

Effect of larval nutrition on maternal mRNA contribution to the *Drosophila* egg.

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Keywords: maternal mRNA deposition, effects of nutrition, gene expression, life history, RNA-Seq

1 **ABSTRACT**

2 Embryonic development begins under the control of maternal gene products,
3 mRNAs and proteins that the mother deposits into the egg; the zygotic genome is
4 activated some time later. The early control of development by the maternal genome is
5 conserved across metazoans, and some flowering plants. The gene products contributed
6 by the mother are critical to many early developmental processes, and they set up
7 trajectories for the rest of development. The maternal deposition of these factors is an
8 often-overlooked aspect of parental investment. If the mother experiences challenging
9 environmental conditions, such as poor nutrition, previous studies have demonstrated a
10 plastic response wherein these mothers may produce fewer, larger eggs, to buffer the
11 offspring against the same difficult environment. This additional investment to produce
12 larger eggs can produce offspring that are more fit in the challenging environment. With
13 this study, we ask whether mothers who experience poor nutrition during their own
14 development plastically change their gene product contribution to the egg. We perform
15 mRNA-Seq on eggs at a stage where all mRNAs are maternally derived, from mothers
16 with different degrees of nutritional limitation. We find that nutritional limitation
17 produces similar transcript changes at all of our degrees of limitation. Genes that are
18 downregulated are those involved in translation, which is likely one of the most
19 energetically costly processes occurring in the early embryo. We find an increase of
20 transcripts for transport and localization of macromolecules. This may be consistent with
21 compensation for the known disruption of these processes in the ovary of nutrition-
22 limited mothers, and also for the critical role these processes play in many early
23 developmental processes such as axis formation. Our findings of upregulation of the
24 electron transport chain may reflect an earlier activation of this source of energy in
25 embryogenesis. The eggs produced by nutrition-limited mothers show a plastic response
26 in mRNA deposition, which may better prepare the future embryo for development in a
27 nutrition-limited environment.

28 **INTRODUCTION**

29 The earliest stages of embryonic development are entirely dependent on
30 maternally deposited RNAs and proteins, until the zygotic genome is activated later in
31 development (SCHIER 2007; TADROS and LIPSHITZ 2009; Langley *et al.* 2014). While
32 the length of the maternally driven portion of early embryogenesis varies amongst
33 species, both in absolute time and relative time of development, the maternal genetic
34 control of early development that sets up trajectories for the rest of development is a
35 conserved feature across animals and some plants (TADROS and LIPSHITZ 2009; LI *et al.*
36 2013; BAROUX and GROSSNIKLAUS 2015; ROBERTSON and LIN 2015; YARTSEVA and
37 GIRALDEZ 2015). Many fundamental developmental processes are initiated by maternal
38 factors, making the maternal contribution to early development highly critical, and
39 therefore has been the subject of considerable study. The composition of gene products
40 that mothers contribute to eggs has been investigated in a number of model systems at a
41 genomic scale (LI *et al.* 2010; HARVEY *et al.* 2013; LAVER *et al.* 2015).

42 The contribution of mRNAs and proteins to the egg by the mother is an often-
43 overlooked aspect of parental investment in offspring fitness. The maternal provisioning
44 of nutrients to offspring and subsequent effect on offspring life history and fitness has
45 been examined in a number of systems (MOUSSEAU and FOX 1998). Mothers that
46 experience poor nutrition will have more limited resources to devote to provisioning to
47 offspring. Thus effect of limited parental nutrition may be detrimental to the fitness of the
48 offspring. However, mothers may be able to alter provisioning when they have
49 experienced unfavorable environmental conditions to enhance offspring performance
50 under the same unfavorable environmental conditions. Life history theory predicts that
51 under stressful conditions, mothers may be expected to shift toward fewer offspring with
52 better provisioning (ROFF 1992). Provisioning is largely viewed through the lens of
53 providing nutrition to the offspring, with egg size or offspring size used as an easily
54 measured proxy.

55 *Drosophila* is a well-studied model system for maternal investment and life
56 history (PRASAD and JOSHI 2003; YADAV and SHARMA 2014; LACK *et al.* 2016;
57 PORCELLI *et al.* 2017), as well as for metabolic studies relevant to human disease
58 (PADMANABHA and BAKER 2014; ALFA and KIM 2016). Poor parental nutrition in
59 *Drosophila* has been demonstrated to result in a mix of potentially maladaptive and
60 adaptive phenotypes in offspring. Flies with poor nutrition at the larval stage become
61 smaller flies, yet lay heavier eggs (PRASAD *et al.* 2003; VIJENDRAVARMA *et al.* 2010).
62 This contrasts with the positive correlation of egg size and body size observed within
63 species (AZEVEDO *et al.* 1997), which would predict that the eggs laid by smaller mothers
64 would be smaller. As egg size is an approximation of maternal investment in offspring in
65 species lacking parental care, this can be viewed as an increase in maternal investment
66 per offspring in these nutritionally deprived mothers. And while all studies reported
67 larger eggs from nutritionally limited mothers, the flies that developed from those eggs
68 were reported to be smaller (VIJENDRAVARMA *et al.* 2010), larger (VALTONEN *et al.*
69 2012), or the same size (PRASAD *et al.* 2003) as those produced by mothers under
70 standard nutritional conditions. Note that while these studies all limited nutrition at the
71 larval stage for mothers, they limited nutrition in different ways, and used strains with
72 different genetic backgrounds. The offspring of nutritionally deprived mothers also have
73 higher viability to adulthood when raised on both standard and poor food in one study

74 (VALTONEN *et al.* 2012) and only when raised on standard food in other studies (PRASAD
75 *et al.* 2003; VIJENDRAVARMA *et al.* 2010). These results imply that the additional
76 investment of the mother experiencing poor nutrition is beneficial to the offspring under
77 some conditions.

