Swansea University
Prifysgol Abertawe

Cronfa
Setting Research Free

Cronfa - Swansea University Open Access Repository

_____

This is an author produced version of a paper published in:
Behavioral Ecology

Cronfa URL for this paper:
http://cronfa.swan.ac.uk/Record/cronfa48268

_____

Paper:

_____

1 **Predictable gene expression related to behavioral**

2 **variation in parenting**

3

4 **Kyle M. Benowitz,[a] Elizabeth C. McKinney,[b] Christopher B.**

5 **Cunningham,[c] and Allen J. Moore[b]**

6

7 [a]Department of Entomology, University of Arizona, Tucson, AZ 85721, USA,

8 [b]Department of Entomology, University of Georgia, Athens, GA 30602, USA,

9 [c]Department of Biosciences, Swansea University, Swansea, SA2 8PP, UK

10

11 Address correspondence to A.J. Moore, Department of Entomology, University of

12 Georgia, 137 Cedar St., Athens, GA, 30606, USA. Telephone: +17065422151. E-mail:

13 ajmoore@uga.edu.

14

15 Running title: "Predicting genes related to variable behavior"

16

17

18

19

20   Differential gene expression has been associated with transitions between

21   behavioral states for a wide variety of organisms and behaviors. Heterochrony,

22   genetic toolkits, and predictable pathways underlying behavioral transitions have

23   been hypothesized to explain the relationship between transcription and

24   behavioral changes. Less studied is how variation in transcription is related to

25   variation within a behavior, and if the genes that are associated with this variation

26   are predictable. Here we adopt an evolutionary systems biology perspective to

27   address two hypotheses relating differential expression to changes within and

28   between behavior. We predicted fewer genes will be associated with variation

29   within a behavior than with transitions between states, and the genes underlying

30   variation within a behavior will represent a narrower set of biological functions.

31   We tested for associations with parenting variation within a state with a set of

32   genes known *a priori* to be differentially expressed between parenting states in

33   the burying beetle *Nicrophorus vespilloides*. As predicted, we found that far fewer

34   genes are differentially expressed related to variation within parenting. Moreover,

35   these were not randomly distributed among categories or pathways in the gene

36   set we tested and primarily involved genes associated with neurotransmission.

37   We suggest that this means candidate genes will be easier to identify for

38   associations within a behavior, as descriptions of behavioral state may include

39   more than a single phenotype.

40

41   *Key words:* **behavior genetics, evolutionary systems biology, gene**

42   **expression, *Nicrophorus vespilloides*, parental care**

43 **INTRODUCTION**

44 Ever since the description of the "phenotypic gambit" (Grafen 1984), evolutionary

45 biologists in general (Travisano and Shaw 2013) and behavioral ecologists specifically

46 (Stamps 1991; Zuk and Balenger 2014) have struggled with the questions of if and when

47 it is necessary to understand the mechanisms underlying behavior. The development of

48 transcriptomic, metabolomic, and proteomic approaches have provided us with new tools

49 to address mechanisms (Moore et al. 2010), and advocates of a genomic approach to

50 behavior have argued that finding the genes underlying behavior can lead us to important

51 insights into the nature of pleiotropy and constraint, selection, and evolutionary

52 convergence (Fitzpatrick et al. 2005; Rittschof and Robinson 2014; Bengston et al. 2018).

53 Furthermore, understanding details of behavioral mechanisms may provide insight into

54 whether behavior plays a unique role in evolution (Bailey et al. 2018). However, what

55 precisely does it mean for a gene to underlie a behavior? Most studies have asked what

56 genes are responsible for producing discrete changes in behavior and have focused on

57 gene expression changes between two behavioral states (Harris and Hofmann 2014;

58 Rittschof and Robinson 2016), particularly on a genome wide scale (Calisi and

59 MacManes 2015). These studies have led to predictions of the types of genes that will be

60 differentially expressed (DE). Two hypotheses have been generally supported to explain

61 change in behavior. First, changes in timing of gene expression (heterochrony) will be

62 associated with behavioral evolution (Linksvayer and Wade 2005). Second, the genes DE

63 will be associated with a "genetic toolkit" (Rittschof and Robinson 2016; Toth and Rehan

64 2017). The toolkit hypothesis states that specific genes may differ between organisms,

65 but the underlying pathway or category altered will be shared across animals displaying

66   similar behavior. These hypotheses are applied to the generation of novel behavioral

67   states and are therefore well-suited to answer questions about the evolutionary origins of

68   behavior and the tradeoffs involved with its production.

