016).

In this study, we examine how variation in transcription is associated with variation in parental provisioning behavior in burying beetles (*Nicrophorus* spp.), an insect taxon with unusually well-developed and well-described parenting behavior (Eggert and Müller 1997, Eggert et al. 1998, Scott 1998). Recent studies in *N. vespilloides* investigated the transcriptional underpinnings of transitions between behavioral states, from solitary to parenting and back to a solitary state, using transcriptomics (Parker et al. 2015, Palmer et al. 2016), candidate gene studies (Cunningham et al 2014, 2016, Roy-Zokan et al. 2015) and proteomics (Cunningham et al. 2017). Parenting and specifically the act of provisioning food by regurgitation to begging offspring is an ideal phenotype for contrasting the mechanisms causing behavioral transitions versus within-state variation because it

is quantifiable, predictable, shows continuous variation, and is heritable (Walling et al. 2008, Benowitz et al. 2016).

To examine the prediction that quantitative gene expression is related to the quantitative expression of provisioning behavior in *Nicrophorus*, we performed RNA-seq comparing gene expression of 20 individuals *a priori* classified as having high or low levels of provisioning behavior based on extensive behavioral observation (Benowitz et al. 2016). Furthermore, we repeated this in both sexes as both males and females are competent parents, performing separate RNA-seq experiments in males and females of two species, *N. vespilloides* and *N. orbicollis*. We broadly expected to find many transcripts associated with variation in provisioning behavior in each of the four samples. Given the strong behavioral (Benowitz et al. 2016) but imperfect genetic (Parker et al. 2015) similarity between sexes, we expected moderate but not complete overlap in the transcripts underlying variation in provisioning between males and females. We predicted we would find even less overlap between species, given the nuanced behavioral differences between them (Benowitz et al. 2016, Benowitz and Moore 2016). Additionally, following Cardoso et al. (2015), we predicted that the transcripts related to quantitative expression within parenting behavior should be different than those related to transitions between parental and non-parental states in *N. vespilloides* (Parker et al. 2015). Moreover, given the trait is quantitative and heritable (Walling et al. 2008), we predicted that more moderate differential expression is involved, as there may be many genes of small effect. Our results show that very nuanced changes in gene expression are associated with provisioning behavior and are shared to a degree across sexes and species. Contrary to our predictions, however, we found that the transcripts related to changes between behavioral states are more likely than random to be also related to within-state variation in provisioning behavior.

Materials and Methods

PHENOTYPING PARENTING

We collected *Nicrophorus vespilloides* and *Nicrophorus orbicollis* from Cornwall, UK and Georgia, USA, respectively, and maintained outbred laboratory colonies for multiple generations as described in Benowitz et al. (2015). Detailed methods of behavioral experimentation are described in Benowitz et al. (2016). All observations were of uniparental adults that were reared and observed in common and controlled laboratory conditions. Briefly, we made concurrent observations of female *N. vespilloides* (n = 57) and *N. orbicollis* (n = 61) during the hours of peak parental care, approximately one day after larval hatching. We scan-sampled each family 80 times over an eight-hour period, recording each time the adult was observed having mouth-to-mouth contact with larvae. We then repeated the experiment at a later date under the same conditions for males of *N. vespilloides* (n = 79) and *N. orbicollis* (n = 78). After we finished observations of each parent, we removed their heads, flash-froze the heads in liquid nitrogen, and stored them at -80°C for later transcriptome sequencing.

SAMPLE COLLECTION, PREPARATION, AND RNA SEQUENCING

After we completed all behavioral observations, we collected (flash freezing) the heads of the 10 highest and 10 lowest caring parents from each species and sex for our RNA-seq experiment. Sampling from the extremes of the phenotypic distribution in this way increases the power to detect associations with quantitative traits (Van Gestel et al. 2000). We collected only heads to limit our tissue types to brain and head fat body, the latter being an endocrine organ in insects. We extracted RNA from these 80 heads by first homogenizing them in liquid nitrogen and Qiazol (Qiagen, Venlo, Netherlands), then adding chloroform (Roy-Zokan et al. 2015). After this step, extractions followed

standard protocols from a Qiagen RNeasy Lipid kit. RNA libraries were then prepared using a TruSeq Stranded RNA LT Kit (Illumina, San Diego, CA) with one-third of the normal reaction volume (Reynolds et al. 2015). Samples were then sequenced on an Illumina NextSeq Mid Output Flow Cell at the Georgia Genomics Facility (GGF), generating paired-end 75 base pair reads. We first sequenced females of each species together, then both species of males together at a later date.