78 In this study, we ask if and how maternal provisioning of gene products
79 (specifically mRNAs) provided to the egg changes with poor maternal nutrition at the
80 larval stage. We limit nutrition only at the larval stage in the parents, thus any observed
81 differences are due only to the parental diet during development. We collected eggs from
82 a developmental stage where all mRNAs present are maternally derived (Bownes' stage
83 2), extracted RNAs, and performed mRNA-Seq. Analysis of the RNA-Seq data shows
84 striking patterns of differential transcript deposition into eggs by nutritionally limited
85 mothers. These mothers deposit fewer transcripts for ribosomes and translation, and more
86 transcripts for macromolecule transport and localization and also for the electron
87 transport chain. These coordinated changes in gene expression across nutrition-limited
88 mothers do not correspond with genes previously identified as responding to nutrition,
89 which is consistent with these individuals not being nutrition-limited at the time of egg
90 production. Instead, we interpret these differences in expression relative to known
91 expression patterns of these genes in oogenesis, to understand the potential benefit or
92 detriment to the eggs development progresses.

93

94

95 MATERIALS AND METHODS

96 Larval diet

97 To raise larvae on food with varying levels of nutritional restriction, we prepared
98 dilutions (VIJENDRAVARMA *et al.* 2010) of a standard cornmeal food recipe. The standard
99 food was melted and diluted with an autoclaved water-agar mixture, to contain 100% (not
100 diluted), 25%, and 5% of the original cornmeal food. This was then portioned into
101 bottles, with at least 2 replicate bottles per food treatment, where it solidified. Then, 50
102 eggs were added to each bottle, from 4-10 day old, population controlled, Oregon-R
103 females, reared at 25 degrees C. Multiple rounds of bottles were set up, and effects on
104 development time and fecundity were observed. The bottles used to collect eggs from
105 were typical for these parameters. The larvae developed in the 100%, 25% and 5% bottles
106 at different rates, with non-nutritionally limited flies beginning to eclose in the 25% and
107 5% bottles days later (2 days later for the 25%, 5 days later for the 5%).

108

109 Sample acquisition

110 Female and male flies (10 each, newly eclosed) reared on 100%, 25%, and 5%
111 food were collected and placed in an egg collection bottle, and supplied with a standard
112 glucose-based egg-laying plate. Females were 2-14 days old at the time of egg collection.
113 Embryos were collected from egg-laying plates (for each collection, a new plate was
114 harvested after ~30 minutes), eggs were dechorionated using 50% bleach, and embryos
115 were moved to a microscope slide with halocarbon oil for visualization. Embryos were
116 observed, imaged (Zeiss AxioImager M2), and harvested as they reached stage 2
117 (Bownes' stages) of development (BOWNES 1975; CAMPOS-ORTEGA and HARTENSTEIN

118 1985). At stage 2, all mRNAs present are maternally derived (ALI-MURTHY *et al.* 2013).
119 This stage is also easy to identify based on morphology, as the vitelline membrane
120 recedes from both the anterior and posterior of the embryo, but the pole cells are not yet
121 visible at the posterior. This allows the collection of the same morphological stage,
122 despite any possible differences in development time between treatments.

123 Once imaged, total RNAs were extracted from embryos as in our previous studies
124 (LOTT *et al.* 2011; LOTT *et al.* 2014; PARIS *et al.* 2015). Briefly, embryos were removed
125 from oil to Parafilm (Bemis), where the oil was removed, and the embryo was rolled into
126 a drop of TRIzol reagent (Ambion), where the embryo was ruptured with a needle, and
127 left to dissolve. Once dissolved, the drop of TRIzol was removed to a tube with more
128 TRIzol, and extracted with a glycogen carrier according to manufacturer's instructions,
129 with the exception of using 1mL of TRIzol per embryo (which is an excess compared to
130 the expected amount of total RNA). Protocol available upon request.

131 On average ~100ng total RNA was extracted from an individual embryo. RNA
132 quantity was measured using a Qubit 2.0 fluorometer (Invitrogen). RNA quality was
133 assessed using an Agilent Bioanalyzer. Total RNA from three individual embryos (three
134 replicates) for each food treatment were chosen, samples were sent to the DNA
135 Technologies Core at the UC Davis Genome Center for mRNA-Seq library construction
136 and sequencing. Libraries were sequenced (150bp, paired-end) pooled in a single lane on
137 an Illumina HS 4000 sequencer.

138

139 **Data processing and differential gene expression analysis**

140 Reads were trimmed and adapters removed using Cutadapt (MARTIN 2011), and
141 gently (PHRED Q<5) trimmed for quality (MACMANES 2014). Mapping was done with
142 the *D. melanogaster* Ensembl genome assembly BDGP6 and associated annotation file.
143 Reads were aligned and transcript abundances quantified (in TPM, Table S1) using
144 Kallisto (BRAY *et al.* 2016). Differential expression analysis at the transcript and gene
145 level was conducted using Sleuth (PIMENTEL *et al.* 2017), and gene level abundance
146 counts (in scaled reads per base) quantified (Table S2). Using Sleuth, we construct two
147 models, one where transcripts/genes are at the same abundance in all samples, and the
148 other where transcripts/gene abundances differ by food percentage, and identify
149 transcripts/genes that have a significantly better fit the latter model using a likelihood
150 ratio test. Using a FDR adjusted p-value (Benjamini-Hochberg) of 0.05, here were 119
151 significantly differentially expressed transcripts (Table S3), and 357 differentially
152 expressed genes (Table S4) between treatments in our dataset. Kallisto allows multiple
153 mapping, so examining the 357 genes, we find 314 genes with unique mapping counts, as
154 the same reads map the same number of times to genes with high degrees of similarity
155 (histone genes, snRNA:U1). For all analyses here, we group all of these multiple
156 mapping genes together (i.e. all His4 with the same number of counts mapping are treated
157 as a single “gene”). Most differentially expressed transcripts are represented in the
158 differentially expressed gene list (86%, or all but 16). Transcripts that are significant at
159 the transcript level but not the gene level are one of a number of transcripts for a given
160 gene, and often have lower abundance than other transcripts for that gene. In contrast, the
161 larger number of significantly differentially expressed genes than transcripts represent

162 multi-isoform genes where the individual isoforms fail to meet statistical significance, but
163 summing expression over isoforms at the gene level does show statistical significance.

164

165 **PCA and clustering analyses**

166 For these analyses, each transcript/gene abundance was standardized to have a
167 mean of zero and a variance of one across embryos. Statistical analysis was performed in
168 R (R DEVELOPMENT CORE TEAM 2017). Principal component analysis (PCA) was
169 performed using the *prcomp* function in R. Hierarchical clustering was performed using
170 the *heatmap* function in R, on the standardized genes we had previously determined to be
171 significantly differentially expressed (*via* the analysis described in the preceding section).