69   Behavior also displays abundant variation within a state, which also must have

70   some mechanistic basis. The genes related to this type of behavioral variation should be

71   more important for understanding responses to contemporary selection; if selection acts

72   to refine behaviors, it should similarly refine levels of transcription associated with

73   specific behavioral values. Less attention has been paid to mechanisms responsible for

74   this type of variation. For instance, the extent that differential gene expression within and

75   between behavioral states overlaps is unknown (Bengston et al. 2018). Many genes

76   undergo massive expression changes when behavior shifts, indicating that behavioral

77   transitions also involve concurrent suites of major physiological changes. We do not

78   know to what degree the same genes influence variation within state, possibly with less

79   extreme differences in expression. Some have argued that the mechanisms controlling

80   the maintenance of specific behavioral phenotypes are likely to be different than those

81   controlling transitions between behaviors (Cardoso et al. 2015). Alternatively, others

82   have predicted more overlap between these mechanisms (Duckworth 2015), perhaps

83   reflecting the expectation that behavioral plasticity and behavioral evolution are

84   mechanistically similar (Pfennig et al. 2010). However, existing data explicitly

85   comparing transitions with variation for a single behavior are sparse. One example comes

86   from Bell et al. (2016), who found notable but limited overlap between mechanisms of

87   induction of aggression and variation in aggression in sticklebacks. They proceed to

88   suggest that the magnitude of such overlap may be less important than the identity of the

89   common genes.

90   Theory from developmental biology may bear on our expectations for the

91   distinction between transitions between behavior states and variation within a behavior

92   and provide an addition to the toolkit and heterochrony hypotheses. Wagner's (2014)

93   makes a distinction between the origin of novel types and modification of existing types.

94   Wagner predicts that the former evolves via sweeping changes in transcriptional

95   programs leading to novel regulatory networks, whereas the latter evolves within existing

96   networks through the modification of expression of "realizer" genes, which are directly

97   related to phenotypic variation. Adapting Wagner's hypothesis to behavior, we make two

98   predictions. First, we propose that only a narrow subset of genes DE in changes between

99   states will be associated with variation within that state. Changing behavioral states

100   involves complex and multifaceted environmental changes, and therefore the need for

101   changes in multiple coordinated sensory, physiological, and neurological processes, while

102   variation within a state occurs in a single social and more uniform biotic and abiotic

103   environment where only a single phenotype may be different among individuals.

104   Therefore, fewer transcriptional changes should be needed. Second, we suggest that this

105   subset of genes influencing variation within a state will fall into functional categories

106   typically displaying causal links to behavior; i.e., neuropeptides (Chandrasekaran et al.

107   2011). Presumably, if the behavior in question is heritable, *cis*-regulatory variants in

108   such causal genes will allow them to vary transcriptionally without generating broad

109   rewiring of transcriptional networks as occurs during transitions (Chandrasekaran et al.

110   2011).

111   Parental care in the burying beetle *Nicrophorus vespilloides* presents an

112   opportunity with which to test these predictions. Adult beetles rear their offspring on

113   prepared vertebrate carcasses (Eggert and Müller 1997; Scott 1998), and the transition

114   from a non-parenting to a parenting state is phenotypically complex and multifaceted.

115   Parents undergo changes in immune function (Cotter and Kilner 2010; Palmer et al. 2016;

116   Ziadie et al. 2019), physiology (Benowitz et al. 2017a), chemical status (Steiger et al.

117   2008; 2009) and multiple behaviors (reviewed in Scott 1998; Royle and Hopwood 2017).

118   Reflecting this, an RNA-seq study comparing non-parenting and parenting individuals

119   identified broad transcriptional differences (> 700 genes) between the two states (Parker

120   et al. 2015). Transcriptional differences have also been defined for responses to social

121   context and for plasticity in male parenting (Cunningham et al. 2019). Thus, we have a

122   clear phenotype with well characterized DE associations.

123   Within the burying beetle parenting state, the primary individual social behavior

124   is provisioning, which consists of direct regurgitation of partially digested food from

125   parents into the mouths of begging offspring (Pukowski 1933; Milne and Milne 1976).

