1   **Title** Autogenous and anautogenous *Culex pipiens* bioforms exhibit insulin-like peptide signaling

2   pathway gene expression differences that are not dependent upon larval nutrition

3   **Running Title** Nutrition and ILP expression in *Cx. pipiens* autogeny

4   Alys M. Cheatle Jarvela[1], Katherine L. Bell[1], Anna Noreuil[1], and Megan L. Fritz[1,2]*

5   [1] Department of Entomology, University of Maryland College Park, 4112 Plant Sciences

6   Building, College Park, MD 20042

7   [2] Institute for Advanced Computer Studies, University of Maryland College Park

8   *corresponding author

9   **Keywords** insulin-like peptides, *Culex pipiens*, form *molestus*, form *pipiens*, autogeny

10

11

12

13 **Abstract**

14 *Culex pipiens* form *pipiens* and *Cx. pipiens* form *molestus* differ in their ability to produce eggs

15 without a bloodmeal. Autogenous mosquitoes, such as the *molestus* bioform of *Cx. pipiens,*

16 depend on nutrition acquired as larvae instead of a bloodmeal to fuel the energy intensive

17 process of vitellogenesis, which requires abundant production of yolk proteins. In

18 anautogenous mosquito systems, ovary ecdysteroidogenic hormone (OEH) and insulin-like

19 peptides (ILPs) transduce nutritional signals and trigger egg maturation in response to a

20 bloodmeal. It is unclear to what extent the process is conserved in autogenous mosquitoes and

21 how the bloodmeal trigger has been replaced by teneral reserves. Here, we measured the

22 effects of a series of nutritional regimens on autogeny, time to pupation, and survival in *Cx.*

23 *pipiens* form *molestus* and form *pipiens*. We find that abundant nutrients never result in

24 autogenous form *pipiens* and extremely poor food availability rarely eliminates autogeny from

25 form *molestus*. However, the number of autogenous eggs generated increases with nutrient

26 availability. Similarly, using qPCR to quantify gene expression, we find several differences in the

27 expression levels of *ilps* between bioforms that are reduced and delayed by poor nutrition, but

28 not extinguished. Changes in *OEH* expression do not explain bioform-specific differences in

29 autogeny. Surprisingly, the source of most of the gene expression differences correlated with

30 autogeny is the abdomen, not the brain. Overall, our results suggest that autogeny is

31 modulated by nutritional availability, but the trait is encoded by genetic differences between

32 forms and these impact the expression of ILPs.

33

34   **Introduction**

35   Blood is a requirement for egg production in most mosquito vectors of disease, and

36   acquisition of blood drives pathogen transmission. This reproductive strategy is known as

37   anautogeny. Yet egg production in the absence of a blood meal, or autogeny, has been

38   described in dozens of mosquito species (Vinogradova, 2000). Autogeny can be obligatory,

39   where females always lay their first (and, in some species, subsequent) batches of eggs without

40   a blood meal, or facultative, where females may lay an autogenous egg raft dependent on their

41   larval environmental conditions (Attardo et al., 2005; Provost-Javier et al., 2010; Tsuji et al.,

42   1990). While it is well-established that autogenous reproduction is under genetic control

43   (Aslamkhan and Laven, 1970; Krishnamurthy and Laven, 1961; Mori et al., 2008; O'Meara et

44   al., 1969; Spielman, 1957; Trpis, 1978), the molecular basis for this unique reproductive

45   strategy remains unknown. Comparing the molecular and physiological tradeoffs underlying

46   autogenous and anautogenous reproduction has strong potential to elucidate the key genetic

47   changes that led to multiple instances of reproductive divergence within and among mosquito

48   species (O'Meara, 1985).

49   Due to its epidemiological relevance, the reproductive physiology of anautogenous

50   mosquitoes has been investigated extensively. Egg development requires that yolk proteins

51   produced by the fat body are deposited into developing oocytes, a process known as

52   vitellogenesis. In anautogenous mosquitoes, vitellogenesis is tightly suppressed until a blood

53   meal is obtained (reviewed in Attardo et al., 2005; Hansen et al., 2014). Four key signaling

54   pathways, juvenile hormone III (JH), ecdysone, insulin-signaling, and target of rapamycin

55   (TOR), interact to regulate the expression of yolk protein precursors (YPPs) in the fat body,

56   vitellogenesis, and ovarian maturation (reviewed in Attardo et al., 2005; Hansen et al., 2014).

57   JH acts on the fat body and ovaries during maturation, influencing the ability of a female to

58   activate *YPP* genes (Noriega, 2004; Zou et al., 2013). Once a female obtains a blood meal, the

59   steroid hormone ecdysone is the major hormone that regulates egg maturation via activation of

60 target genes, such as the predominant *YPP* gene, *vitellogenin* (*Vg*) (reviewed in Roy et al.,

61 2016). Ecdysone is regulated synergistically by ovary ecdysteroidogenic hormone (OEH) and

62 insulin-signaling. After feeding, stretch receptors in the gut trigger the release of OEH from the

63 brain into the hemolymph, which stimulates the production and release of ecdysone by follicle

64 cells of the ovary (Dhara et al., 2013; Hagedorn et al., 1979, 1975). Insulin-like peptides (ILPs),

65 a group of evolutionarily conserved peptide hormones, may also influence *YPP* gene expression

66 both directly and indirectly (Hansen et al., 2014).Together, insulin pathway and ecdysone

67 signaling activate *YPP* expression in the fat body (Roy et al., 2007). In *Aedes aegypti*, one

68 member of the insulin-like peptide family, ILP3, has been demonstrated to be essential for the

69 production of ecdysone, providing indirect reinforcement of *YPP* expression (Brown et al., 2008,

70 Dhara et al. 2013, Vogel et al. 2015). ILP3 also stimulates serine protease activity in the midgut

71 for blood meal digestion, contributing to the availability of amino acids required to produce YPPs

72 (Gulia-Nuss et al., 2012). The TOR signaling pathway detects these increases in amino acid

73 levels and contributes to the activation of *YPP* gene expression (Hansen et al., 2004).

74 Ultimately, vitellogenesis is not initiated in anautogenous females until these nutrient sensing

75 pathways signal the availability of yolk building blocks after a blood meal.

76   In contrast to anautogenous reproduction, the physiology of ovarian maturation in

77 autogenous mosquitoes has received less attention (Gulia-Nuss et al., 2015, 2012; Kassim et

78 al., 2012; Provost-Javier et al., 2010). One of the most well-studied autogenous species is the

79 rockpool mosquito, *Georgecraigius atropalpus* (formerly *Aedes* and *Ochlerotatus*)(Gulia-Nuss et

80 al., 2015, 2012; Telang et al., 2013, 2006). *G. atropalpus* females that are decapitated within 6

81 hours of pupal emergence fail to initiate vitellogenesis, demonstrating that hormones secreted

82 from the brain are critical to autogenous reproduction in this species. When injected, OEH fully

83 restores and ILP3 partially restores reproductive maturation in these decapitated females

84 (Gulia-Nuss et al., 2012), demonstrating that the roles of these hormones in *G. atropalpus* are

85   analogous to what has been found in the primarily anautogenous *Ae. aegypti* after they have

86   acquired a blood meal.

87   In *G. atropalpus* and other autogenous mosquitoes, nutritional status interacts with

88   genetic factors, including those underlying hormonal secretion from the brain, to influence

89   autogenous egg production. Females emerging from crowded, or nutrient poor larval habitats

90   may fail to reproduce autogenously or have low fecundity (Kassim et al., 2012; Krishnamurthy

91   and Laven, 1961; Lounibos et al., 1982; O'Meara and Krasnick, 1970; Trpis, 1978). Such

92   nutritional control of trait expression is observed in other insect species (Casasa and Moczek,

93   2018; Chandra et al., 2018; Wheeler and Frederik Nijhout, 1983), and in some cases, the

94   molecular mechanism underlying this nutritional regulation is known. For example, horn size in

95   taurus scarab beetles (*Onthophagus taurus*) is dependent on nutritional state, which is

96   transduced by insulin signaling (Casasa and Moczek, 2018). In ants, upregulation of *insulin-like*

97   *peptide 2* (*ilp2*) occurs in reproductive as opposed to non-reproductive individuals and larval

98   nutrition can influence adult ILP2 expression levels (Chandra et al., 2018). In these and other

99   examples, an emerging theme is that insulin signaling is a critical component of the mechanism

100   that ties phenotype to nutritional status (reviewed in Nijhout and McKenna, 2018). In

101   autogenous mosquitoes, it is unclear how and whether the nutritional state of females impacts

102   the expression of ILPs and other genes underlying induction of vitellogenesis and ovarian

103   maturation.

104   Here, we examine the interaction between nutrition, gene expression, and ovarian

105   maturation in a species of mosquito which is polymorphic for autogeny, *Culex pipiens*. Within

106   the species, there are two interfertile and morphologically indistinguishable bioforms that exhibit

107   divergent reproductive strategies (*Culex pipiens* form *pipiens* and form *molestus*, hereafter

108   *pipiens* and *molestus*) (Harbach et al., 1984; Spielman, 2001; Spielman and Wong, 1973). The

109   form *molestus* is thought to be facultatively autogenous, while the form *pipiens* is anautogenous

110   (Roubaud, 1929). Although these populations are genetically distinct (Fonseca et al., 2004;

111 Kent et al., 2007; Yurchenko et al., 2020), the molecular mechanisms underlying their divergent

112 reproductive strategies have not been identified.

113 We begin to address this knowledge gap by quantifying changes in expression of key

114 nutrient-sensing pathway genes for the autogenous *molestus*, as compared to the

115 anautogenous *pipiens*. Multiple genes previously demonstrated to play a critical role in ovarian

116 maturation, including *oeh*, the full suite of *ilp*s, their downstream effector *foxo,* as well as *Vg1b*,

117 were examined for expression level differences in female heads and abdomens for four days

118 following adult emergence. We also measured gene expression in females that experienced

119 nutrient rich and poor larval conditions, which produced dramatic changes in time spent as

120 larvae and fecundity in autogenous *molestus*. Expression patterns for several of these genes

121 differed temporally, by form, and in a body segment specific way. Interestingly, insulin-signaling

122 pathway genes whose expression differed by form also show delayed or dampened expression

123 in poorly-fed *molestus* individuals, but never matched the expression levels observed in well-fed

124 *pipiens*. This demonstrates the relatively greater importance of genotype over nutrition in

125 autogenous egg production for *Cx. pipiens*. Furthermore, this work establishes a system that

126 will allow investigation of the key genetic variants which halt ovarian maturation in some females

127 yet facilitate it in others. Such variants would make attractive targets for novel genetic control

128 measures that limit ovarian maturation, egg production, and ultimately vector population growth.