NICROPHORUS ORBICOLLIS GENOME SEQUENCING AND ASSEMBLY

We assembled the *N. orbicollis* genome *de novo* from Illumina sequencing to produce comparable mapping resources for transcriptome analysis. The *N. vespilloides* genome was recently sequenced, assembled, and annotated (Cunningham et al. 2015). For genome sequencing, we chose a single larva that was the product of two generations of inbreeding. We extracted its DNA using a sodium dodecyl sulfate (SDS) lysis buffer (Chen et al. 2010) and a phenol chloroform extraction. Two libraries, one 350 bp PCR-free library and one 6.5kb mate-pair library were made using Illumina manufacturer's instructions, and sequenced on an Illumina NextSeq Mid Output Flow Cell at GGF generating paired-end 75 base pair reads. We next trimmed reads for quality using Trimmomatic 0.32 (Bolger et al. 2014), removing 4bp windows with average quality less than 15 and removing reads under 25bp. We assembled the trimmed reads with Platanus (Kajitani et al. 2014) under the same parameters as for *N. vespilloides* (Cunningham et al. 2015). We then used DeconSeq (Schmieder and Edwards 2011) to remove any possible contaminants from the genome assembly. Genome assembly was 193 mb, with a scaffold N50 of 178.7 kb. BUSCO analysis (Simão et al. 2015) showed the assembly had complete sequence for 82.9% of conserved insect orthologs, and partial sequence for 88.6%.

TRANSCRIPTOME ASSEMBLY AND DIFFERENTIAL EXPRESSION ANALYSIS

We trimmed all RNA reads for quality using Trimmomatic 0.32 as above (Bolger et al. 2014) and mapped them to their respective reference genome using Tophat 2 (Kim et al. 2013). We then assembled reads for each species into transcripts using Cufflinks (Roberts et al. 2011), taking the longest isoform provided. We then remapped reads to the assembled and reduced transcriptome and generated gene level counts using RSEM (Li and Dewey 2011). Descriptive statistics for each assembled transcriptome are provided in Table S1. We then used edgeR (Robinson et al. 2010) to filter transcripts by abundance and perform normalization. We used normalized counts to perform hierarchical clustering analysis using the R package heatmap under default parameters. We then performed differential expression (DE) analysis in edgeR between high and low caring parents. We analyzed each species and sex separately, thus creating four separate DE analyses, assessing significance after multiple testing using FDR (Benjamini and Hochberg 1995) with a cutoff of 0.05 to match a closely related RNA-seq study (Parker et al. 2015). Our DE analysis in *N. orbicollis* females uncovered three samples with relatively high expression values for some transcripts (see Table S2). We therefore checked to determine if they were outliers by comparing these genes to overall expression profiles. Only the single gene was unusually highly expressed in each sample, and so these individuals were retained in the analysis because their overall expression profiles were not unusual (Fig. S1).

GENE SET ENRICHMENT ANALYSIS

We used Gene Set Enrichment Analysis (GSEA) to examine the similarity between DE transcripts in our four independent datasets. GSEA has the ability to test a predicted set of transcripts across the

entire transcriptome, as opposed to similar methods such as hypergeometric statistics that compare the overlap between specified subsets of the transcriptome. Following Subramanian et al. (2005), we selected the top 100 differentially expressed transcripts from one dataset (*N. vespilloides* females) to serve as a predictive set of transcripts. These 100 transcripts intentionally included both significantly and not significantly DE transcripts, as a major part of the rationale behind our GSEA was to assess the biological significance of genes with low-fold changes (and thus not called significant in edgeR). To examine this set of genes in another dataset, we calculated a running-sum statistic going down the entire list of genes (ordered from most DE to least DE). This statistic increases each time a gene in the set is encountered (weighted by the amount of DE) and decreases by a constant amount each time a gene outside of the set is encountered. The test statistic, or E-score, is the global maximum of this running sum statistic. The significance of the E-score was calculated by comparing it against 10,000 E-scores generated from gene lists with randomized phenotypic permutations. Therefore, this analysis asks whether the predictive gene set displays more DE using the real phenotypic information than random phenotypic information, a conservative GSEA methodology (Subramanian et al. 2005, Ackermann and Strimmer 2009). Directionality of DE was not considered to preserve consistency between real and permuted datasets (which do not contain directionality due to the lack of phenotypic information). For the set of 100 genes taken from *N. vespilloides* females, we performed this analysis in *N. vespilloides* males, *N. orbicollis* females, and *N. orbicollis* males. We also repeated the same analysis using the top 100 differentially expressed genes from *N. orbicollis* females. This predictive gene set was analyzed in *N. orbicollis* males, *N. vespilloides* females, and *N. vespilloides* males. We performed all GSEA analysis in R using homemade scripts.