172

173 **GO and network analysis**

174 Gene ontology analysis was performed using PANTHER (MI *et al.* 2017), using
175 the statistical overrepresentation test on default settings, using the GO complete
176 annotations for biological process, molecular function, and cellular component (Table
177 S5). We performed the analysis on the genes previously identified as significantly
178 differentially expressed between our treatment (25%, 5%) and control (100%) groups.
179 We ran the upregulated genes and downregulated genes separately, and compared them
180 to a list of all genes with transcripts present at this developmental stage. This was
181 determined by requiring >1 TPM for all replicates of either the 100% or the 5% samples.
182 The results from this PANTHER analysis with a Bonferroni corrected p-value less than
183 0.05 can be found in Table S5. Visualization of this data was created using the GOpot
184 package (WALTER *et al.* 2015) in R using the GOpot plotting function. Further analysis,
185 including KEGG pathway analysis (KANEHISA *et al.* 2017) was performed in STRING
186 (SZKLARCZYK *et al.* 2017).

187

188 **Data Accessibility**

189 All sequencing data from this study have been submitted to NCBI/GEO, and are
190 accessible at accession number: *not yet available*. Processed data are available as
191 supplemental tables.

192

193

194 **RESULTS**

195 In order to investigate the effect of parental nutrition limitation on maternal
196 mRNA deposition into the egg, we raised larvae in differing nutritional conditions,
197 allowed those larvae to develop into adults, and collected their eggs for analysis.
198 Consistent with some previous studies (VIJENDRAVARMA *et al.* 2010), we produced
199 differences in nutrition by diluting the “standard” cornmeal food, so that larvae were
200 supplied with either 100%, 25%, or 5% of normal food. Flies were from an Oregon-R
201 laboratory stock of *D. melanogaster*, and the limited food treatment was restricted to the
202 larval stage of the parents. Stage 2 embryos were selected for RNA extraction, as this
203 stage contains only maternal RNA, and has distinct morphological characteristics to
204 assure collection of the correct stage regardless of development time. Total RNA was

205 extracted from individual embryos, 3 individuals per treatment, for both experimental and
206 biological replication (see Methods), and sent for mRNA-Seq library preparation and
207 sequencing. The mRNA-Seq libraries (150bp, paired-end) were sequenced on a single
208 lane on an Illumina HS4000 sequencer.

209 Resulting sequencing reads were processed (see Methods) using Kallisto (BRAY *et*
210 *al.* 2016) for mapping to the *D. melanogaster* transcriptome and Sleuth (PIMENTEL *et al.*
211 2017) for differential expression analysis at the transcript level and gene level. Both
212 transcript level and gene level abundance measurements demonstrate high correlations
213 between all samples. At the transcript level, Spearman rank correlations for transcript
214 abundances over all transcripts compared pairwise between all samples range from 0.976-
215 0.995 (TPMs, Table S7). For all transcript abundances at the gene level, correlations
216 range from 0.989-0.997 (scaled reads per base, Table S7). Both the highest and lowest
217 correlations are within a treatment group, as the highest correlations are between two of
218 the replicates whose mothers were raised on 100% food, and the lowest are between two
219 replicates with mothers raised on 5% food. Thus, the difference in transcript and gene
220 abundance between our treatment groups is relatively small as compared to the amount of
221 transcriptomic similarity between these samples. This is perhaps unsurprising, given that
222 these samples are embryos at the same developmental stage from the same inbred genetic
223 background.

224 Next, we examined changes in transcript abundance per gene over our varying
225 nutrition treatment groups and replicates using principal component analysis (PCA).
226 Figure 1 represents the first two principal components, together representing 58 percent
227 of the variance (39% and 18% respectively). Here, the samples from parents raised on
228 100% food group together, while all the 25% and 5% samples group together more
229 loosely. This indicates that the changes in the transcriptome when nutrition is restricted
230 may be similar at 25% and 5% of standard food. If we perform the PCA analysis with
231 only the genes we identify as differentially expressed (described below), the 100%
232 samples are clearly separated from the 25% and 5% samples (Figure S1), and the first
233 principal component alone explains 81% of the variance.

234

235 **mRNA levels of many maternally deposited genes change with different nutritional 236 conditions**

237 We identified 119 transcripts (Table S3) and 314 genes (Table S4) as differing in
238 abundance between our different levels of nutritional limitation (25%, 5%) and controls
239 (100%), using Sleuth (PIMENTEL *et al.* 2017). Almost all of the differentially expressed
240 (DE) transcripts corresponded to genes we identified as differentially expressed in the
241 gene-level analysis, but the gene level list is longer due to power gained by summing
242 over transcripts in cases where genes have multiple transcripts. Of the 314 genes with
243 significantly different transcript levels, 150 of them are downregulated and 164 are
244 upregulated in the limited nutrition samples (25%, 5%) as compared to those whose
245 mothers were raised on standard food. Using hierarchical clustering on genes identified
246 as differentially expressed (Figure 2), the 100% samples group together, while one of the
247 5% samples is closer to the 25% samples than the other 5% samples. Figure 2 highlights
248 the main feature of this group of genes, that the transcript level of the 100% samples is
249 high where the 5% level is low and *vice versa* with some number of genes having an

intermediate level for the 25%. That the 5% and 25% samples are so similar in transcript levels makes it difficult to determine which genes likely have an intermediate transcript level in the 25% as compared to the 5% and 100%.

253

254 Types of maternal transcripts that change with nutrition

The genes with the most significantly different transcript levels between the eggs from mothers on standard and reduced diets are represented in Figure 3. We present the top 15 most significantly downregulated and upregulated genes in this figure, but note that in the list of significantly differentially expressed genes, upregulated genes dominate amongst the most significant (i.e. of the top 10 differentially expressed genes, 9 of them are upregulated). Among the most highly downregulated genes represented in Figure 3, we do not see many of the examples of what will characterize the largest group of downregulated genes, such as genes involved in translation (only *JhI-1* among the top 15 downregulated genes). We do observe the downregulation of *midway* (*mdw*), which is a known regulator of lipid metabolism that is critical in oogenesis, indeed, oogenesis is not completed in *mdw* mutants (BUSZCZAK *et al.* 2002). The amount of transcription of *mdw* in our limited nutrition mothers is apparently sufficient to complete oogenesis, but this aspect of lipid metabolism is downregulated in these mothers. Among the top 15 downregulated genes are two snRNPs, this category is not significantly enriched overall, but these particular splicesomal components are downregulated. There are also a number of genes of unknown function that are downregulated, some of which (CG31898, CG31517) are most highly expressed in the early embryo (GRAVELEY *et al.* 2010; GELBART and EMMERT 2013).