129

130 **Results and Discussion**

131 *Bioinformatic and phylogenetic analyses reveal six* Culex ilp *orthologs*

132 At the start of this work, three *ilps* had been identified in *Cx. pipiens* (named *ilp1*, *ilp2*,

133 and *ilp5*)(Sim and Denlinger, 2009), but based on the numbers in *Aedes aegypti* (n = 8; (Riehle

134 et al., 2006)), *Anopheles gambiae* (n = 5; (Riehle et al., 2002)), *Anopheles stephensi* (n = 5;

135 (Marquez et al., 2011)), and *Drosophila melanogaster* (n = 8; Brogiolo et al., 2001; Colombani et

136 al., 2012; Garelli et al., 2012; Grönke et al., 2010), it was predicted that more remained to be

137   discovered (Sharma et al., 2019; Sim and Denlinger, 2009). We identified six *Culex ilp* genes

138   by performing a HMMR analysis in Vectorbase using an alignment of all eight *Drosophila* and

139   *Aedes ilps* against the CpipJ2.4 geneset. A phylogenetic analysis of these six *Culex* ILPs

140   determined their orthology to established sequences from *Drosophila*, *Aedes*, and *Anopheles*

141   (Fig. 1). We confirmed the identity of previously isolated *Cpip-ilp1* and *-ilp5* genes, as well as

142   those from the *Culex quinquefasciatus* Johannesburg genome that were included in other's

143   phylogenetic analyses (Marquez et al., 2011; Sim and Denlinger, 2009). One gene previously

144   named *Cpip-ilp2* (Genbank accession ACM66967.1) was orthologous to *ilp3* in other mosquito

145   species. Comparison of "*Cpip-ilp2*" to *Cqui-ilp2* and *Cqui-ilp3* further supports its orthology with

146   *ilp3* (Fig. S1). Therefore, we refer to this sequence as *Cpip-ilp3* in this work.

147   One new *ilp* gene was identified and had likely been overlooked in previous work

148   because it is unique to *Culex* (XP_001868260.1). We named this gene *ilp4* because it is

149   syntenic with *ilp*s *1-3*, as is the case in other Dipteran genomes (Fig. S2). It is not a clear

150   ortholog of any other Dipteran *ilp4*, however, and likely arose by a lineage-specific gene

151   duplication event. All of the genes in this syntenic cluster have sequence features that define

152   them as "insulin-like peptides", such as a long, cleavable C peptide (Okamoto and Yamanaka,

153   2015). *Cpip-ilp5* and other mosquito *ilp5* orthologs are not syntenic in the genome with other *ilp*s

154   and contain distinct sequence features that define them as orthologs of *Dmel-ilp7* (Krieger et al.,

155   2004; Okamoto and Yamanaka, 2015; Riehle et al., 2006). Our phylogenetic analysis upholds

156   this grouping (Fig. 1). Similarly, *Cqui-ilp6* is in a clade with *Aaeg-ilp6* in our analysis and both

157   are located elsewhere in the genome, not in a syntenic cluster with other ILP genes. This is

158   also true for *Dmel-ilp6*, which is considered "IGF-like" based on sequence features such as its

159   short C peptide. Although our phylogeny does not group mosquito *ilp6* genes with *Dmel-ilp6*,

160   the short C peptides, extended C-terminus following the A domain, and genomic locations

161   suggest that they can also be considered "IGF-like" genes (Riehle et al., 2006). In sum, there

162   are six *ilp* genes in *Cx. pipiens* assemblage species: *ilps1-4* are true insulin-like peptides, *ilp5* is

163   similar to *Drosophila*'s atypical *ilp7*, and *ilp6* is best described as an IGF-like peptide.

164

165   *Autogenic ovarian maturation is enhanced by a nutrient rich larval environment*

166   Prior to quantifying gene expression, we confirmed that the timing and degree of *Culex*

167   ovarian maturation was consistent with previous observations of autogenous and anautogenous

168   mosquito populations. Dissections of adult *molestus* and *pipiens* females conducted over 96h

169   post emergence (PE) showed that ovarian maturation in *molestus* progressed beyond that of

170   *pipiens* by 48h PE and was complete by 96h PE, regardless of mating status (Fig. 2). Failure of

171   mating status to impact autogenous ovarian maturation agreed well with previous studies of

172   another *molestus* population and *G. atropalpus* (Gulia-Nuss et al., 2012; Kassim et al., 2012;

173   Spielman, 1957). Furthermore, our time course of ovarian maturation was consistent with

174   studies showing *molestus* follicle length reaches its maximum between 80-105h post-

175   emergence (Spielman, 1957), and egg deposition begins at 120h (Kassim et al., 2012).

176   Nutrient rich and poor aquatic environments are known to impact body size, larval

177   development, and mortality, as well as egg production in autogenous mosquitoes (Kassim et al.,

178   2012; Lounibos et al., 1982; O'Meara and Krasnick, 1970; Telang and Wells, 2004). Here, we

179   examined both anautogenous *pipiens* and autogenous *molestus* for these traits in a

180   comparative framework under identical environmental conditions. Rather than rearing larvae

181   together in the same environment, as was previously done (Kassim et al., 2012; Spielman,

182   1957), we reared each larva in a single well of cell culture plate to avoid the confounding effects

183   of competition on trait expression. Each larva was consistently fed one of four diet treatments

184   ranging from 0.25mg desiccated liver powder (LP) + 0.14mg dry yeast (DY) per larva (extra-low)

185   to 1.07mg LP + 0.53mg DY (high) every other day. For both forms, larval survivorship was high

186   (> 85%) and did not differ by diet treatment (Fig. S3; Tables S1 & S2). Wing length, which

187   serves as a proxy measure of female body size and teneral reserves (Telang et al., 2006) was

188   positively correlated with larval nutrient availability, however (Table 1; Tables S3 & S4).

189   Together, this indicated that our diet treatments effectively manipulated larval nutrition without

190   inducing mortality.

191   As expected based on previous work (Attardo et al., 2005), larval development time was

192   negatively correlated with nutrient availability for both forms (Fig. S4; Tables S5 & S6). When

193   fed the highest diet treatment, form *pipiens* and *molestus* larvae developed at similar rates

194   (Tables S5 & S7), where mean days to pupation were 7.2 (s.d. = 0.8, n = 208) and 7.8 (s.d. 1.1,

195   n = 222), respectively. At the lowest diet treatments, larval development times lengthened to

196   10.2 (s.d. = 1.9, n = 202) and 13.1 (s.d. 2.6, n = 200) days, respectively, and there was higher

197   variation in development time for *molestus*. We reasoned that sex-specific differences in

198   nutrient acquisition, particularly for *molestus,* could account for this additional variation. In a

199   separate experiment, we quantified male and female development times for both *molestus* and

200   *pipiens*, and our results revealed a three-way interaction between sex, form, and diet treatment

201   (Fig. 3; Table S8 & S9). Female *molestus* larvae compensated for very low nutrient availability

202   by lengthening their development time beyond what we observed for *pipiens* females and males

203   of both forms (Fig. 3). This was consistent with previous studies of autogenous mosquitoes

204   (Lounibos et al. 1982, Kassim et al. 2012), but unlike our results, *molestus* larvae from previous

205   studies suffered increased mortality (up to 38.7%), likely due to the more severe starvation

206   conditions imposed for the lowest diet treatments (Kassim et al., 2012).

207   Ovarian dissections revealed that larval nutrition was positively correlated with the

208   numbers of elongated follicles produced by each *molestus* female (Tables S10 & S11), while

209   *pipiens* females never showed signs of ovarian maturation (Table S12). Form *molestus* females

210   raised at the highest diet treatment matured, on average, 47.6 (s.d. = 14.3, n = 62) follicles

211   whereas those from the lowest diet treatment matured only 14.3 (s.d. = 9.6, n = 51; Table S12,

212   Fig. S4). We also quantified the probability that *molestus* females failed to produce any mature

213   follicles according to diet treatment. From the highest to lowest diet treatment, 1.6 %, 3.2%,

214   2.8% and 19.6% of *molestus* females showed no signs of ovarian maturation. Our statistical

215   analysis indicated that the lowest diet treatment had greater potential to halt ovarian maturation

216   in *molestus* females than did the other diet treatments (Tables S13 & S14), confirming that

217   expression of autogeny is the result of a genotype by environment interaction. Finally, our

218   observation that poorly nourished *molestus* females lengthened larval development time led us

219   to quantify the relationship between time spent as larvae and the numbers of elongated follicles

220   produced. We tested for a positive correlation between development time and numbers of

221   elongated follicles produced by females reared under the low and extra low diet treatments but

222   found little evidence of this (Fig. S5; Tables S15 & S16).

223   Altogether, these results support and add to previous work, showing that autogenic

224   follicle development in *Culex* is a form-specific trait that is quantitatively impacted and

225   sometimes eliminated by larval diet (Fig. S4). Poor nutritional conditions lengthen larval

226   development in a pronounced, sex-specific way for autogenous female *molestus*, compared to

227   anautogenous *pipiens* reared under identical conditions (Fig. 3; Table S8). Yet increased larval

228   developmental time does not necessarily result in strong increases in the numbers of elongated

229   follicles produced by poorly nourished females. These results suggest there is a threshold level

230   of nutrient acquisition that must be met prior to pupation in autogenous *molestus* females. In

231   our hands, when nutritional requirements were met, and female *molestus* pupated, most

232   initiated follicle maturation upon eclosion. Extended larval development times observed for

233   *molestus* females appeared to me more important to the progression from larva to pupa than for

234   increasing reproductive output, however (Fig. S5).