MAPPING AND DIFFERENTIAL EXPRESSION USING PREVIOUSLY PUBLISHED TRANSCRIPTOME

Previous research on *N. vespilloides* compared gene expression profiles of individuals before they were parents, to individuals that were actively parenting (regurgitating food to begging offspring), to individuals who were no longer parenting (Parker et al. 2015). In order to compare our results directly to those of Parker et al. (2015) we mapped our *N. vespilloides* RNA-seq reads directly to their published transcriptome using Tophat2 (Kim et al. 2013) and generated read counts using RSEM (Li and Dewey 2011). Gene level counts were then filtered and normalized in edgeR as above. We then specifically examined the 867 (752 after abundance filtering) genes differentially expressed during parenting in *N. vespilloides* (Parker et al. 2015). First, we examined whether these transcripts displayed greater variability among parenting than non-caring transcripts by comparing the average squared coefficient of variance (CV^2) to that of two random transcript sets of equal size and average abundance using analysis of variance. We then performed GSEA using the same methodology as above to examine whether these genes displayed unusual patterns of differential expression between parents with high versus low provisioning phenotypes.

Results and Discussion

BEHAVIORAL PHENOTYPE

The complete description of behavioral variation in both species and both sexes is given in Benowitz et al. (2016). For *N. orbicollis*, the top 10 females caring for offspring selected for further analysis provided (mean \pm SD) 39.8 ± 5.7 bouts of care during the observation period. The lowest 10 *N.*

orbicollis females provided 11.7 ± 4.5 , bouts of care during the observation period. For *N. vespilloides*, the top 10 females caring for offspring provided 72.5 ± 2.5 bouts of care during the observation period. The lowest 10 *N. vespilloides* females provided 13.6 ± 9.7 bouts of care during the observation period. In males, in *N. orbicollis*, the top 10 parents provided 33.9 ± 4.4 bouts of care while the lowest 10 provided 11.8 ± 1.3 bouts of care. For *N. vespilloides* males the top 10 provided 72.9 ± 3.1 bouts of care while the lowest 10 provided 12.6 ± 8.6 bouts of care. These individuals provided the samples for analysis of transcriptional variation.

TRANSCRIPTOME-WIDE ANALYSIS OF PARENTAL VARIATION

We first examined whether an individual's overall transcriptomic profile predicted its parental provisioning phenotype. Therefore, we used hierarchical clustering to group all 20 samples in each of the four RNA-seq experiments per the similarity of their genome-wide expression profile. In none of our four experiments did samples of similar phenotype group together (Fig. S1-S4), thus, there is no obvious overall transcriptional signature of being a high or low provisioning parent. We next examined two possibilities: that variation in provisioning behavior is either associated with a few genes of large effect or with many genes of small effect.

To test the first of these possibilities, that variation in provisioning is associated with a few large effect transcripts, we performed standard analysis for differential expression (DE) in edgeR. DE analysis turned up a small number of significantly DE transcripts in three of our four RNA-seq experiments but with no obvious patterns (Table S2). Consistent with Parker et al. (2015), more variation in transcription was associated with females but there was no overlap between the species. In males, there was only one transcript that was significantly differentially expressed between high and low provisioning parents *N. orbicollis* and none in *N. vespilloides*. Because uniparental

provisioning behavior is remarkably similar between sexes at both the phenotypic (Benowitz et al. 2016) and molecular (Parker et al. 2015) levels, we expected the DE transcripts found in males and females of a species to at least partially replicate each other. Thus, there is no suggestion that these transcripts are evolutionarily associated with variation in provisioning behavior.