126   The amount of parental provisioning exhibited in *N. vespilloides* is highly variable

127   (Benowitz et al. 2016a), heritable (Walling et al. 2008) and important for offspring

128   development and fitness (Eggert et al. 1998; Lock et al. 2004). Variation in the extent of

129   provisioning has been investigated in an RNA-seq study comparing genome wide

130   transcriptional variation between ten high-caring and ten low-caring parents (Benowitz et

131   al. 2017b). This study broadly supported the expectation of overlap between genes

132   involved in transitions and within-state variation but could not specify the number or the

133   identity of the genes affecting the variability of parental provisioning (Benowitz et al.

134   2017b). This likely reflects a basic limitation of using RNA-seq to investigate subtle

135   behavioral differences; if small phenotypic changes are accompanied by small expression

136   changes, important signals may be swamped by noise and costly multiple corrections.

137   Here, to further probe our hypotheses in *N. vespilloides*, we take a complementary

138   approach to Benowitz et al. (2017b) but with an *a priori* candidate gene method rather

139   than RNA-seq, creating a more focused and therefore more powerful test. We examine

140   specific candidates shown to be significantly DE when switching from a non-parenting to

141   a parenting state in earlier studies (Table 1, S1), and ask which of these genes also

142   display differential expression between very high and very low caring mothers.

143   Furthermore, we specifically chose to examine genes spanning a range of well-

144   documented functions, including several neurotransmitters, in order to ask whether the

145   genes associated with behavioral variation are more likely to display certain

146   functionalities. There are certainly more genes than these involved; however, most DE

147   genes identified in transcriptomic studies are unannotated and therefore the function is

148   unknown (Parker et al. 2015).

149

150   **METHODS**

151   *Nicrophorus vespilloides* were collected and maintained as an outbred colony as

152   described by Cunningham et al. (2014). For this study, our sample consisted of 57 female

153   adult beetles on 19-21g mouse carcasses in a uniparental context. We observed parental

154   provisioning, defined as mouthpart-to-mouthpart contact by parents and offspring, on the

155   first day after larvae hatched. Following Benowitz et al. (2016) we made 80 scan samples

156   for provisioning behavior over the course of an 8-hour period. Within the entire dataset,

157   parental care was roughly uniformly distributed (Benowitz et al. 2016a) with a mean and

158   standard deviation of 47.53 ± 21.52 observations of feeding (out of 80 scans) (Benowitz

159   et al. 2016b). Immediately after observations we removed the heads, which were flash-

160   frozen in liquid nitrogen and stored at -80°C.

161   Following the method of Benowitz et al. (2017b) we selected the 12 highest and

162   12 lowest caring female parents for RNA extraction. Mean and standard deviation of

163   parenting was 11.67 ± 8.33 observations of feeding for the low group and 72.42 ± 2.18

164   observations of feeding for the high group, indicating substantial quantitative behavioral

165   differences (Benowitz et al. 2016b). We analyzed head tissue, which contains both brain

166   and fat body, following previous studies that identified differential expression of genes

167   associated with parenting in females (Parker et al. 2015; Cunningham et al. 2014, 2016,

168   2019; Roy-Zokan et al. 2015; Benowitz et al. 2017a,b). We performed phenol-

169   chloroform extractions using Qiagen RNeasy Lipid Kits (Qiagen, Venlo, Netherlands)

170   and synthesized cDNA using qScript (Quantabio, Beverly, MA) reverse transcriptase

171   (Parker et al. 2015; Roy-Zokan et al. 2015). We designed quantitative real-time PCR

172   (qRT-PCR) primers for 23 genes (Table S1) identified in other independent experiments

173   to be DE in transition from non-parenting to parenting *N. vespilloides* females. Seventeen

174   of these genes were identified in an RNA-seq experiment comparing parenting and non-

175   parenting *N. vespilloides*, while the other six were identified in qRT-PCR experiments

176   making the identical comparison (Cunningham et al. 2016; 2017; unpub. data). These

177   genes have functional annotation and can be categorized into neurotransmission, energy

178   acquisition and usage, immunity, and hormones (Table 1). This is not an exhaustive

179   identification of any genes differing between parental states in *N. vespilloides*, but rather

180   those that are annotated, DE when comparing parenting and non-parenting states, and fall

181   into different known functional categories. Therefore, this set is coarsely representative