235

236   *Gene expression changes associated with autogeny are frequently observed in abdominal*

237   *tissue samples and are damped or delayed under poor nutritional conditions*

238   After observing significant, yet non-lethal impacts of diet on larval development and

239   reproduction in our *Culex* forms, we compared the expression patterns of nine genes involved in

240   nutrient-sensing and ovarian maturation for females raised under nutrient rich and poor

241   conditions using our previous experimental design. These gene candidates included *ilps 1-6*,

242   *foxo*, *Vg1b,* and *oeh*. Autogenous *molestus* and anautogenous *pipiens* were reared individually

243   under the highest or lowest diet treatments as described above, and non-bloodfed females were

244   collected at 24-hour intervals PE in groups of ten, according to form and diet treatment. Heads

245   and abdomens were divided into pooled tissue samples for RNA extraction, cDNA synthesis,

246   and qPCR analysis. Relative fold changes in gene expression are reported with respect to 0-

247   24h PE well-fed form *pipiens* (Fig. 4-6)*.* Statistical significance of fold change differences

248   between forms for each time point were always examined by two-way ANOVA with multiple

249   comparisons testing. This also allowed us to assess significance of fold change differences

250   between nutritional regimens within a bioform. With two discussed exceptions, we found no

251   significant differences associated with diet treatment.

252   Previous work suggested that insulin signaling is an attractive candidate pathway for

253   understanding the regulation of autogeny. For example, *ilp1* and *ilp5* are downregulated in

254   diapausing *Cx. pipiens* females, which shuts down their ovarian maturation (Sim and Denlinger,

255   2009). RNAi knock-down of *ilp1* also results in cessation of ovarian development in females not

256   programmed for diapause (Sim and Denlinger, 2009). Furthermore, ILP3 promotes ovarian

257   maturation in both the autogenous *G. atropalpus* (Gulia-Nuss et al., 2012), as well as

258   anautogenous *Ae. aegypti* following a blood meal (Brown et al., 2008). Brain medial

259   neurosecretory cells are the dominant source of ILP expression not only in mosquitoes, but in

260   insects in general (reviewed by Okamoto and Yamanaka, 2015). While ILP activity is controlled

261   primarily by the release of these neuropeptides into the hemolymph, we predicted that

262   genetically encoded regulatory changes between *Culex* bioforms could affect transcription

263   levels, and subsequently, ILP activity. Therefore, we expected to see upregulation of *ilps*,

264   especially *ilp3*, in the heads of *molestus* females, with the strongest upregulation occurring in

265   those that were well-fed.

266   Females of both bioforms expressed *ilp1, ilp2, ilp3, ilp4* and *ilp6* in the head tissues,

267   regardless of larval feeding conditions (Fig. 4A-E, Fig. 6). *ilp5* was the only insulin-like peptide

268   that was undetectable in the head (data not shown). Expression of *ilp1* and *ilp6* increased over

269   time but did so to the same extent in all groups, showing no correlation with autogenous egg

270   production (Fig. 4A and E). Expression levels of *ilp1, ilp3, ilp4,* and *ilp6* did not differ between

271   bioforms or feeding conditions in head tissues (Fig. 4A-E), even though ILP3 release from the

272   brain is associated with mosquito reproduction in other systems (Brown et al., 2008). One

273   exception is that *ilp1* is significantly different between well-fed and poorly-fed *molestus* at 48-

274   72h PE (log2 = -0.57 vs. 0.5, p = 0.018)(Fig. 4A). This likely reflects a slight delay initiating

275   upregulation of *ilp1* in poorly-fed *molestus*, which is ultimately upregulated in both diet

276   treatments at 72-96h PE (log2 = 1.67 vs. 1.94). Interestingly, *ilp2* experienced a ten-fold

277   downregulation in well-fed *molestus* at 24-48h PE vs. well-fed *pipiens* at the same time point

278   (log2 = -3.03 vs. log2 = 0.33, p = 0.014), and more modest downregulation in poorly-fed

279   *molestus* (log2 = -1.64)(Fig. 4B). The downstream effector of ILP signaling, *foxo*, was also

280   detected in all head samples (Fig. 4F). Well-fed *molestus* downregulated *foxo* by nine-fold at

281   24-48h PE with respect to equivalently staged *pipiens* (log2 = -2.7 vs. 0.47, p = 0.021) and at

282   48-72h PE (log2 = -1.81 vs. 1.31, p = 0.023). Weaker downregulation of *foxo* was observed in

283   poorly-fed *molestus* at those same time points (log2 = -1.57 and -0.52).

284   We also screened abdominal tissues as a potential alternative source of *ilp* expression.

285   While not as well-known, there is a precedent for *ilp* expression originating from ovaries, fat

286   body, and other abdominal tissues in many diverse insects (Okada et al., 2019; Okamoto et al.,

287   2009a, 2009b). Insects can express ILPs directly from their fat bodies to signal nutritional

288   conditions in a post-feeding stage. For example, in the beetle *Gnatocerus cornutus*, instead of

289   the fat body signaling to the brain to synthesize and release ILPs in response to nutritional input,

290   the fat body directly produces ILP to stimulate post-feeding growth (Okada et al., 2019). In

291   mosquitoes, ILP expression of abdominal origin has also been characterized. For example,

292   expression of ILP4 and ILP7 are detectable in ovary tissue in *Aedes* (Riehle et al., 2006).

293   Furthermore, in female *Anopheles gambiae*, an antibody against ILP1/3/4 detected expression

294   in abdominal ganglia that run along the body wall (Marquez et al., 2011). This same study

295   identified ILP1/3/4 immunoreactivity in neuronal axons along the midgut and observed changes

296   in the expression of several ILPs in the abdomen and midgut in response to sugar deprivation

297   and exposure to human insulin via a bloodmeal. We reasoned that *ilp*s of similar abdominal or

298   neural origin may be expressed in *molestus* prior to bloodfeeding and quantified gene

299   expression in whole abdomens (Fig. 5). *ilp1* was significantly upregulated at 72-96h PE in both

300   well and poorly-nourished *molestus* (log2 = 1.5 and 1.37 respectively) with respect to well-fed

301   *pipiens* of the same age (log2 = -1.78) (p = 0.0014 vs. p = 0.02) (Fig. 5A). *ilp3* expression

302   increased 53-fold in well-fed *molestus* compared to well-fed *pipiens* at 48-72h PE (log2 = 1.91

303   vs. -3.83, p = 0.003). The *ilp3* expression level differences between well-fed *molestus* and

304   *pipiens* gradually rose to 97-fold by 72-96h PE (log2 = 3.39 vs. -3.21, p = 0.0002) (Fig. 5C).

305   Poorly-nourished *molestus* significantly upregulated *ilp3* starting at 48-72h PE (16-fold, log2 =

306   0.24, p = 0.048), but expression levels did not reach those of well-fed *molestus* until 72h PE

307   (60-fold, log2 = 2.71, p = 0.002). Oddly, the expression of *ilp3* in *molestus* was not significantly

308   different when compared to poorly nourished *pipiens*. No differences in *ilp2, ilp4* or *ilp6*

309   expression were observed between bioforms or feeding conditions (Fig. 5B-F).   Finally, as was

310   the case for head tissues, *foxo* expression was also briefly downregulated in the abdomens of

311   well-fed *molestus.* We observed over three-fold lower expression in well-fed *molestus* vs. well-

312   fed *pipiens* at 0-24h PE (log2 = -1.86 vs. 0, p = 0.040) (Fig. 5G).

313   Interestingly, *ilp5* was only expressed in abdominal tissues, and primarily by the *pipiens*

314   bioform (Fig. 5E). Predominantly abdominal expression is conserved in *Aedes* and *Anopheles*

315   mosquitoes (Krieger et al., 2004; Riehle et al., 2006), so it was unsurprising that *ilp5* was not

316   detectable in head tissues. At any given time, expression was three to five times lower in

317   *molestus* than well-fed *pipiens* of the same stage (log2 on average was approximately -2 vs 0, p

318   < 0.05 in each case). While expression was equally low in *molestus* irrespective of nutritional

319   regimen, in *pipiens*, poor nutrition was associated with modest, but not significant, upregulation

320   of *ilp5*. These form and dietary differences in *ilp5* expression are especially intriguing. Previous

321   work found that the expression of *ilp5* was associated with a non-diapause state in *Culex*

322   *pipiens* (Sim and Denlinger, 2009). Indeed, one of the primary differences between the *pipiens*

323   and *molestus* bioforms is the ability to undergo diapause (Denlinger and Armbruster, 2014;

324   Vinogradova, 2000), although it is notable that knock-down of ILP5 had no effect on diapause

325   phenotypes in previous work (Sim and Denlinger, 2009). Currently, the functional role of *ilp5* in

326   *pipiens* is not well understood, but clues from *Aedes* suggest it is critical to acquisition of teneral

327   reserves. For example, CRISPR-Cas9 ablation of *ilp5* in *Aedes* resulted in bigger mosquitoes

328   with larger stores of lipids accumulated during larval stages (Ling and Raikhel, 2018). Because

329   increases in female size and teneral reserves are critical for autogeny (Chambers and Klowden,

330   1994; Gulia-Nuss et al., 2015; Telang et al., 2006), the significant reduction in *ilp5* expression

331   that we observed in the *molestus* bioform is potentially important for explaining a gain of

332   autogenic ability.

333   To summarize our major findings for insulin signaling pathway genes, we observed brief

334   downregulation of *ilp2* and *foxo* in the heads of females beginning at 24h PE. Upregulation of

335   *ilp1* and *ilp3* occurred in the abdomens of molestus females after 48h PE, the latter of which

336   experienced more dramatic upregulation. Abdominal expression of *foxo* was mildly

337   downregulated in well-fed *molestus* females. Interestingly, *ilp5*, whose function is currently not

338   well understood in *Cx. pipiens*, was only detected in the abdominal tissue, and strongly

339   downregulated in *molestus* throughout the course of our experiment.