Standard analyses of differential expression, as performed above, often do a poor job of finding transcripts with small expression differences between treatments because of multiple testing across the entire transcriptome (Bullard et al. 2010). Therefore, to test whether such subtle differences might be associated with behavioral phenotype, we asked whether broader patterns of differential expression were similar across the four experiments. To answer this question, we used Gene Set Enrichment Analysis (GSEA; Subramanian et al. 2005) to assess whether the top DE genes from the standard analysis above displayed unusual patterns of DE in the other three RNA-seq datasets. This should only be the case if two things are true: that there are real transcriptional effects associated with provisioning behavior, and that those effects were at least partially shared across sexes and/or species. Therefore, the GSEA tested our hypothesis that even transcripts not considered statistically significant by edgeR might still be biologically related to parenting. Because females have more robust gene expression changes during parenting (Parker et al. 2015) we used the most DE transcripts from females of each species as a set of “predictor” transcripts, and following Subramanian et al. (2005) we *a priori* restricted the analysis to the top 100 DE transcripts. The top 100 DE transcripts from *N. vespilloides* females showed a high degree of DE in *N. vespilloides* males ($E = 0.582$, $p = 0.0393$; Fig. 1A), no unusual DE in *N. orbicollis* females ($E = 0.506$, $p = 0.2944$; Fig. 1B), and a moderate degree of DE in *N. orbicollis* males ($E = 0.578$, $p = 0.0605$; Fig. 1C). The top 100 DE transcripts from *N. orbicollis* females showed a moderate degree of DE in *N. orbicollis* males ($E = 0.625$, $p = 0.0775$; Fig. 2A), significant DE in *N. vespilloides* females ($E = 0.628$, $p = 0.0112$; Fig. 2B), and no unusual DE in *N. vespilloides* males ($E = 0.444$, $p = 0.4294$; Fig. 2C). *N. vespilloides*

males and females display much greater behavioral variation than *N. orbicollis* parents (Benowitz et al. 2016), which may account for the greater transcriptional similarity we observed within a species.

Our data largely recapitulate the results of other QTT-like studies, which typically demonstrate hundreds of transcripts with significant associations with phenotype (Mackay et al. 2009). It is unclear why we saw weak statistical associations between phenotype and individual transcripts, though that may simply be a consequence of the messiness of parental care as a phenotype with low but non-zero heritability (Walling et al. 2008), moderate repeatability (Benowitz et al. 2016), the inability of RNA-seq to measure potentially important classes of transcripts such as neuropeptides (Valcu and Kempenaers 2014), or the possibility that large and causal transcriptional differences might have occurred prior to our sampling time. Despite this, we suggest that the transcriptomic basis of variation in *Nicrophorus* provisioning behavior is multifaceted yet extremely subtle, with many transcripts showing slight associations with phenotype.

The differential expression of any transcript related to parenting may reflect either environmental plasticity or genetic variation. Our dataset likely includes transcripts of both types, and cannot distinguish between them. However, given that beetles were reared in common and controlled environments, we suggest that plasticity is at most playing a minor role in generating phenotypic and associated transcriptional differences between individuals. Instead, we argue that it is more likely that many of the transcriptional associations we find reflect genetic differences between individuals. Such genetic differences may be causing variation in behavior or may simply be correlated with it, perhaps reflecting variation in energy demands. Again, it is probable that our results capture transcripts of both types. However, either way, our data reflect the idea that even large differences in behavioral phenotype are associated with modest differences in the mechanisms related to that behavior.

These results have implications for the longstanding question of whether phenotypic variation is underpinned by a few genes of large effect or many genes of small effect (Rockman 2012). Though

our RNA-seq approach cannot directly answer the question of how many loci are responsible for producing phenotypic variation, it is suggestive of how many variables might be involved. eQTL studies suggest two broad possible explanations for our data. One is that the many expression differences we see could be caused by an eQTL hotspot, a single locus with causal effects on hundreds of transcriptional phenotypes (Schadt et al. 2003, Mehrabian et al. 2005, West et al. 2007). However, hotspots are not a ubiquitous feature of eQTL studies (Monks et al. 2004, Morley et al. 2004, Smith and Kruglyak 2008, Rockman et al. 2010), which are more likely to uncover *cis*-acting variants affecting individual genes (Gilad et al. 2008). Therefore, we suggest that a more likely possibility is that the transcriptional phenotypes in our study themselves display polygenic inheritance (Petretto et al. 2006).

Furthermore, we find no evidence of any genes whose transcription has major effects on phenotype. Thus, we argue that our results support an infinitesimal model of quantitative inheritance (Fisher 1918, Bulmer 1972), with many genes contributing an individually undetectable effect on phenotypic expression.