182   of the types of genes DE between states, and most importantly allows us to test our

183   hypotheses. We performed qRT-PCR using each primer pair on each of the 24 samples

184   with a Roche LightCycler 480 (Roche, Basel, Switzerland) with *alpha-tubulin* (Table S1)

185   used as an endogenous reference gene. We calculated gene expression for each sample

186   as $2\char94-(Cp_{exp} – Cp_{ref})$, where Cp_{exp} is the average cycle number of three technical

187   replicates of each experimental gene and Cp_{ref} is the average cycle number of three

188   technical replicates for *alpha-tubulin*. Following this, DE was calculated between high

189   and low care using separate one-way ANOVAs for each experimental gene. Because

190   each gene represents a distinct, *a priori* specified hypothesis, conservative multiple

191   corrections are inappropriate (Rice 1989). We used a Fisher's Exact Test to test whether

192   genes *a priori* classified as having roles in neurotransmission were more likely to be DE

193   than genes with other functional roles. The data are publicly available in Dryad

194   (Benowitz et al. 2018).

195

196   **RESULTS**

197   Four of seven neurotransmission genes (*serotonin receptor 2*, *octopamine/tyramine*

198   *receptor 2*, *tachykinin*, and *glutamate receptor*) show statistically different expression

199   between high vs. low caring *N. vespilloides* mothers (Table 1). Furthermore, the

200   direction of differential expression followed *a priori* expectations based on our previous

201   studies; genes that were upregulated during the transition into parental care were also

202   upregulated in high caring mothers, and vice versa. However, of the other genes we

203   examined, which included hormones and genes related to energy, immunity, and general

204   behavior, only one (*fatty acyl-CoA synthetase*) of 16 was DE between high and low

205   caring mothers (Table 1), and in the opposite direction as the change with a transition to

206   parenting. Neurotransmission genes were statistically more likely to be associated with

207   provisioning variation than other genes (p = 0.017).

208

209   **DISCUSSION**

210   Differences in gene expression are suggested to be fundamental contributors to the

211   evolution of social behavior (Calisi and McManes 2015; Rittschof and Robinson 2016;

212   Toth and Rehan 2017; Kronauer and Libbrecht 2018). Despite this, we currently lack a

213   nuanced understanding of how transcription is related to selectable variation in social

214   behavior. Here, we examined how gene expression relates to quantitative variation in

215   parental provisioning behavior involving direct regurgitation of food in the burying beetle

216   *Nicrophorus vespilloides*. Rather than examining transcriptional differences associated

217   with transitions between behavioral states (e.g., Harris and Hoffmann 2014; Rittschof and

218   Robinson 2016), we determined which of the genes previously associated with this

219   transition in *N. vespilloides* parenting were also related to variation in parenting within a

220   state. Transitions often involve large changes in physiology, feeding, aggression and

221   reproduction (Kronauer and Libbrecht 2018), and so many different pathways and genes

222   should be involved. We specifically tested our predictions that far fewer genes will be

223   involved in variation within a behavioral state, and for behavior they will be involved in

224   neurotransmission rather than other physiological processes. Focusing on those genes

225   that we know are associated with changes in parenting state provides a powerful test of

226   the hypothesis. That is, we bias our examination toward genes known to change, rather

227   than looking for associations of genes and behavior, thereby reducing extraneous

228   correlations and irrelevant tests. What we cannot determine from this approach is the

229   contribution to variation of genes that do not see transcriptional changes across

230   behavioral transitions. Behavioral syndromes theory (Sih et al. 2004) predicts the

231   existence of large suites of genetically correlated behaviors, suggesting that common

232   regulators may control behavior in a non-specific fashion, and therefore might not be

233   involved in behavioral transitions. If such genes exist and are important, they may be

234   difficult to identify by either *a priori* or genome-wide approaches.

235   Four of seven neurotransmitter genes associated with changes in parenting state

236   were also associated with variation in parenting, while only one of 16 not associated with

237   neurotransmission also influenced parental variation. This result suggests that many

238   immune, energetic, and hormonal processes altered upon induction of parental behavior

239   are not further changed in association with behavioral variation. We suggest this pattern

240   is generalizable, and that this methodology eliminates many of the confounding

241   transcriptional effects produced when comparing between different behaviors. Here, we

242   predicted fewer transcriptional differences within parenting because the number of

243   changes required to transition from a non-parenting to parenting state include those

244   associated with changing biotic and abiotic conditions as well as changes in parenting.