340   We then determined the time course of *Vg1b* expression to determine whether insulin-

341   signaling pathway genes could be regulators of its expression. In anautogenous systems, *Vg*

342   expression is repressed until a bloodmeal is acquired (Hansen et al., 2014). FOXO is the

343   downstream effector of insulin signaling. It is a forkhead box transcription factor that represses

344   the expression of *Vg* in many insects until the insulin signaling pathway is activated (Sheng et

345   al., 2011). Insulin signaling triggers phosphorylation of FOXO and deportation from the nucleus

346   to the cytoplasm, relieving the repression of *Vg* genes.   In *molestus*, *Vg1b* was strongly

347   upregulated (p < 0.0001) in abdominal tissues immediately upon eclosion in well-nourished

348   samples and by 24-48h PE in poorly-nourished samples (Fig. 5H). Well-fed and poorly-fed

349   *molestus* are significantly different at 0-24h PE (p = 0.011), owing to the delayed onset of

350   upregulation that occurs in the poorly-fed sample. This agrees well with previous work in *Cx.*

351   *tarsalis* which demonstrated robust upregulation of this gene within a day of emergence in

352   autogenous females (Provost-Javier et al., 2010). Both well- and poorly-nourished *molestus*

353   samples ultimately attained changes in expression greater than 65,000 times that of newly

354   eclosed *pipiens*. It is notable that *Vg1b* expression peaks at 24-48h PE (log2 = 15.29) in well-

355   fed *molestus* but is delayed until 48-72h PE (log2 = 16) in poorly-fed *molestus.*   No

356   upregulation occurs in *pipiens* in any feeding condition or at any time point (Fig. 5H). It is

357   interesting to note that *Vg1b* is detected before adulthood in *Cx. tarsalis* and that in *Ae.*

358   *albopictus, Vg* has an additional role in repressing blood-seeking behavior in young, sugar-fed

359   females (Dittmer et al., 2019; Provost-Javier et al., 2010). We can infer that peak *Vg*

360   transcription must occur before 48h PE because ovary maturation is visible by this point (Fig. 2),

361   but it may also be that very early upregulation of *Vg1b* serves to block blood-seeking behavior

362   prior to or in addition to egg-producing functions.

363   We next examined expression patterns of insulin-signaling pathway genes in light of

364   *Vg1b*'s temporal dynamics. A notable early-acting difference of potential importance for rapid

365   *Vg1b* activation is the downregulation of *foxo* in 0-24h PE well-fed *molestus* abdomens.

366   Downregulation of this conserved repressor of vitellogenesis genes could make it easier to

367   activate *Vg1b* expression without bloodmeal-induced activation, even though it is typically

368   regulated at the post-transcriptional level. However, the difference is only seen in well-fed

369   *molestus* while autogeny occurs in both feeding regimens. Downregulation of *ilp5* also occurs

370   early in *molestus*, but is observed in both diets tested, making it a better candidate regulator of

371   *Vg1b*. In contrast, *ilp1* and *ilp3,* while significantly upregulated in the abdomens of *molestus*

372   females*,* are not likely to be involved in upregulation of *Vg1b.* We did not observe notable

373   differences between *molestus* and *pipiens* females until after autogenic egg production was in

374   progress (Fig. 5A, 5C, Fig. 6). *ilp3* was not significantly upregulated until 48h PE in well-fed

375   *molestus* and did not achieve a similar fold increase until 72h PE in poorly-fed *molestus.* By this

376   point, *Vg1b* expression had passed its peak in both feeding conditions. Likewise, upregulation

377   of *ilp1* specific to *molestus* did not occur until 72h PE. It may still reinforce the process once it is

378   in progress since this *ilp* gene has already been shown to regulate ovarian development in

379   *Culex* mosquitoes. Knock-down of expression of ILP1 has been demonstrated to halt ovarian

380   development in non-diapausing *pipiens* and lower endogenous expression levels are observed

381   in diapausing females (Sim and Denlinger, 2009). It is possible that changes in *ilp1* and *ilp3*

382   expression have some important function in autogeny unrelated to *Vg1b* activation that will be

383   determined by additional functional studies.

384   In *Aedes*, OEH, a neuroparsin type neuropeptide, activates the insulin signaling pathway

385   in response to a bloodmeal, but bypasses the insulin receptor to do so (Dhara et al., 2013).

386   More recent work demonstrated ecdysone signaling influences *ilp* expression in response to a

387   bloodmeal, suggesting that it could also be important during autogeny (Ling and Raikhel, 2021).

388   Based on previous predictions, we expected that OEH stimulation of egg production would be a

389   conserved feature of mosquito reproduction (Vogel et al., 2015). We measured expression of

390   *OEH* in the heads of females from both forms for 0-96h PE, with the expectation that it would be

391   uniquely upregulated in *molestus* females. We did not detect expression in abdomens during

392   pilot experiments. Although it was implicated in autogenous reproduction in other mosquito

393   species (Brown et al., 2008; Dhara et al., 2013; Gulia-Nuss et al., 2015, 2012), *OEH* underwent

394   very modest upregulation in the heads of *molestus* females throughout our time course relative

395   to equivalently-staged *pipiens* (Fig. 4G, Fig. 6). OEH upregulation in the heads of *molestus*

396   females preparing for autogenous egg production occurs after *Vg1b* upregulation and is not

397   statistically significant compared to expression in equivalent-staged *pipiens* females (Fig. 4G,

398   Fig. 6). In *G. atropalpus,* ecdysteroid is secreted by the ovaries immediately upon eclosion and

399   peaks during the first day of emergence under high-food conditions, and experience a ~12 hour

400   delay of peak secretion under low-food conditions (Telang et al., 2006). This is controlled by

401   OEH from the brain released within 6 hours of eclosion and produced during pupation and the

402   first few hours post-eclosion (Fuchs et al., 1980; Gulia-Nuss et al., 2012). It is possible that our

403   wide 0-24 hour sampling window prevented detection of this early peak in OEH expression, or

404   that OEH peak expression occurs during pupation for *molestus.* It may also be that OEH

405   activity is predominantly regulated by release of peptide, and that transcriptional regulation is

406   not a good indicator of its potential role in *molestus* autogeny.

407   Here, we characterized the effects of nutrition on time to pupation, autogenous egg

408   production, and expression of genes connected to sensing of nutritional state in *Cx. pipiens.*

409   We confirmed that genetic factors interacted with nutritional status to regulate autogenous egg

410   production in *molestus*, while even well-fed *pipiens* never produced eggs autogenously. In

411   agreement with this trait expression data, we observed strong and significant bioform-specific

412   gene expression differences, which were only mildly influenced by nutrition. The comparison

413   between autogenous *molestus* and anautogenous *pipiens* revealed changes in gene expression

414   patterns in the abdomen (*ilp1*, *ilp3*, *ilp5*), head (*ilp2*), or both (*foxo*) (Fig. 6). Some of these

415   coincided with dramatic upregulation of *Vg1b* (ex. *foxo*, *ilp5*), while others occurred afterward

416   (ex. *ilp1*, *ilp3*). Gene expression differences in well- vs. poorly-nourished *molestus* could be

417   characterized as dampened or delayed rather than statistically significant changes in

418   expression, in agreement with incomplete loss of autogeny in our lowest diet treatment. Future

419   work on the functional consequences of these gene expression differences, especially that of

420   *ilp5*, will enhance our understanding of the evolution of autogeny and loss of diapause as

421   bioform-specific traits.

422

Data Availability

Scripts and data used to examine expression of autogeny under different dietary conditions can be found at https://github.com/mcadamme/Culex_Nutrition_Exp. Data from gene expression analysis can be found at: link to be added following acceptance.

427

Experimental Procedures

Gene identification and orthology

We identified six *Culex ilp* genes by performing a HMMR analysis in Vectorbase (https://legacy.vectorbase.org/hmmer) using an alignment of all eight *Drosophila* and *Aedes ilps* against the CpipJ2.4 geneset (Arensburger et al., 2010; Giraldo-Calderón et al., 2015). We next constructed phylogenetic trees to establish gene orthology. Putative *Culex* ILPs plus previously characterized insect ILP sequences were aligned in MUSCLE (https://www.ebi.ac.uk/Tools/msa/muscle/)(Edgar, 2004). Tree topology was determined by TOPALI v2.5 using both a MrBayes algorithm (Model: JTT + G, Runs: 2 Generations: 500000, Sample Freq.: 10, Burnin: 25%) and maximum PhyML algorithm (Model: JTT + G, 100 bootstrap runs)(Anisimova and Gascuel, 2006; Milne et al., 2004; Ronquist and Huelsenbeck, 2003). Synteny of *ilp* genes was examined using Genomicus Metazoa (web-code version: 2014-07-06, database version: 30.01, https://www.genomicus.biologie.ens.fr/genomicus-metazoa-30.01/cgi-bin/search.pl)(Louis et al., 2013).

442

Mosquitoes

A below-ground population (form *molestus*) of *Cx. pipiens* was obtained from Calumet, IL, and has been in colony since 2009 (Mutebi and Savage, 2009) and maintained following Fritz et al. (Fritz et al., 2015, 2014). An above-ground population (form *pipiens*) of *Cx. pipiens* was obtained from Evanston, IL, and has been in colony since 2016 (Noreuil and Fritz 2021).

448   Our laboratory-reared *molestus* had been cultured for 8 years without requiring a blood meal,

449   but our *pipiens* never produced egg rafts prior to blood feeding during its 2 years in culture. All

450   adults and larvae were maintained in an environmental chamber with a L:D photo period of

451   16h:8h, temperature of 25±1◦C, and humidity of 50±10%. Adults for each form were kept in

452   separate 60 x 60 x 60 cm white BugDorm-2 insect rearing cages (Megaview Science Education

453   Services Co., Taichung, Taiwan) and were provided with a 10% sucrose solution at all times.

454

455   *Imaging of ovarian development*

456   Pupae were picked from respective larval rearing pans, placed in individual tubes until

457   eclosion, then moved to cages containing a 10% sucrose resource where females were allowed

458   to age with or without males for one to four days. Ovaries were dissected under a

459   stereomicroscope (Olympus Corporation, model SZ61, Center Valley, PA, USA) at 3.0-4.5 X

460   magnification on a petri dish filled with 70% ethanol. Images were made using the Olympus

461   cellSens Entry microscope-mounted imaging system.