Among the most highly differentially deposited genes that we observe to be upregulated in eggs of nutritionally limited mothers are those involved in the electron transport chain (*mt:ND1*, *mt:ND2*, *mt:ND3*, *mt:ND5*), transport of macromolecules (*Nup154*), genes we might expect to be affected by nutrition (*Pfk*, *FASN1*, *Oga*), as well as some genes of unknown function (CG31759, CG42307). With *Phosphofructokinase* (*Pfk*) and *Fatty acid synthase 1* (*FASN1*), we find highly significantly upregulated genes involved in both glycolysis and fatty acid synthesis. *O*-Linked *N*-acetylglucosamine (*O-GlcNAc*) is a post-translational modification of proteins that functions as a nutrient sensing mechanism, *O-GlcNAcase* (*Oga*) removes *O-GlcNAc* from proteins (AKAN *et al.* 2016). Thus a number of our top differentially expressed genes might be expected to be affected by nutrition.

To categorically analyze the types of genes upregulated and downregulated in their maternal deposition due to differences in nutrition, we performed gene ontology analysis on the differentially expressed genes using PANTHER, GO categories complete (MI *et al.* 2017). The significantly enriched GO categories associated with our differentially expressed gene lists (upregulated and downregulated) as compared to the set of all genes maternally deposited in the embryo (present at stage 2 in our samples) are represented in Figure 4. Genes whose transcription is downregulated in the nutritionally limited mothers are those involved in ribosomes and translation, peptide and amide biosynthetic processes, and peptide metabolism. Transcripts provided by the mother to the egg at a higher level in nutritionally limited conditions are involved in localization and transport of macromolecules in the cell and the production of ATP via the electron

295 transport chain. These findings were further reinforced by analysis of enriched KEGG
296 pathway ontology (KANEHISA *et al.* 2017) using STRING (SZKLARCZYK *et al.* 2017). As
297 reported in Table S6, the ribosome is the only significantly downregulated pathway,
298 while oxidative phosphorylation, metabolic pathways, and RNA transport were all
299 upregulated. Protein network interactions for significantly differentially expressed genes
300 belonging to the most significantly differentially KEGG pathways are pictured in Figure
301 5.

302

303 **Comparison of changes in maternal deposition due to nutrition limitation to other 304 studies**

305 In order to understand whether the changes in maternal transcript deposition we
306 observe are usual cellular responses to starvation, we compare our data to previous
307 studies. Comparing our list of 314 genes with significant changes in maternal transcript
308 deposition due to parental nutrition limitation to the 177 genes listed under the GO term
309 “response to starvation” (ASHBURNER *et al.* 2000; CARBON *et al.* 2009), we find an
310 overlap of eight genes (*Bruce*, *CG9422*, *Mat89Ba*, *Nmd3*, *Ns1*, *p53*, *Pten*, *Rack1*). We
311 also find very little overlap between our gene list and the 126 transcripts affected by
312 starvation in a genomic study of 16 hour starved adults (MOSKALEV *et al.* 2015), with
313 only two of the same genes implicated as differentially expressed (*ade3*, *Spat*). Despite
314 the low number of genes implicated in both studies, we find some of the same GO
315 categories upregulated in starved conditions in both studies, such as oxidation-reduction
316 process and metabolic process (MOSKALEV *et al.* 2015). We also compared our data to
317 two previous microarray studies, one that identified transcription changes in starved larva
318 (ERDI *et al.* 2012), the other in starved adults (HARBISON *et al.* 2005). These studies
319 identified far more differentially expressed genes, 2819 in (ERDI *et al.* 2012) and 3451 in
320 (HARBISON *et al.* 2005). Of our differentially expressed genes, 67 or 22% of our genes
321 were in common with the Erdi *et al.* 2012 study, and 83 or 26% of our genes were in
322 common with the Harbison et al. study (Table S8). There were 16 genes on that were in
323 common between our study and both of these microarray studies on nutrition-limited flies
324 and larvae (HARBISON *et al.* 2005; ERDI *et al.* 2012), some with known roles relating to
325 nutrition: *ade3*, *ATPCL*, *CG11275*, *CG13631*, *CG15098*, *CG4733*, *Cyp6a17*, *GNBP3*,
326 *Hsc70-5*, *mrt*, *Rab4*, *rdgBbeta*, *RpL13*, *Spat*, *Sps2*, and *Tif-IA*.

327 To expand our comparisons beyond previous studies of nutrition limitation, we
328 also compared our list of differentially expressed genes to other pathways that we might
329 expect to respond to starvation. We compared our data to components of the
330 insulin/insulin-like growth factor signaling (IIS) and target of rapamycin (TOR) pathways
331 and found again limited overlap, with only four genes (*p53*, *Pten*, *TifIA*, *tbl*) in common
332 between our data and 52 core IIL/TOR components (STANLEY *et al.* 2017). We noticed
333 that some of the overlapping genes in all comparisons were general stress response
334 factors, so we investigated genes annotated under the GO term “response to stress”
335 (ASHBURNER *et al.* 2000; CARBON *et al.* 2009). We found 45 genes in common (Table
336 S8), which corresponds to 14% of the genes in our list. Over all comparisons to all listed
337 studies and GO categories, we report 37 genes present in two or more of these lists in
338 common with our data (Table S8).

339 In general, while there are some commonalities in genes previously implicated as
340 differentially regulated upon starvation, part of the IIS/TOR pathways, or as part of a
341 general stress response, our set of differentially expressed genes is largely distinct. This is
342 likely due to examining the effect of nutrition deprivation during development on the
343 maternal investment in the next generation. The mothers are not currently experiencing
344 starvation or stress themselves, but may be provisioning their offspring to face limited
345 resources during their development.