245   This highlights a further problem studying behavior: terminology. The labels widely used

246   to group behaviors into categories (e.g. parenting) actually describe composites that are

247   necessarily more complex than the individual phenotypes that are contained within them

248   (Rittschof and Robinson 2016).   However, it is these individual phenotypes that are

249   actually quantified, and often considered to be proxies for the entire category, which may

250   lead to an oversimplification (Wenzel 1992).

251   The neurotransmission genes related to provisioning variation included serotonin,

252   octopamine, glutamate, and tachykinin signaling genes. These pathways are all known to

253   influence behavior, and expression of an octopamine/tyramine receptor has previously

254   been shown to affect behavior quantitatively in *C. elegans* (Bendesky et al. 2011).

255   Furthermore, modification of neural signaling pathways leading to variable brain function

256   is predicted to be a general mechanism for the evolution of social behavior (Baran et al.

257   2017). Interestingly, the single other gene found to be differentially expressed was the

258   lipid metabolism gene *fatty acyl CoA synthetase*, which was upregulated in high caring

259   parents. This raises the possibility that body condition could be linked to variation in

260   parenting, presenting precisely the sort of confound our study was designed to avoid.

261   Because plastic changes of this nature likely require additional gene expression changes

262   in order to incorporate environmental inputs into behavior, it will be interesting to see in

263   the future whether plastic variation is more transcriptionally complex than genetic

264   variation. We hypothesize that this will be the case, and therefore that more heritable

265   behaviors will be associated with fewer DE genes.

266   There have been several attempts to provide a framework for predicting specific

267   genes or pathways underlying behavior given the accessibility of modern molecular

268   approaches, from heterochrony (Linksvayer and Wade 2005) to genetic toolkits (Toth and

269   Rehan 2017), to hypotheses derived from ethological principles (Cunningham et al. 2016,

270   2017; Kronauer and Libbrecht 2018). We suggest that molecules predicted from an

271   evolutionary systems biology approach (Chandrasekaran et al. 2011; Wagner 2014)

272   coupled with predictions from ethology, allowing targeted transcriptional comparison

273   between individuals with quantitatively different behavioral phenotypes, presents a

274   promising methodology for finding genes that may be related to behavioral variation.

275   The identities of these molecules, and their regulation in other behavioral contexts, will

276   help inform important questions on the constraints and possibilities of behavioral

277   evolution.

278

279

280   **FUNDING**

282

283   **ACKNOWLEDGMENTS**

287

288   **AUTHORS' CONTRIBUTIONS**

289   K.M.B., E.C.M., and A.J.M. conceived the study and analyzed the data, E.C.M. and

290   C.B.C. collected the data, and K.M.B. and A.J.M wrote the paper. All authors approved

291   the version to be published and agree to be accountable for all aspects of the research.

292

293   **DATA ACCESSIBILITY**

294   Analyses reported in this article can be reproduced using the data provided by Benowitz

295   et al. (2018).

296

COMPETING INTERESTS.

298   We declare no competing interests.

299

ETHICAL STATEMENT

301   All institutional, national, and international guidelines for the care and use of insects for

302   research were followed.

REFERENCES

Bailey NW, Marie-Orleach L, Moore AJ. 2018. Indirect genetic effects in behavioral ecology: does behavior play a special role in evolution? Behav Ecol. 29:1-11.

Baran NM, McGrath PT, Streelman JT. 2017. Applying gene regulatory logic to the evolution of social behavior. Proc Natl Acad Sci USA. 114:5886-5893.

Bell AM, Bukhari SA, Sanogo YO. 2016. Natural variation in brain gene expression profiles of aggressive and nonaggressive sticklebacks. Behaviour. 153:1723-1743.

Bendesky A, Tsunozaki M, Rockman MV, Kruglyak L, Bargmann CI. 2011. Catecholamine receptor polymorphisms affect decision-making in *C. elegans*. Nature. 472:313-318.

Bengston SE, Dahan RA, Donaldson Z, Phelps SM, van Oers K, Sih A, Bell AM. 2018. Genomic tools for behavioural ecologists to understand repeatable individual differences in behaviour. Nat Ecol Evol. 2:944-955.