462

463   *Impacts of genotype and nutrition on survivorship, larval development and ovarian maturation*

464   To produce sufficient mosquitoes for experimentation, each colony was blood fed at 12-

465   14 days of age using an artificial membrane feeder. Goose blood (Na-heparinated, obtained

466   from Lampire Biological) sweetened with 50% sucrose solution (8:1 ratio) was provided through

467   a pork sausage casing for a minimum of 1 hour. Approximately 72 hours after blood feeding

468   females were provided with a cup of dechlorinated tap water and allowed to lay eggs for 48

469   hours. For each colony, the resulting egg rafts were placed in 27.0 x 19.4 x 9.5 cm plastic pans

470   with 800ml of dechlorinated tap water and were supplied with 0.16g of liver powder (BD

471   DifcoTM Desiccated, Powdered Beef Liver) and 0.08g of powdered inactive dry yeast (Genesee

472   Scientific) suspended in 8ml of RO water. After 48 hours, 60 individuals from each colony were

473   placed in one of four diet treatments using a 3mL plastic transfer pipette. Those treatments were

474   as follows: extra low (0.25mg desiccated liver powder (LP), 0.14mg dry yeast (DY), 4ml RO

475   water per larva), low (0.53mg LP, 0.27mg DY, 4ml RO water per larva), medium (0.80mg LP,

476   0.40mg DY, 4ml RO water per larva), and high (1.07mg LP, 0.53mg DY, 4ml RO water per

477   larva). To avoid competition between individuals, larvae were placed in a single well of a 12-well

478   culture plate. This resulted in 5 plates (12 x 5 = 60 individuals) per treatment, per form. Plates

479   were held in an environmental chamber with the same conditions as described above.

480   Individuals were fed every other day, diet fed on days subsequent to the initial loading of the

481   plates was the same as described above but with 100µl of RO water instead of 4ml. Water was

482   changed completely, and the diet was re-loaded as initially described at day 8 or 10 of the

483   experiment to avoid build-up of waste within each well. The number of pupae present was

484   recorded daily, and mortality was recorded every other day during feeding. Pupae were

485   removed from the well and placed in a small plastic cup within a 30 x 30 x 30 white Bugdorm-2

486   cage. Adults were provided with a 10% sucrose solution and held under the previously

487   described environmental conditions. Wing and ovarian dissections were performed on adult

488   females, beginning at 4 days PE. A single wing was removed from each of 3 females per diet

489   treatment, per form and photographed using an Olympus cellSens Entry microscope-mounted

490   imaging system. Wing length measurements were made from the allular notch to the tip of the

491   wing using ImageJ software (Schneider et al., 2012). Ovaries were also removed from at least

492   20 *molestus* females and 5 *pipiens* females per diet treatment, and the numbers of follicles

493   advanced beyond Christopher's stage IIb present were recorded (O'Meara and Krasnick, 1970).

494   This experiment was repeated four times.

495   In a separate experiment, we measured the impacts of diet, form, and sex on larval

496   development time. For each bioform and diet treatment (high or extra low), 60 larvae were

497   placed into wells of the five 12-well cell culture plates and allowed to develop as described

498   above. Upon pupation, individuals were transferred to a 2ml clear plastic lysis tube plugged

499   with cotton wool. We monitored emergence every 48h and scored both larval development time

500   and sex of the emerged adult. This experiment was repeated three times.

501

502   *Statistical analysis of mortality, pupation, and ovarian maturation*

503   Bayesian generalized linear models (GLMs) were always fit using the package BRMS (v.

504   2.12.0 (Bürkner, 2017)) in R (R Core Team, 2016). Posterior probability distributions of model

505   parameters were estimated using Markov Chain Monte Carlo (MCMC). For each model, we ran

506   4 chains with 6,000 steps, a burn-in of 2,000 steps, and saved every other step resulting in a

507   total of 8,000 samples drawn from the posterior probability distribution. To ensure a stable

508   sampling distribution had been reached, we visually examined trace plots, estimated effective

509   sample size, and calculated Gelman and Rubin's convergence diagnostic (Gelman and Rubin,

510   1992). Details of the models and their underlying distributions can be found in Tables S1-6, S8-

511   11, and S13-16.

512

513   *RNA isolation and qPCR*

514   To compare gene expression levels between species, developmental time points, and

515   nutritional states, we first extracted total RNA from pools of tissue derived from ten like

516   individuals using TRIzol Reagent (Invitrogen, Carlsbad, CA) according to the manufacturer's

517   instructions. Larvae from each bioform were reared individually in 12-well cell culture plates and

518   fed either high or extra-low diet treatments. Pupae were placed in individual 2mL lysis tubes,

519   allowed to emerge, and adult females were collected every 24 hours. From these females,

520   replicate pools of ten heads (bearing intact chemosensory appendages) and ten abdomens

521   were separately dissected on a small petri dish filled with dry ice for each diet treatment. Pooled

522   tissue was stored in sterile 1.5 mL microcentrifuge tubes containing TRIzol Reagent at -80°C

523   until RNA isolation was performed. One minor modification to this is that we back-extracted the

524   aqueous RNA-containing layer with chloroform an additional time to remove trace amounts of

525   TRIzol components that interfered with downstream steps. Once the resultant RNA pellet was

526   dissolved in 20 µL of nuclease-free water, the RNA was treated to remove residual genomic

527   DNA contamination with the Turbo DNA-free kit (Invitrogen, Carlsbad, CA) according to the

528   manufacturer's protocol. RNA yield was assessed following genomic DNA removal using a

529   Nanodrop Lite spectrophotometer (Thermo Fisher Scientific, Waltham, MA). 250ng of RNA was

530   used to synthesize cDNA with the iScript kit (BioRad, Hercules, CA). cDNA was diluted five-fold

531   in nuclease-free water for use as a qPCR template, and total RNA was diluted to an equivalent

532   dilution factor (2ng/µL) for –RT controls. qPCR was performed on a LightCycler 480 real-time

533   PCR cycler (Roche, Basel, CH) using Luna Universal qPCR master mix (NEB, Ipswich, MA).

534   Primer sequences can be found in Table S17. Results were analyzed by the ΔΔCt method

535   (Livak and Schmittgen, 2001). Reference gene (*EF1A*) was selected using the Normfinder

536   algorithm (Andersen et al., 2004) from a panel of six candidate reference genes selected from

537   relevant literature (Ling and Salvaterra, 2011; Sim and Denlinger, 2009; Van Hiel et al., 2009).

538   Statistical significance of qPCR data determined by 2-way ANOVA of ΔΔCts with Tukey's

539   multiple comparisons test using Graphpad software.

548

549 **References**
550
551 Andersen, C.L., Jensen, J.L., Ørntoft, T.F., 2004. Normalization of Real-Time Quantitative
552   Reverse Transcription-PCR Data: A Model-Based Variance Estimation Approach to
553   Identify Genes Suited for Normalization, Applied to Bladder and Colon Cancer Data
554   Sets. Cancer Res. 64, 5245–5250. https://doi.org/10.1158/0008-5472.CAN-04-0496
555 Anisimova, M., Gascuel, O., 2006. Approximate likelihood-ratio test for branches: A fast,
556   accurate, and powerful alternative. Syst. Biol. 55, 539–552.
557   https://doi.org/10.1080/10635150600755453
558 Arensburger, P., Megy, K., Waterhouse, R.M., Abrudan, J., Amedeo, P., Antelo, B.,
559   Bartholomay, L., Bidwell, S., Caler, E., Camara, F., Campbell, C.L., Campbell, K.S.,
560   Casola, C., Castro, M.T., Chandramouliswaran, I., Chapman, S.B., Christley, S., Costas,
561   J., Eisenstadt, E., Feschotte, C., Fraser-Liggett, C., Guigo, R., Haas, B., Hammond, M.,
562   Hansson, B.S., Hemingway, J., Hill, S.R., Howarth, C., Ignell, R., Kennedy, R.C., Kodira,
563   C.D., Lobo, N.F., Mao, C., Mayhew, G., Michel, K., Mori, A., Liu, N., Naveira, H., Nene,
564   V., Nguyen, N., Pearson, M.D., Pritham, E.J., Puiu, D., Qi, Y., Ranson, H., Ribeiro,
565   J.M.C., Roberston, H.M., Severson, D.W., Shumway, M., Stanke, M., Strausberg, R.L.,
566   Sun, C., Sutton, G., Tu, Z.J., Tubio, J.M.C., Unger, M.F., Vanlandingham, D.L., Vilella,
567   A.J., White, O., White, J.R., Wondji, C.S., Wortman, J., Zdobnov, E.M., Birren, B.,
568   Christensen, B.M., Collins, F.H., Cornel, A., Dimopoulos, G., Hannick, L.I., Higgs, S.,
569   Lanzaro, G.C., Lawson, D., Lee, N.H., Muskavitch, M.A.T., Raikhel, A.S., Atkinson,
570   P.W., 2010. Sequencing of Culex quinquefasciatus establishes a platform for mosquito
571   comparative genomics. Science 330, 86–88. https://doi.org/10.1126/science.1191864
572 Aslamkhan, M., Laven, H., 1970. Inheritance of autogeny in the Culex pipiens complex. Pak. J.
573   Zool. 2, 121–147.
574 Attardo, G.M., Hansen, I.A., Raikhel, A.S., 2005. Nutritional regulation of vitellogenesis in
575   mosquitoes: Implications for anautogeny. Insect Biochem. Mol. Biol., Genetic
576   manipulation of insects 35, 661–675. https://doi.org/10.1016/j.ibmb.2005.02.013
577 Brogiolo, W., Stocker, H., Ikeya, T., Rintelen, F., Fernandez, R., Hafen, E., 2001. An
578   evolutionarily conserved function of the Drosophila insulin receptor and insulin-like
579   peptides in growth control. Curr. Biol. CB 11, 213–221. https://doi.org/10.1016/s0960-
580   9822(01)00068-9
581 Brown, M.R., Clark, K.D., Gulia, M., Zhao, Z., Garczynski, S.F., Crim, J.W., Suderman, R.J.,
582   Strand, M.R., 2008. An insulin-like peptide regulates egg maturation and metabolism in
583   the mosquito Aedes aegypti. Proc. Natl. Acad. Sci. U. S. A. 105, 5716–5721.
584   https://doi.org/10.1073/pnas.0800478105
585 Bürkner, P.-C., 2017. brms: An R Package for Bayesian Multilevel Models Using Stan. J. Stat.
586   Softw. 80, 1–28. https://doi.org/10.18637/jss.v080.i01
587 Casasa, S., Moczek, A.P., 2018. Insulin signalling's role in mediating tissue-specific nutritional
588   plasticity and robustness in the horn-polyphenic beetle Onthophagus taurus. Proc. R.
589   Soc. B Biol. Sci. 285, 20181631. https://doi.org/10.1098/rspb.2018.1631
590 Chambers, G.M., Klowden, M.J., 1994. Nutritional Reserves of Autogenous and Anautogenous
591   Selected Strains of Aedes albopictus (Diptera: Culicidae). J. Med. Entomol. 31, 554–
592   560. https://doi.org/10.1093/jmedent/31.4.554
593 Chandra, V., Fetter-Pruneda, I., Oxley, P.R., Ritger, A.L., McKenzie, S.K., Libbrecht, R.,
594   Kronauer, D.J.C., 2018. Social regulation of insulin signaling and the evolution of
595   eusociality in ants. Science 361, 398–402. https://doi.org/10.1126/science.aar5723
596 Clifton, M.E., Noriega, F.G., 2012. The fate of follicles after a blood meal is dependent on
597   previtellogenic nutrition and juvenile hormone in Aedes aegypti. J. Insect Physiol. 58,
598   1007–1019. https://doi.org/10.1016/j.jinsphys.2012.05.005
599 Colombani, J., Andersen, D.S., Léopold, P., 2012. Secreted peptide Dilp8 coordinates