346

347

348

349 DISCUSSION

350 Poor environmental conditions for a parent may have profound effects on the
351 offspring. These effects can be detrimental if they have impacts such as reducing the
352 investment that a parent is able to make in an individual offspring. But we also
353 sometimes observe parents preparing offspring to experience similar stresses, for
354 example by investing more resources in a smaller number of offspring (MOUSSEAU and
355 FOX 1998). In *Drosophila*, parents with reduced nutrition produce larger eggs
356 (VIJENDRAVARMA *et al.* 2010), and in some cases grow into larger adults (VALTONEN *et*
357 *al.* 2012), which results from provisioning more resources to each offspring despite
358 having fewer resources to begin with.

359 In this study, we asked what effect reduced nutrition in mothers would have on
360 provisioning of mRNAs to the egg. As these maternally supplied gene products are the
361 basis of genetic control of the organism up until zygotic genome activation, this is an
362 opportunity for the mother to set up developmental trajectories that will influence the rest
363 of development for this offspring. For example, perhaps the mother can supply her
364 offspring with more of particular transcripts that can help to offset the detrimental effect
365 of future poor nutrition on her offspring. Or, perhaps the production of transcripts (or
366 particular transcripts) is costly to the mother. In this case, a mother who experienced
367 limited nutrition during development would be unable to supply the same number of
368 transcripts (or particular transcripts) to the offspring. Our data do not address whether the
369 process of producing these transcripts is itself costly to the mother, but we did not find
370 any systematic bias in the total amount of RNA in the egg during the extraction process
371 to make RNA-Seq libraries. The cost to a cell of protein production is often considered to
372 be more than transcription (LYNCH and MARINOV 2015), but transcription can also be as
373 limiting under certain conditions (KAFRI *et al.* 2016). Our observation of no differences
374 in amount of total RNA indicates that transcription itself may not be limited in these
375 mothers with reduced nutrition during their own development, likely because they were
376 nutrient limited only during larval stage. This suggests that we can potentially view the
377 differences in transcript levels in eggs we observe from a life-history perspective as
378 nutrient limited mothers preparing their offspring for nutrition limitation during
379 development.

380 Nutrition limited mothers deposit fewer transcripts for many genes involved in
381 translation. Many genes involved in biogenesis of ribosomes, translation, and the
382 biosynthesis and metabolism of proteins are downregulated in mothers who experienced
383 limited nutrition as larvae. As translation is a major cost to the cell, this downregulation

384 in translation-related transcripts may reduce the proportion of the energy devoted to these
385 processes. One prediction of this hypothesis is that this would likely slow development
386 time, as translation would become rate-limiting. Yet in the study on which we modeled
387 our nutritional limitation method (food dilution for parents in the larval stage) the
388 offspring of nutrition limited mothers showed no difference in development time when
389 raised on standard food (VIJENDRAVARMA *et al.* 2010). However, with a different
390 nutrition limitation method, another study (VALTONEN *et al.* 2012) did find a longer
391 development time for offspring whose parents were both raised on limited food.

392 To understand the implications for the transcripts differentially deposited in eggs
393 by mothers with different nutritional conditions, we must consider the egg as the end
394 product of the process of oogenesis. Considerable study has been devoted to
395 understanding the effect of nutrition limitation on oogenesis in *Drosophila*. Starvation
396 conditions halt oogenesis and induce apoptosis of developing oocytes at a developmental
397 stage before mothers devote resources to yolk and eggshell production, and slow down
398 the production and development of new egg chambers through reducing stem cell
399 proliferation (DRUMMOND-BARBOSA and SPRADLING 2001; BELLES and PIULACHS 2015).
400 Egg chambers early in embryogenesis reorganize their microtubules in response to
401 nutrient stress, this disrupts transport from the nurse cells which provide mRNAs and
402 proteins to the oocyte, leading to mislocalization, which can be rapidly reversed upon
403 introduction of food or the addition of exogenous insulin (SHIMADA *et al.* 2011). Thus,
404 *Drosophila* oogenesis is sensitive to nutrition, and responds with a number of coordinated
405 processes designed to pause the process of producing mature oocytes and instead
406 preserve investment for future offspring if and when nutritional conditions improve.

407 Our experiment addresses a different scenario, where the mother experienced
408 nutritional deficiency as a larva, but has access to adequate food as an adult. In our data,
409 we found an increase in transcript levels for factors involved in the localization and
410 transport of biomolecules. Genes involved in the transport of proteins and genes involved
411 in the transport and localization of RNAs were particularly enriched amongst those
412 upregulated by these mothers. Due to previous study of the effect of nutrition on
413 oogenesis, it is known that nutrition limitation in the mother leads to microtubule
414 reorganization in early oocytes and mislocalization of mRNAs and proteins (SHIMADA *et*
415 *al.* 2011). While these mothers have adequate nutrition at the point they are undergoing
416 oogenesis, it is possible that additional mRNAs for localization of biomolecules are
417 transcribed as a hedge against future poor nutrition during oogenesis. This would imply
418 that these mRNAs are in the egg as a result of their potential function during oogenesis.
419 On the other hand, early embryogenesis is the time when positional information is being
420 established for the rest of development, so perhaps additional transcripts for genes
421 involved in transport and localization of mRNAs and proteins are supplied so that this
422 critical process does not fail. Additionally, if transport and localization is occurring in a
423 larger embryo, possibly for a longer period of time, more transcripts may be needed.