Benowitz KM, McKinney EC, Moore AJ. 2016a. Difference in parenting in two species of burying beetle, *Nicrophorus orbicollis* and *Nicrophorus vespilloides*. J Ethol. 34:315-319.

Benowitz KM, McKinney EC, Moore AJ. 2016b. Data from: Difference in parenting in two species of burying beetle, *Nicrophorus orbicollis* and *Nicrophorus vespilloides*. Dryad Digital Repository. https://doi.org/10.5061/dryad.25rm2

Benowitz KM, McKinney EC, Roy-Zokan EM, Cunningham CB, Moore AJ. 2017a. The role of lipid metabolism in parental care in two species of burying beetle (*Nicrophorus* spp.). Anim Behav. 129:143-149.

Benowitz KM, McKinney EC, Cunningham CB, Moore AJ. 2017b. Relating quantitative variation within a behavior to variation in transcription. Evolution.71:1999-2009.

Benowitz KM, McKinney EC, Cunningham CB, Moore AJ. 2018. Data from: Predictable gene
    expression related to behavioral variation in parenting. Behavioral Ecology.
    doi:10.5061/dryad.n4d73sg.

Calisi RM, MacManes MD. 2015. RNAseq-ing a more integrative understanding of animal behavior.
    Curr Op Behav Sci. 6:65-68.

Cardoso SD, Teles MC, Oliveira RF. 2015. Neurogenomic mechanisms of social plasticity. J Exp Biol.
    218:140-149.

Chandrasekaran S, Ament SA, Eddy JA, Rodriguez-Zas SL, Schatz BR, Price ND, Robinson GE. 2011.
    Behavior-specific changes in transcriptional modules lead to distinct and predictable
    neurogenomic states. Proc Natl Acad Sci USA. 108:18020-18025.

Cotter SC, Kilner RM. 2010. Sexual division of antibacterial resource defence in burying beetles,
    Nicrophorus vespilloides. J Anim Ecol. 79:35-43.

Cunningham CB, Douthit MK, Moore AJ. 2014. Octopaminergic gene expression and flexible social
    behaviour in the subsocial beetle, *Nicrophorus vespilloides*. Ins Mol Biol. 23:391-404.

Cunningham CB, VanDenHeuvel K, Khana DB, McKinney EC, Moore AJ. 2016. The role of
    neuropeptide F in a transition to parental care. Biol Lett. 12:20160158.

Cunningham CB, Badgett M, Meagher RB, Orlando R, Moore AJ. 2017. Ethological principles predict
    the neuropeptides co-opted to influence parenting. Nat Comm. 8:14225.

Cunningham CB, Ji L, McKinney EC, Benowitz KM, Schmitz RJ, Moore AJ. 2019. Changes of gene
    expression but not cytosine methylation are associated with male parental care reflecting
    behavioural state, social context, and individual flexibility. J Exp Biol. In press.

Duckworth RA. 2015. Neuroendocrine mechanisms underlying behavioral stability: implications for the
    evolutionary origin of personality. Ann NY Acad Sci. 1360:54-74.

Eggert A-K, Müller JK. 1997. Biparental care and social evolution in burying beetles: lessons from the
    larder. In: Choe JC, Crespi BJ, editors. Social behavior in insects and arachnids. Cambridge, UK:
    Cambridge University Press. p. 216-236.

Eggert A-K, Reinking M, Müller JK. 1998. Parental care improvise offspring survival and growth in burying beetles. Anim Behav. 55:97-107.

Fitzpatrick MJ, Ben-Shahar Y, Smid HM, Vet LEM, Robinson GE, Sokolowski MB. 2005. Candidate genes for behavioural ecology. Trends Ecol Evol. 20:96-104.

Grafen A. 1984. Natural selection, kin selection, and group selection. In : Krebs JR, Davies NB, editors. Behavioural ecology: an evolutionary approach. 2nd ed. Oxford: Blackwell Scientific. p. 62-84.

Harris RM, Hofmann HA. 2014. Neurogenomics of behavioral plasticity. In: Landry R, Aubin-Horth N, editors. Ecological genomics. Dondrecht, Netherlands: Springer Science. p. 149-168.

Kronauer DJC, Libbrecht R 2018. Back to the roots: the importance of using simple insect societies to understand the molecular basis of complex social life. Curr Op Ins Sci. 28:33-39.