600 Drosophila tissue growth with developmental timing. Science 336, 582–585.
601 https://doi.org/10.1126/science.1216689
602 Denlinger, D.L., Armbruster, P.A., 2014. Mosquito diapause. Annu. Rev. Entomol. 59, 73–93.
603 https://doi.org/10.1146/annurev-ento-011613-162023
604 Dhara, A., Eum, J.-H., Robertson, A., Gulia-Nuss, M., Vogel, K.J., Clark, K.D., Graf, R., Brown,
605 M.R., Strand, M.R., 2013. Ovary ecdysteroidogenic hormone functions independently of
606 the insulin receptor in the yellow fever mosquito, Aedes aegypti. Insect Biochem. Mol.
607 Biol. 43, 1100–1108. https://doi.org/10.1016/j.ibmb.2013.09.004
608 Dittmer, J., Alafndi, A., Gabrieli, P., 2019. Fat body–specific vitellogenin expression regulates
609 host-seeking behaviour in the mosquito Aedes albopictus. PLOS Biol. 17, e3000238.
610 https://doi.org/10.1371/journal.pbio.3000238
611 Edgar, R.C., 2004. MUSCLE: multiple sequence alignment with high accuracy and high
612 throughput. Nucleic Acids Res. 32, 1792–1797. https://doi.org/10.1093/nar/gkh340
613 Feinsod, F.M., Spielman, A., 1980. Nutrient-mediated juvenile hormone secretion in
614 mosquitoes. J. Insect Physiol. 26, 113–117. https://doi.org/10.1016/0022-
615 1910(80)90050-5
616 Fonseca, D.M., Keyghobadi, N., Malcolm, C.A., Mehmet, C., Schaffner, F., Mogi, M., Fleischer,
617 R.C., Wilkerson, R.C., 2004. Emerging vectors in the Culex pipiens complex. Science
618 303, 1535–1538. https://doi.org/10.1126/science.1094247
619 Fritz, M.L., Walker, E.D., Miller, J.R., Severson, D.W., Dworkin, I., 2015. Divergent host
620 preferences of above- and below-ground Culex pipiens mosquitoes and their hybrid
621 offspring. Med. Vet. Entomol. 29, 115–123. https://doi.org/10.1111/mve.12096
622 Fritz, M.L., Walker, E.D., Yunker, A.J., Dworkin, I., 2014. Daily blood feeding rhythms of
623 laboratory-reared North American Culex pipiens. J. Circadian Rhythms 12, 1.
624 https://doi.org/10.1186/1740-3391-12-1
625 Fuchs, M.S., Sundland, B.R., Kang, S.-H., 1980. In vivo induction of ovarian development in
626 Aëdes atropalpus by a head extract from Aëdes aegypti. Int. J. Invertebr. Reprod. 2,
627 121–129. https://doi.org/10.1080/01651269.1980.10553347
628 Garelli, A., Gontijo, A.M., Miguela, V., Caparros, E., Dominguez, M., 2012. Imaginal discs
629 secrete insulin-like peptide 8 to mediate plasticity of growth and maturation. Science
630 336, 579–582. https://doi.org/10.1126/science.1216735
631 Gelman, A., Rubin, D.B., 1992. Inference from Iterative Simulation Using Multiple Sequences.
632 Stat. Sci. 7, 457–472. https://doi.org/10.1214/ss/1177011136
633 Giraldo-Calderón, G.I., Emrich, S.J., MacCallum, R.M., Maslen, G., Dialynas, E., Topalis, P.,
634 Ho, N., Gesing, S., VectorBase Consortium, Madey, G., Collins, F.H., Lawson, D., 2015.
635 VectorBase: an updated bioinformatics resource for invertebrate vectors and other
636 organisms related with human diseases. Nucleic Acids Res. 43, D707-713.
637 https://doi.org/10.1093/nar/gku1117
638 Grönke, S., Clarke, D.-F., Broughton, S., Andrews, T.D., Partridge, L., 2010. Molecular
639 Evolution and Functional Characterization of Drosophila Insulin-Like Peptides. PLOS
640 Genet. 6, e1000857. https://doi.org/10.1371/journal.pgen.1000857
641 Gulia-Nuss, M., Elliot, A., Brown, M.R., Strand, M.R., 2015. Multiple factors contribute to
642 anautogenous reproduction by the mosquito Aedes aegypti. J. Insect Physiol. 82, 8–16.
643 https://doi.org/10.1016/j.jinsphys.2015.08.001
644 Gulia-Nuss, M., Eum, J.-H., Strand, M.R., Brown, M.R., 2012. Ovary ecdysteroidogenic
645 hormone activates egg maturation in the mosquito Georgecraigius atropalpus after adult
646 eclosion or a blood meal. J. Exp. Biol. 215, 3758–3767.
647 https://doi.org/10.1242/jeb.074617
648 Hagedorn, H.H., O'Connor, J.D., Fuchs, M.S., Sage, B., Schlaeger, D.A., Bohm, M.K., 1975.
649 The ovary as a source of alpha-ecdysone in an adult mosquito. Proc. Natl. Acad. Sci. U.
650 S. A. 72, 3255–3259. https://doi.org/10.1073/pnas.72.8.3255

Hagedorn, H.H., Shapiro, J.P., Hanaoka, K., 1979. Ovarian ecdysone secretion is controlled by a brain hormone in an adult mosquito. Nature 282, 92–94. https://doi.org/10.1038/282092a0

Hansen, I.A., Attardo, G.M., Park, J.-H., Peng, Q., Raikhel, A.S., 2004. Target of rapamycin-mediated amino acid signaling in mosquito anautogeny. Proc. Natl. Acad. Sci. U. S. A. 101, 10626–10631. https://doi.org/10.1073/pnas.0403460101

Hansen, I.A., Attardo, G.M., Rodriguez, S.D., Drake, L.L., 2014. Four-way regulation of mosquito yolk protein precursor genes by juvenile hormone-, ecdysone-, nutrient-, and insulin-like peptide signaling pathways. Front. Physiol. 5. https://doi.org/10.3389/fphys.2014.00103

Harbach, R.E., Harbach, R.E., Harrison, B.A., Gad, A.M., 1984. Culex (Culex) Molestus Forskål (Diptera: Culicidae): neotype designation, description, variation, and taxonomic status. Proc. Entomol. Soc. Wash. 86, 521–542.

Kassim, N.F.A., Webb, C.E., Russell, R.C., 2012. Is the expression of autogeny by Culex molestus Forskal (Diptera: Culicidae) influenced by larval nutrition or by adult mating, sugar feeding, or blood feeding? J. Vector Ecol. 37, 162–171. https://doi.org/10.1111/j.1948-7134.2012.00213.x

Kent, R.J., Harrington, L.C., Norris, D.E., 2007. Genetic Differences Between Culex pipiens f. molestus and Culex pipiens pipiens (Diptera: Culicidae) in New York. J. Med. Entomol. 44, 50–59.

Krieger, M.J.B., Jahan, N., Riehle, M.A., Cao, C., Brown, M.R., 2004. Molecular characterization of insulin-like peptide genes and their expression in the African malaria mosquito, Anopheles gambiae. Insect Mol. Biol. 13, 305–315. https://doi.org/10.1111/j.0962-1075.2004.00489.x

Krishnamurthy, B.S., Laven, H., 1961. A Note on Inheritance of Autogeny in Culex Mosquitos. Bull. World Health Organ. 24, 675–677.

Ling, D., Salvaterra, P.M., 2011. Robust RT-qPCR Data Normalization: Validation and Selection of Internal Reference Genes during Post-Experimental Data Analysis. PLoS ONE 6. https://doi.org/10.1371/journal.pone.0017762

Ling, L., Raikhel, A.S., 2021. Cross-talk of insulin-like peptides, juvenile hormone, and 20-hydroxyecdysone in regulation of metabolism in the mosquito Aedes aegypti. Proc. Natl. Acad. Sci. 118. https://doi.org/10.1073/pnas.2023470118

Ling, L., Raikhel, A.S., 2018. Serotonin signaling regulates insulin-like peptides for growth, reproduction, and metabolism in the disease vector Aedes aegypti. Proc. Natl. Acad. Sci. 115, E9822–E9831. https://doi.org/10.1073/pnas.1808243115

Livak, K.J., Schmittgen, T.D., 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods San Diego Calif 25, 402–408. https://doi.org/10.1006/meth.2001.1262

Louis, A., Muffato, M., Roest Crollius, H., 2013. Genomicus: five genome browsers for comparative genomics in eukaryota. Nucleic Acids Res. 41, D700-705. https://doi.org/10.1093/nar/gks1156

Lounibos, L.P., Van Dover, C., O'Meara, G.F., 1982. Fecundity, autogeny, and the larval environment of the pitcher-plant mosquito, Wyeomyia smithii. Oecologia 55, 160–164. https://doi.org/10.1007/BF00384482

Marquez, A.G., Pietri, J.E., Smithers, H.M., Nuss, A., Antonova, Y., Drexler, A.L., Riehle, M.A., Brown, M.R., Luckhart, S., 2011. Insulin-like peptides in the mosquito Anopheles stephensi: Identification and expression in response to diet and infection with Plasmodium falciparum. Gen. Comp. Endocrinol. 173, 303–312. https://doi.org/10.1016/j.ygcen.2011.06.005