424 The other group of upregulated transcripts in our eggs from nutritionally deprived
425 mothers relate to the electron transport chain (ETC). The number of mitochondria
426 increase in late oogenesis (COX and SPRADLING 2003; HURD *et al.* 2016), but display low
427 levels of activity that increases through embryogenesis following egg activation
428 (DUMOLLARD *et al.* 2007; VAN BLERKOM 2011; RAMALHO-SANTOS and AMARAL 2013).
429 A recent study (SIEBER *et al.* 2016) showed that the downregulation of insulin signaling

430 that occurs in late oocytes results in the low activity state of mitochondria due to
431 remodeling of the electron transport chain complexes. This results in an accumulation of
432 glycogen late in oogenesis that is critical for the development of the oocyte, and that
433 mitochondrial activity is upregulated again as embryogenesis proceeds (SIEBER *et al.*
434 2016). As the downregulation of ETC complexes during oogenesis is necessary for
435 progression through oogenesis and presumably into embryogenesis, we are left with two
436 possibilities to explain our upregulated ETC transcripts: one, that the shut down of ECT
437 during oogenesis was not as strong as in well-fed mothers; or two, that mitochondria are
438 active earlier in embryogenesis in eggs from nutrition-limited mothers. If the ECT was
439 not downregulated as much during embryogenesis, this would predict that the eggs would
440 contain less glycogen, which would be inconsistent with the idea that these mothers are
441 better provisioning fewer eggs. Alternatively, mitochondria may become active earlier in
442 development in the eggs from these mothers. A previous study (TENNESSEN *et al.* 2014)
443 found a switch in gene expression in mid-embryonic development (~12 hours after egg
444 lay) to glycolytic gene expression, but in contrast to the canonical aerobic glycolytic
445 pathway, genes involved in the TCA cycle and the electron transport chain are also
446 upregulated. Evidence points restoration of mitochondrial membrane potential as early as
447 blastoderm stage (Bownes' stage 5, mitotic cycle 14) of embryogenesis (SIEBER *et al.*
448 2016), which is after the activation of the zygotic genome. But our study examines
449 embryos earlier stages (stage 2, >1 hour earlier than blastoderm stage begins, > 6 hours
450 before the mid-embryonic stage discussed above), thus may require earlier reactivation of
451 mitochondrial activity in embryos from mothers with reduced nutrition.

452

453 **Conclusions**

454 In this study, we demonstrate that maternal deposition of mRNA into the egg is affected
455 by the nutritional status of the mother during her development. We characterize which the
456 transcripts are affected and what processes these transcripts are involved in. The fitness
457 consequences of these changes in transcript representation in the egg remains to be
458 determined, and will need to be considered in the context of the other life history traits
459 effecting development.

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466 **ACKNOWLEDGEMENTS**

467 We thank James Yacoub for his work on food dilution protocols, and Sherri Wykoff-
468 Clary for stock maintenance and care. We thank the Lott lab and the UC Davis fly
469 community for comments and advice. We acknowledge the support provided by the UC
470 Davis Research Scholars Program in Insect Biology.

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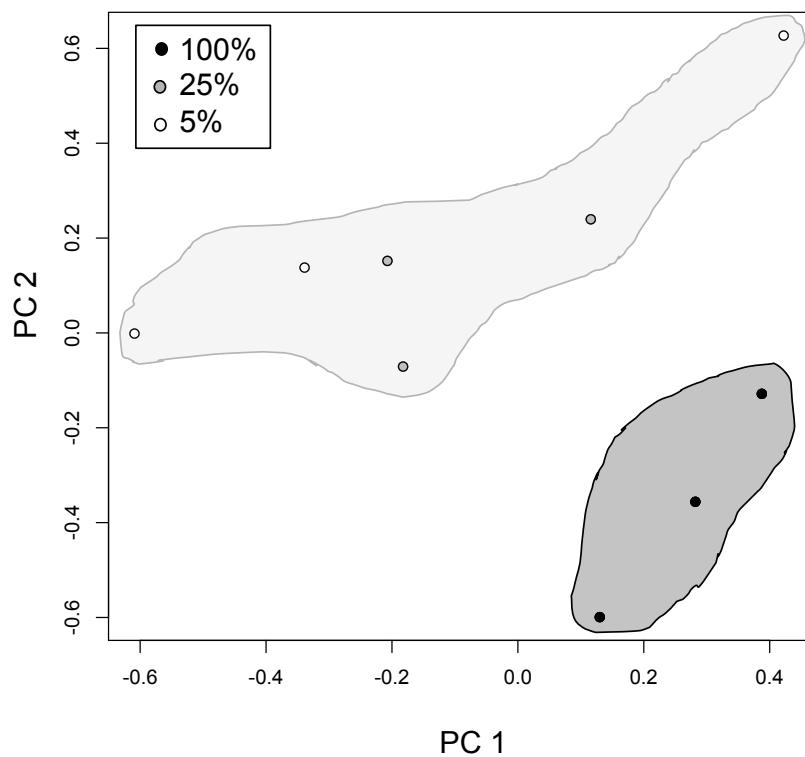


Figure 1. PCA plot of transcript abundances for all genes. The samples from mothers with standard food (100%, black points) group together, while the samples from mothers with reduced nutrition (25%, 5%; grey and white points) all group together.