Linksvayer TA, Wade MJ. 2005. The evolutionary origin and elaboration of sociality in the aculeate Hymenoptera: maternal effects, sib-social effects and heterochrony. Q Rev Biol. 80:317–336.

Lock JE, Smiseth PT, Moore AJ. 2004. Selection, inheritance, and the evolution of parent-offspring interactions. Am Nat. 164:13-24.

Moore AJ, Székely T, Komdeur J. 2010. Prospects for research on social behaviour: systems biology meets behaviour. In: Székely T, Moore AJ, Komdeur J, editors. Social Behaviour: Genes, Ecology and Evolution. Cambridge University Press, UK. p. 538-550.

Palmer WJ, Duarte A, Schrader M, Day JP, Kilner R, Jiggins FM. 2016. A gene associated with social immunity in the burying beetle *Nicrophorus vespilloides*. Proc R Soc Lond B. 283:20152733.

Parker DJ, Cunningham CB, Walling CA, Stamper CE, Head ML, Roy-Zokan EM, McKinney EC, Ritchie MG, Moore AJ. 2015. Transcriptomes of parents identify parenting strategies and sexual conflict in a subsocial beetle. Nat. Comm. 6:8449.

Pfennig DW, Wund MA, Snell-Rood EC, Cruickshank T, Schlichting CD, Moczek AP. 2010. Phenotypic plasticity's impacts on diversification and speciation. Trends Ecol Evol. 25:459-467.

Rice WR. 1989. Analyzing tables of statistical tests. Evolution. 43:223-225.

Rittschof CC, Robinson GE. 2014. Genomics: moving behavioural ecology beyond the phenotypic gambit. Anim Behav. 92:263-270.

Rittschof CC, Robinson GE. 2016. Behavioral genetic toolkits: towards the evolutionary origins of complex phenotypes. Curr Top Dev Biol. 119:157-204.

Royle NJ, Hopwood PE. 2017. Covetable corpses and plastic beetles – the socioecological behavior of burying beetles. In: Naguib M, Podos J, Simmons LW, Barrett L, Healy SD, Zuk M, editors. Advances in the study of behavior. Elsevier, Amsterdam, Netherlands. p. 101-146.

Roy-Zokan EM, Cunningham CB, Hebb LE, McKinney EC, Moore AJ. 2015. Vitellogenin and vitellogenin receptor gene expression is associated with male and female parenting in a subsocial insect. Proc R Soc Lond B. 282:20150787.

Scott MP. 1998. The ecology and behavior of burying beetles. Annu Rev Entomol. 43:595-618.

Sih A, Bell A, Johnson JC. 2004. Behavioral syndromes: an ecological and evolutionary overview. Trends Ecol Evol. 19:372-378.

Stamps J. 1991. Why evolutionary issues are reviving interest in proximate behavioral mechanisms. Integrative Comp Biol. 31:338-348.

Steiger S, Peschke K, Müller JK. 2008. Correlated changes in breeding status and polyunsaturated cuticular hydrocarbons: the chemical basis of nestmate recognition in the burying beetle *Nicrophorus vespilloides*. Behav Ecol Sociobiol. 62:1053-1060.

Steiger S, Whitlow S, Peschke K, Müller JK. 2009. Surface chemicals inform about sex and breeding status in the burying beetle *Nicrophorus vespilloides*. Ethology. 115:178-185.

Toth AL, Rehan SA. 2017. Molecular evolution of insect sociality: an eco-evo-devo perspective. Annu Rev Entomol. 62:419–442.

Travisano M, Shaw RG. 2013. Lost in the map. Evolution. 67:305-314.

Wagner GP. 2014. Evolutionary innovations and novelties: Let us get down to business! Zool Anz. 256:75-81.

Wenzel J. Behavioral homology and phylogeny. Ann Rev Ecol Syst. 23:361-381.

Ziadie MA, Ebot-Ojong F, McKinney EC, Moore AJ. 2019. Evolution of personal and social immunity in

the context of parental care. Am Nat. https://dx.doi.org/10.1086/701122.

Zuk M, Balenger SL. 2014. Behavioral ecology and genomics: new directions, or just a more detailed

map? Behav Ecol. 25:1277-1282.

Table 1. Classification of genes and their patterns of differential expression between high and low cari

mothers.