DIRECT COMPARISON OF TRANSCRIPTIONAL VARIATION WITHIN AND BETWEEN BEHAVIORS

We next asked whether the transcripts involved in transitioning into parental care behavior are also likely to be involved in generating quantitative variance in provisioning behavior. Parker et al. (2015) identified a set of 867 transcripts that were significantly differentially expressed in *N. vespilloides* between parents and non-parents. Using the published transcriptome from Parker et al. (2015), we asked whether this transcript set displayed unusual patterns of differential expression between high

and low provisioning *N. vespilloides*. First, we examined the variability of these transcripts by comparing the average squared coefficient of variance of transcripts in this set from that of two random transcript sets of equal abundance. We found that, for males ($F_{2,2250} = 31.595$, $p < 0.0001$) as well as females ($F_{2,2250} = 22.337$, $p < 0.0001$), the caring transcript set displays higher variability between samples than random genes (Fig. 3). Next, to see if this high level of variation was related to parental behavior, we used the same GSEA methodology as above. We found that these caring transcripts were significantly DE in males ($E = 0.569$, $p = 0.0263$; Fig. 4A) and moderately DE in females ($E = 0.570$, $p = 0.0898$; Fig. 4B).

Thus, it appears that a subset of the transcripts required to turn on parenting behavior are also modified further in association with quantitative expression of that same behavior. This suggests that parenting fits a model wherein specific behavioral phenotypes are obtained by simply modulating the extent of the initial response creating the behavior. In turn, this supports the idea that transitions between behaviors do not rely on specific transcriptional changes. Instead, it appears that only broad expression changes are required, and that these genes retain a high degree of potential variability after initiation of behavior. This may be further related to the relatively high flexibility of behavior; if the genes involved in producing behaviors are relatively unconstrained, they may be able to produce a broad range of phenotypes in response to environmental variation.

CONCLUSIONS

We present three fundamental insights into the transcriptional architecture of variation in behavior, using parental care in *Nicrophorus* beetles as our model. First, many transcripts appear to be related

to variation in parenting behavior, each with subtle effects. Second, the mechanisms governing variation in parenting share some commonality across both sexes and species, as expected. Third, the transcripts related to transitions into and out of parenting behavior are likely to be related to producing variation within parenting behavior. Together, these results not only provide basic mechanistic information on the construction of specific behavioral phenotypes, but may also lead to insight as to why behavior is uniquely flexible and evolvable.

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FIGURE LEGENDS

Figure 1. Gene set enrichment analysis of transcripts predicted from *N. vespilloides* females. The top 100 differentially expressed transcripts from *N. vespilloides* females are (A) significantly DE in *N. vespilloides* males, (B) not DE in *N. orbicollis* females, and (C) moderately DE in *N. orbicollis* males.

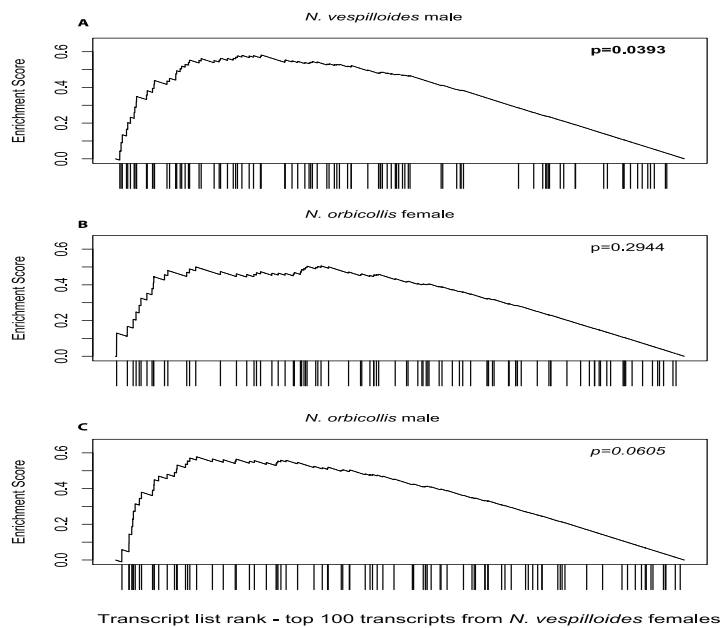


Figure 2. Gene set enrichment analysis of transcripts predicted from *N. orbicollis* females. The top 100 differentially expressed transcripts from *N. orbicollis* females are (A) moderately DE in *N. orbicollis* males, (B) significantly DE in *N. vespilloides* females, and (C) not DE in *N. vespilloides* males.