Milne, I., Wright, F., Rowe, G., Marshall, D.F., Husmeier, D., McGuire, G., 2004. TOPALi: software for automatic identification of recombinant sequences within DNA multiple

702   alignments. Bioinforma. Oxf. Engl. 20, 1806–1807.
703   https://doi.org/10.1093/bioinformatics/bth155
704 Mori, A., Romero-Severson, J., Black, W.C., Severson, D.W., 2008. Quantitative trait loci
705   determining autogeny and body size in the Asian tiger mosquito (Aedes albopictus).
706   Heredity 101, 75–82. https://doi.org/10.1038/hdy.2008.32
707 Mostowy, W., Foster, W., 2004. Antagonistic effects of energy status on meal size and egg-
708   batch size of Aedes aegypti (Diptera: Culicidae). J. Vector Ecol. J. Soc. Vector Ecol. 29,
709   84–93.
710 Mutebi, J.-P., Savage, H.M., 2009. Discovery of Culex pipiens pipiens form molestus in
711   Chicago. J. Am. Mosq. Control Assoc. 25, 500–503. https://doi.org/10.2987/09-5910.1
712 Nijhout, H.F., McKenna, K.Z., 2018. The distinct roles of insulin signaling in polyphenic
713   development. Curr. Opin. Insect Sci. 25, 58–64.
714   https://doi.org/10.1016/j.cois.2017.11.011
715 Noriega, F.G., 2004. Nutritional regulation of JH synthesis: a mechanism to control reproductive
716   maturation in mosquitoes? Insect Biochem. Mol. Biol., Molecular and population biology
717   of mosquitoes 34, 687–693. https://doi.org/10.1016/j.ibmb.2004.03.021
718 Okada, Y., Katsuki, M., Okamoto, N., Fujioka, H., Okada, K., 2019. A specific type of insulin-like
719   peptide regulates the conditional growth of a beetle weapon. PLOS Biol. 17, e3000541.
720   https://doi.org/10.1371/journal.pbio.3000541
721 Okamoto, N., Yamanaka, N., 2015. Nutrition-dependent control of insect development by
722   insulin-like peptides. Curr. Opin. Insect Sci., Global change biology * Molecular
723   physiology 11, 21–30. https://doi.org/10.1016/j.cois.2015.08.001
724 Okamoto, N., Yamanaka, N., Satake, H., Saegusa, H., Kataoka, H., Mizoguchi, A., 2009a. An
725   ecdysteroid-inducible insulin-like growth factor-like peptide regulates adult development
726   of the silkmoth Bombyx mori. FEBS J. 276, 1221–1232. https://doi.org/10.1111/j.1742-
727   4658.2008.06859.x
728 Okamoto, N., Yamanaka, N., Yagi, Y., Nishida, Y., Kataoka, H., O'Connor, M.B., Mizoguchi, A.,
729   2009b. A fat body-derived IGF-like peptide regulates postfeeding growth in Drosophila.
730   Dev. Cell 17, 885–891. https://doi.org/10.1016/j.devcel.2009.10.008
731 O'Meara, G.F., 1985. Gonotrophic Interactions in Mosquitoes: Kicking the Blood-Feeding Habit.
732   Fla. Entomol. 68, 122–133. https://doi.org/10.2307/3494335
733 O'meara, G.F., Jr, C., B, G., 1969. Monofactorial inheritance of autogeny in Aedes atropalpus.
734   Mosq. News 29.
735 O'Meara, G.F., Krasnick, G.J., 1970. Dietary and Genetic Control of the Expression of
736   Autogenous Reproduction in Aedes Atropalpus (Coq.) (Diptera: Culicidae)1. J. Med.
737   Entomol. 7, 328–334. https://doi.org/10.1093/jmedent/7.3.328
738 Provost-Javier, K.N., Chen, S., Rasgon, J.L., 2010. Vitellogenin gene expression in autogenous
739   Culex tarsalis. Insect Mol. Biol. 19, 423–429. https://doi.org/10.1111/j.1365-
740   2583.2010.00999.x
741 R Core Team, 2016. R: A language and environment for statistical computing. R Foundation for
742   Statistical Computing.
743 Riehle, M.A., Fan, Y., Cao, C., Brown, M.R., 2006. Molecular characterization of insulin-like
744   peptides in the yellow fever mosquito, Aedes aegypti: expression, cellular localization,
745   and phylogeny. Peptides 27, 2547–2560. https://doi.org/10.1016/j.peptides.2006.07.016
746 Riehle, M.A., Garczynski, S.F., Crim, J.W., Hill, C.A., Brown, M.R., 2002. Neuropeptides and
747   Peptide Hormones in Anopheles gambiae. Science 298, 172–175.
748   https://doi.org/10.1126/science.1076827
749 Ronquist, F., Huelsenbeck, J.P., 2003. MrBayes 3: Bayesian phylogenetic inference under
750   mixed models. Bioinformatics 19, 1572–1574.
751   https://doi.org/10.1093/bioinformatics/btg180
752 Roubaud, E., 1929. Autogenous Cycle of Winter Generations of Culex pipiens L. C. r. Acad. Sci.

188.

Roy, S., Smykal, V., Johnson, L., Saha, T.T., Zou, Z., Raikhel, A.S., 2016. Chapter Five - Regulation of Reproductive Processes in Female Mosquitoes, in: Raikhel, Alexander S. (Ed.), Advances in Insect Physiology, Progress in Mosquito Research. Academic Press, pp. 115–144. https://doi.org/10.1016/bs.aiip.2016.05.004

Roy, S.G., Hansen, I.A., Raikhel, A.S., 2007. Effect of insulin and 20-hydroxyecdysone in the fat body of the yellow fever mosquito, Aedes aegypti. Insect Biochem. Mol. Biol. 37, 1317–1326. https://doi.org/10.1016/j.ibmb.2007.08.004

Schneider, C. A., Rasband, W. S., Eliceiri, K. W., 2012. NIH Image to ImageJ: 25 years of image analysis. Nat. Methods, 9(7), 671–675.

Sharma, A., Nuss, A.B., Gulia-Nuss, M., 2019. Insulin-Like Peptide Signaling in Mosquitoes: The Road Behind and the Road Ahead. Front. Endocrinol. 10. https://doi.org/10.3389/fendo.2019.00166

Sheng, Z., Xu, J., Bai, H., Zhu, F., Palli, S.R., 2011. Juvenile Hormone Regulates Vitellogenin Gene Expression through Insulin-like Peptide Signaling Pathway in the Red Flour Beetle, Tribolium castaneum. J. Biol. Chem. 286, 41924–41936. https://doi.org/10.1074/jbc.M111.269845

Sim, C., Denlinger, D.L., 2009. A shut-down in expression of an insulin-like peptide, ILP-1, halts ovarian maturation during the overwintering diapause of the mosquito Culex pipiens. Insect Mol. Biol. 18, 325–332. https://doi.org/10.1111/j.1365-2583.2009.00872.x

Spielman, A., 2001. Structure and seasonality of nearctic Culex pipiens populations. Ann. N. Y. Acad. Sci. 951, 220–234. https://doi.org/10.1111/j.1749-6632.2001.tb02699.x

Spielman, A., 1957. The inheritance of autogeny in the Culex pipiens complex of mosquitoes.. Am. J. Epidemiol. 65, 404–425. https://doi.org/10.1093/oxfordjournals.aje.a119878

Spielman, A., Wong, J., 1973. Environmental Control of Ovarian Diapause in Culex pipiens. https://doi.org/10.1093/AESA/66.4.905

Telang, A., Li, Y., Noriega, F.G., Brown, M.R., 2006. Effects of larval nutrition on the endocrinology of mosquito egg development. J. Exp. Biol. 209, 645–655. https://doi.org/10.1242/jeb.02026

Telang, A., Rechel, J.A., Brandt, J.R., Donnell, D.M., 2013. Analysis of ovary-specific genes in relation to egg maturation and female nutritional condition in the mosquitoes Georgecraigius atropalpus and Aedes aegypti (Diptera: Culicidae). J. Insect Physiol. 59, 283–294. https://doi.org/10.1016/j.jinsphys.2012.11.006

Telang, A., Wells, M.A., 2004. The effect of larval and adult nutrition on successful autogenous egg production by a mosquito. J. Insect Physiol. 50, 677–685. https://doi.org/10.1016/j.jinsphys.2004.05.001

Trpis, M., 1978. Genetics of hematophagy and autogeny in the Aedes scutellaris complex (Diptera: Culicidae). J. Med. Entomol. 15, 73–80. https://doi.org/10.1093/jmedent/15.1.73

Tsuji, N., Okazawa, T., Yamamura, N., 1990. Autogenous and Anautogenous Mosquitoes: a Mathematical Analysis of Reproductive Strategies. J. Med. Entomol. 27, 446–453. https://doi.org/10.1093/jmedent/27.4.446

Van Hiel, M.B., Van Wielendaele, P., Temmerman, L., Van Soest, S., Vuerinckx, K., Huybrechts, R., Broeck, J.V., Simonet, G., 2009. Identification and validation of housekeeping genes in brains of the desert locust Schistocerca gregaria under different developmental conditions. BMC Mol. Biol. 10, 56. https://doi.org/10.1186/1471-2199-10-56

Vinogradova, A.B., 2000. Culex Pipiens Pipiens Mosquitoes: Taxonomy, Distribution, Ecology, Physiology, Genetics, Applied Importance and Control. Pensoft Publishers, Sofia.

Vogel, K.J., Brown, M.R., Strand, M.R., 2015. Ovary ecdysteroidogenic hormone requires a receptor tyrosine kinase to activate egg formation in the mosquito Aedes aegypti. Proc.