Gene	Functional Role	Evidence for DE in transition between parenting states	Expression in high (mean ± sd)	Expression in low (mean ± sd)	Comp of exp in high low ca
Thaumatin	Immune	Parker et al. 2015	1.177 ± 1.051	1.143 ± 1.787	$(F_{1,22} =$ $p = 0.9$
Peptidoglycan recognition protein SC2-like	Immune	Parker et al. 2015	3.447 ± 2.743	2.926 ± 3.519	$(F_{1,22} =$ $p = 0.6$
Peptidoglycan recognition protein A	Immune	Parker et al. 2015	2.578 ± 1.136	2.742 ± 0.995	$(F_{1,22} =$ $p = 0.7$
Defensin	Immune	Parker et al. 2015	9.564 ± 4.923	9.219 ± 5.237	$(F_{1,22} =$ $p = 0.8$
Prophenoloxidase	Immune	Parker et al. 2015	4.293 ± 2.098	3.942 ± 1.585	$(F_{1,22} =$ $p = 0.6$
Serine protease 93	Immune	Parker et al. 2015	0.885 ± 0.663	0.660 ± 0.638	$(F_{1,22} =$ $p = 0.4$
Beta glucosidase	Digestion/Immune	Parker et al. 2015	4.294 ± 2.822	3.881 ± 5.626	$(F_{1,22} =$ $p = 0.8$
Fatty acyl-CoA synthetase	Lipid synthesis	Parker et al. 2015	0.00927 ± 0.00401	0.00495 ± 0.00273	$(F_{1,22} =$ **p = 0.0**
Vitellogenin 1	Lipid transport/Hormone	Parker et al. 2015; Roy-Zokan et al. 2015	135.800 ± 85.387	113.003 ± 38.542	$(F_{1,22} =$ $p = 0.8$
Hexamerin 3	Energy storage/Hormone Transport	Parker et al. 2015	0.0201 ± 0.00863	0.0253 ± 0.014	$(F_{1,22} =$ $p = 0.2$

Hexamerin 4	Energy storage/Hormone Transport	Parker et al. 2015	0.0677 ± 0.104	0.0238 ± 0.0240	(F$_{1,22}$ = p = 0.1
Apolipophorin-III	Lipid transport	Parker et al. 2015; Benowitz et al. 2017b	135.430 ± 55.438	117.294 ± 25.408	(F$_{1,22}$ = p = 0.3
Yellow 3	Hormone/Pigment/Immune	Parker et al. 2015	0.802 ± 0.277	0.628 ± 0.302	(F$_{1,22}$ = p = 0.1
Insulin like peptide 3	Hormone	Parker et al. 2015	0.0598 ± 0.0136	0.0589 ± 0.0175	(F$_{1,22}$ = p = 0.8
Neuropeptide F	Neurotransmission	Cunningham et al. 2016	0.0735 ± 0.0212	0.0916 ± 0.0601	(F$_{1,22}$ = p = 0.3
Serotonin receptor 7	Neurotransmission	Unpub. data	0.400 ± 0.108	0.454 ± 0.0941	(F$_{1,22}$ = p = 0.2
Serotonin receptor 2	Neurotransmission	Unpub. data	0.0233 ± 0.00336	0.0286 ± 0.00742	(F$_{1,22}$ = **p = 0.0**
Glutamate receptor	Neurotransmission	Parker et al. 2015	0.179 ± 0.0261	0.0257 ± 0.0679	(F$_{1,22}$ = 13.97 **0.001)**
Natalisin	Neurotransmission	Cunningham et al. 2017	0.00964 ± 0.00509	0.0208 ± 0.0259	(F$_{1,22}$ = p = 0.1
Octopamine/Tyramine receptor 2	Neurotransmission	Unpub. data	0.0250 ± 0.00637	0.0326 ± 0.00931	(F$_{1,22}$ = **p = 0.0**
Tachykinin	Neurotransmission	Unpub. data	0.431 ± 0.183	0.292 ± 0.136	(F$_{1,22}$ = **p = 0.0**
Takeout	Circadian behavior/Feeding	Parker et al. 2015	0.0499 ± 0.0321	0.0435 ± 0.0338	(F$_{1,22}$ = p = 0.6
Pickpocket	Sodium channels/Olfaction	Parker et al. 2015	0.0834 ± 0.0655	0.0559 ± 0.0176	(F$_{1,22}$ = p = 0.1

Table S1. Primers and accession numbers of the genes used in this study.