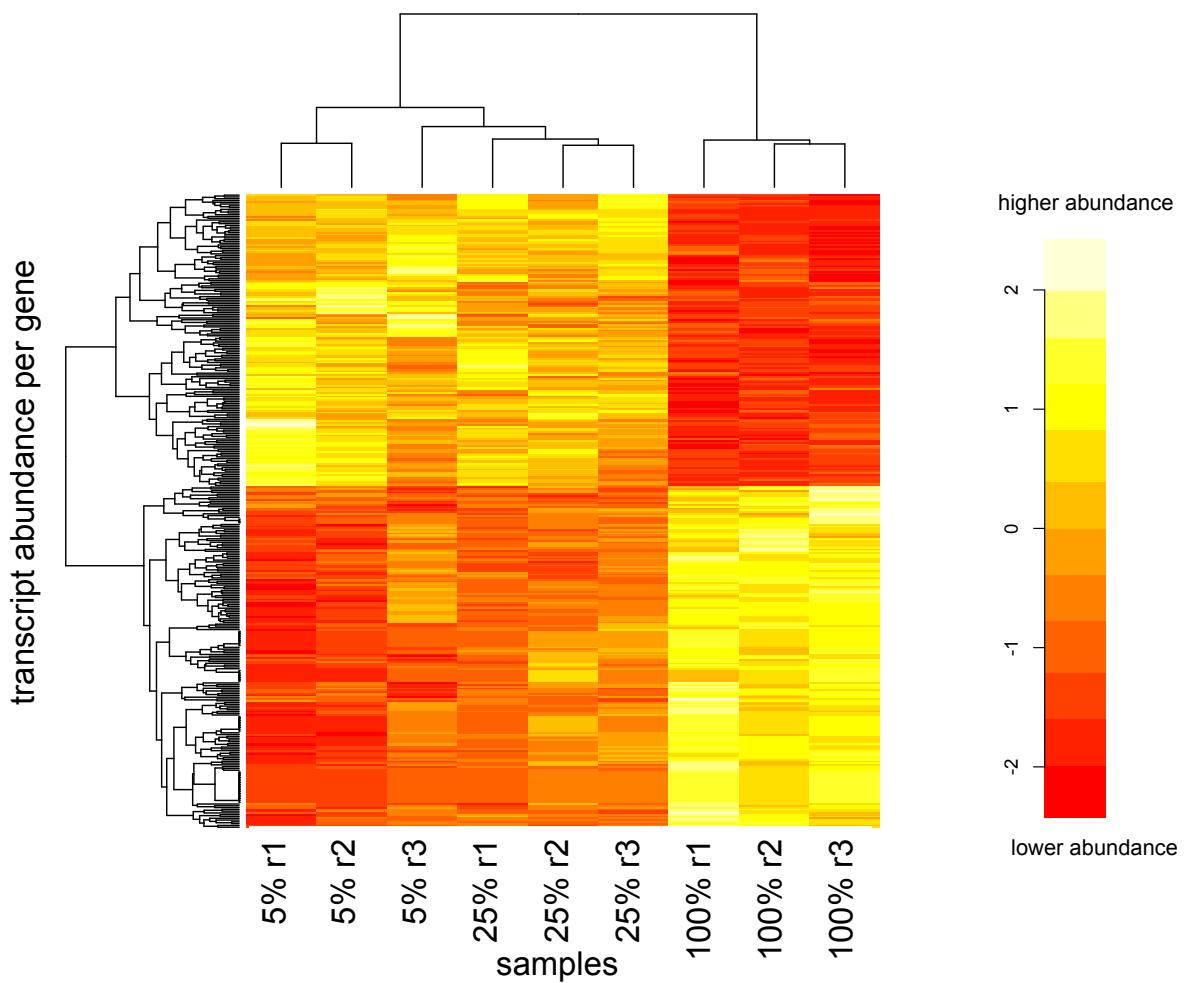


Figure 2. Clustering of data shows differential transcript levels of maternally deposited genes from mothers with limited nutrition. Hierarchical clustering was performed on standardized levels of genes identified as being differentially expressed between food percentage groups. Each row shows transcript abundance per gene. The r1-r3 indicates replicate number for each food percentage. The 100% samples from mothers with standard food cluster together, while the 25% and 5% samples cluster together.

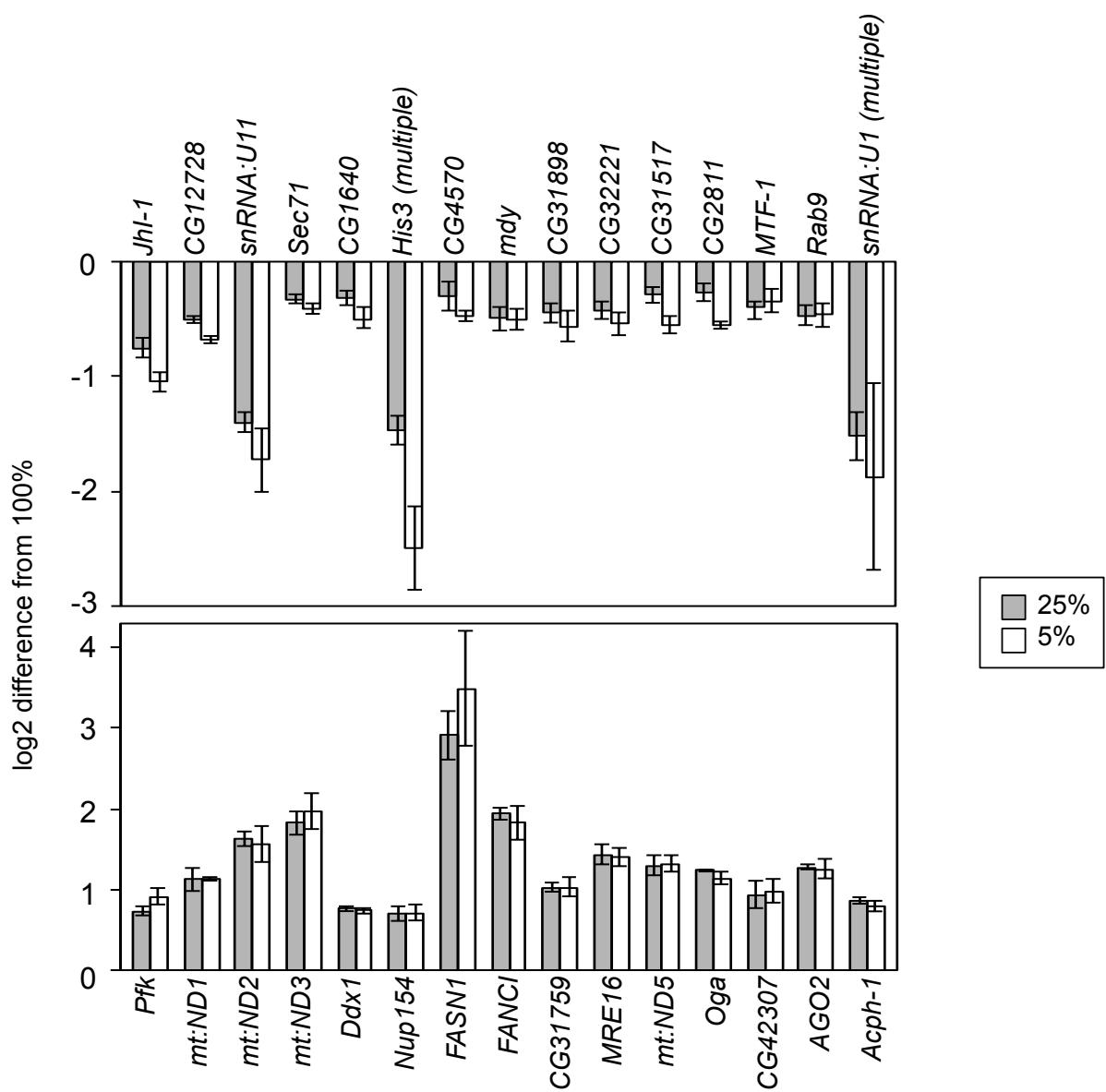


Figure 3. Genes most significantly differentially maternally deposited between mothers with standard nutrition and mothers with reduced nutrition. Transcript levels are the difference between the log₂ scaled reads per base counts in eggs from the reduced food mothers (25%, 5%) and the standard food controls (100%). In the case of the *His3* and *snRNA:U1* genes, these are multi-copy genes with similar coding sequence, so transcripts map to multiple copies of these genes, levels here are reads mapped to a single copy.