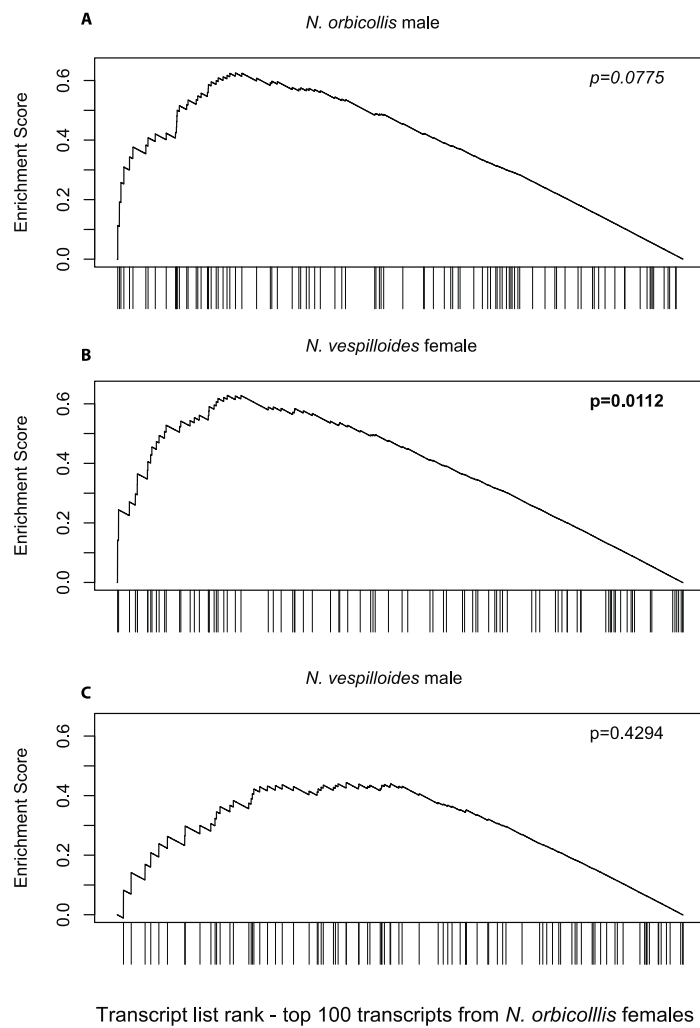


Figure 3. The variance of transcripts implicated in caring versus random genes. Transcripts differentially expressed between caring and non-caring *N. vespilloides* (Parker et al. 2015) show higher expression variance among caring parents than random transcripts in both (A) *N. vespilloides* females and (B) *N. vespilloides* males.

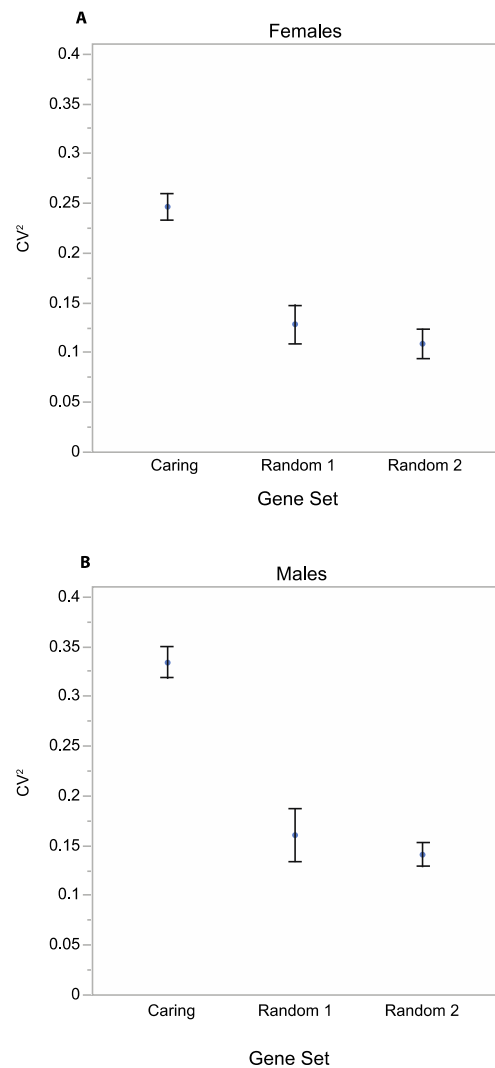


Figure 4. Gene set enrichment analysis of transcripts previously implicated in caring. The 752 transcripts found in our data set that were differentially expressed between caring and non-caring *N. vespilloides* from Parker et al. (2015) are (A) moderately DE in *N. vespilloides* females, and (B) significantly DE in *N. vespilloides* males.