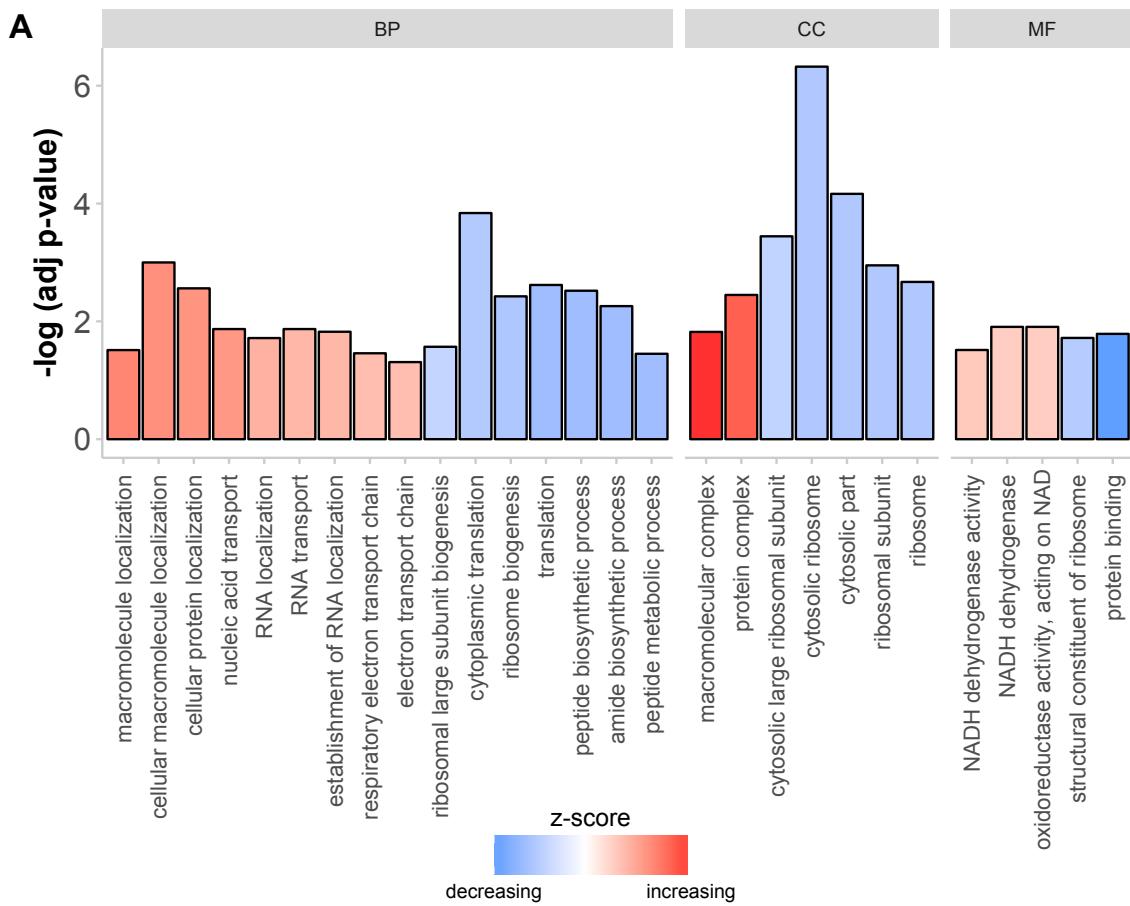


Figure 4. GO term analysis. A) Significantly enriched GO terms are pictured, grouped by category (BP: biological process, CC: cellular component, MF: molecular function). Those with increasing z-score (red) describe genes upregulated in the nutritionally limited mothers (25% and 5% food) as compared to mothers supplied with standard nutrition, while those with a decreasing z-score (blue) are those downregulated in nutritionally limited mothers.

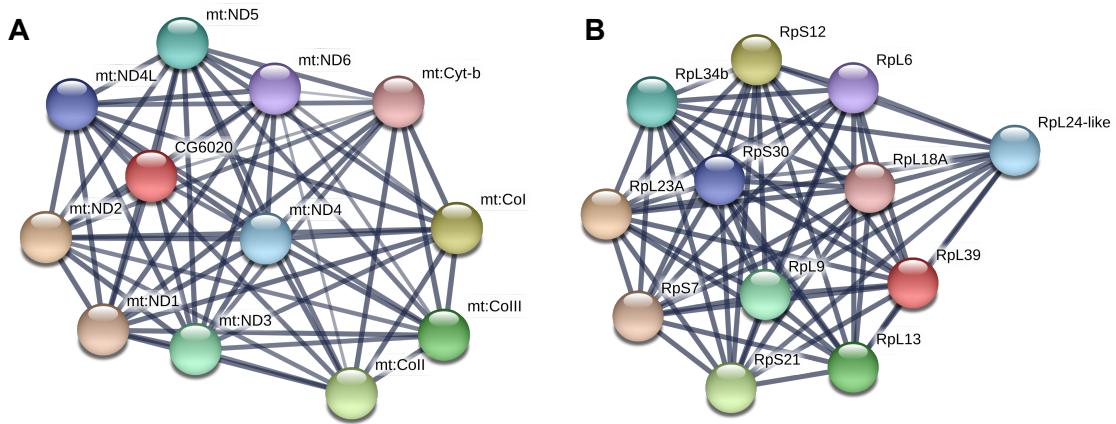


Figure 5. Protein network interaction diagrams for genes in most significantly enriched KEGG pathways. Edges represent protein-protein associations; line width indicates the strength of data support. A) Protein network for genes upregulated in nutritionally limited mothers involved in the oxidative phosphorylation pathway. B) Protein network for genes downregulated in nutritionally limited mothers involved in the ribosome.