804   Natl. Acad. Sci. 112, 5057–5062. https://doi.org/10.1073/pnas.1501814112
805   Wheeler, D.E., Frederik Nijhout, H., 1983. Soldier determination in Pheidole bicarinata: Effect of
806   methoprene on caste and size within castes. J. Insect Physiol. 29, 847–854.
807   https://doi.org/10.1016/0022-1910(83)90151-8
808   Yurchenko, A.A., Masri, R.A., Khrabrova, N.V., Sibataev, A.K., Fritz, M.L., Sharakhova, M.V.,
809   2020. Genomic differentiation and intercontinental population structure of mosquito
810   vectors Culex pipiens pipiens and Culex pipiens molestus. Sci. Rep. 10.
811   https://doi.org/10.1038/s41598-020-63305-z
812   Zou, Z., Saha, T.T., Roy, S., Shin, S.W., Backman, T.W.H., Girke, T., White, K.P., Raikhel, A.S.,
813   2013. Juvenile hormone and its receptor, methoprene-tolerant, control the dynamics of
814   mosquito gene expression. Proc. Natl. Acad. Sci.
815   https://doi.org/10.1073/pnas.1305293110
816
817
818

819 **Tables**

820

821 **Table 1: Mean wing length is positively correlated with nutrient availability in larval *Cx.***
822 ***pipiens.*** Means and standard deviations were calculated using one wing for 12 individuals per
823 form, per diet treatment. Correlations between wing length and larval nutrition were determined
824 using a Bayesian generalized linear model (Tables S3 & S4).

825

Form	Diet Treatment	Mean Wing Length (µm)	Standard Deviation
Cx. pipiens form *molestus*	High	34.3	2.1
	Medium	32.2	1.0
	Low	30.6	1.1
	Extra Low	28.4	0.9
Cx. pipiens form *pipiens*	High	36.6	1.5
	Medium	35.1	1.6
	Low	33.2	1.5
	Extra Low	31.6	1.4

826

827   **Figures**

828
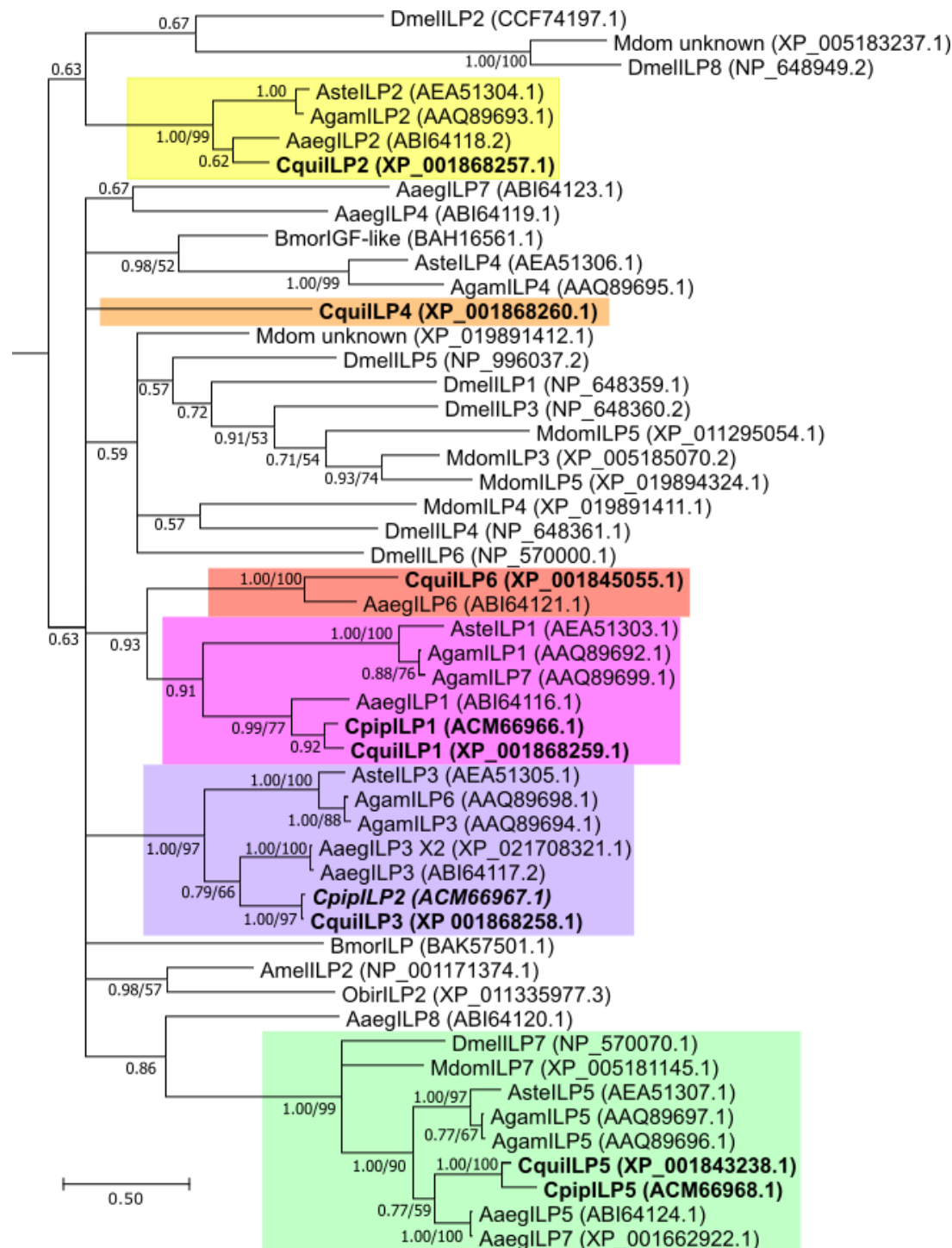
829   **Figure 1: Phylogenetic Tree of Dipteran ILPs establishes *Culex ilp* orthology.** Six distinct

830   *Culex ilp* genes (bold) were assigned orthology based on clustering with their closest homologs

831   in other species, indicated by colored boxes around each clade. *Cqui-ilp4* is a lineage-specific

832   gene with no homology to other Dipteran *ilp* genes (orange box). Only *Cqui-ilp5* has well

833   supported orthology to a *Dmel-ilp* gene (*ilp7*) (green box). Tree topology determined by TOPALI

834   v2.5 using a MrBayes algorithm. Posterior probabilities listed at nodes to indicate statistical

835   support. When the node was also supported by a maximum likelihood tree (>50) (TOPALI,

836   PhyML algorithm, JTT + G, 100 bootstrap runs), the bootstrap support is listed as the second

837   value at the node. Genbank accession numbers listed next to gene names.

838
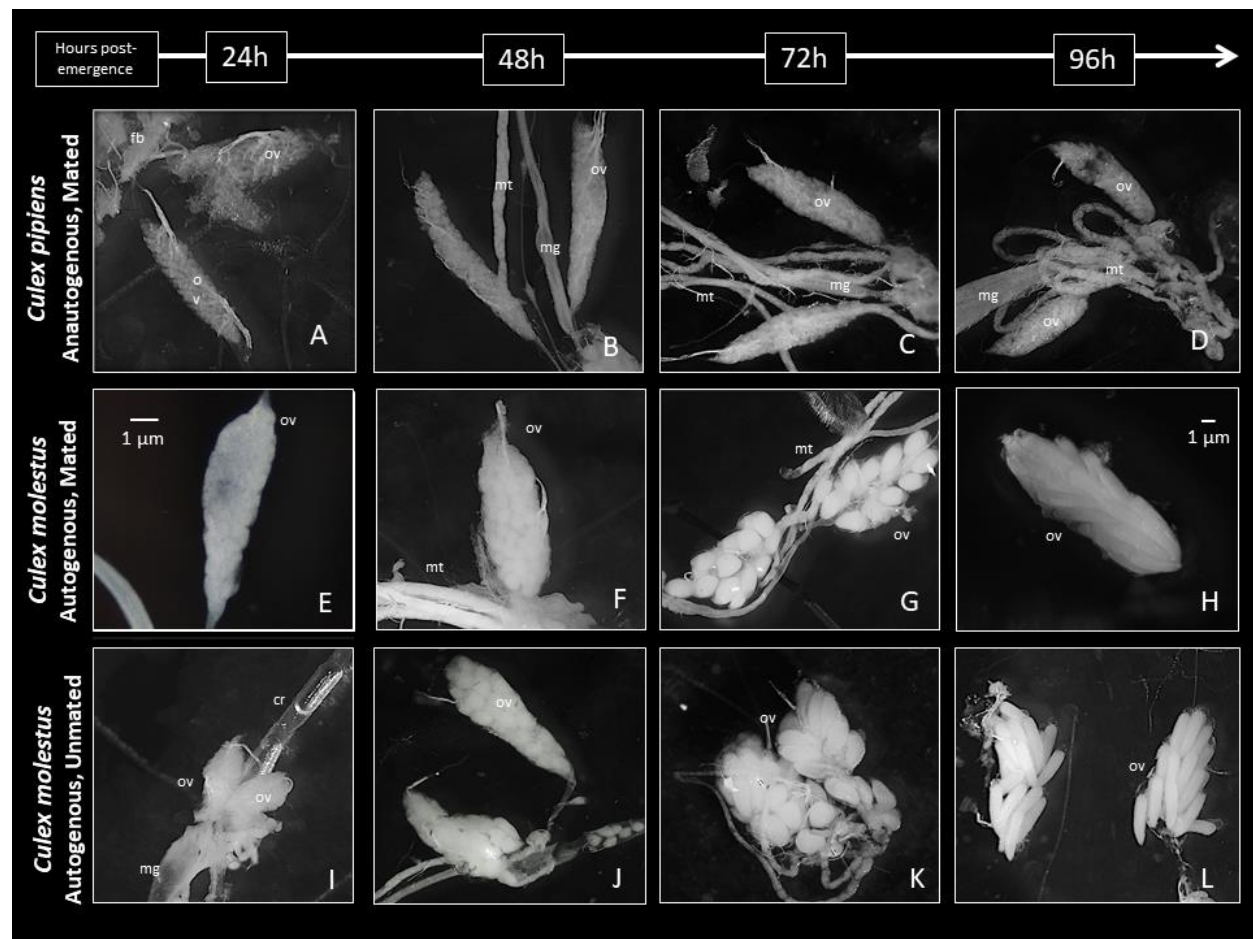
Figure 2: Ovarian development up to 96 hours post adult emergence in anautogenous
***pipiens* and autogenous *molestus* females.** Panels A-D depict ovarian development in
anautogenous, form *pipiens* females in which follicles remain in resting stage (Christopher's
Stage - IIb) until a blood meal is taken. Panels E-H depict autogenous ovarian development for
form *molestus* females provided the opportunity to mate. Panels I-L show ovarian development
in autogenous *molestus* females denied a mating opportunity. 1 μm scale shown in panels E
and H for reference. ov, ovary; mg, midgut; fb, fatbody; mt, malpighian tubules; cr, crop.

846
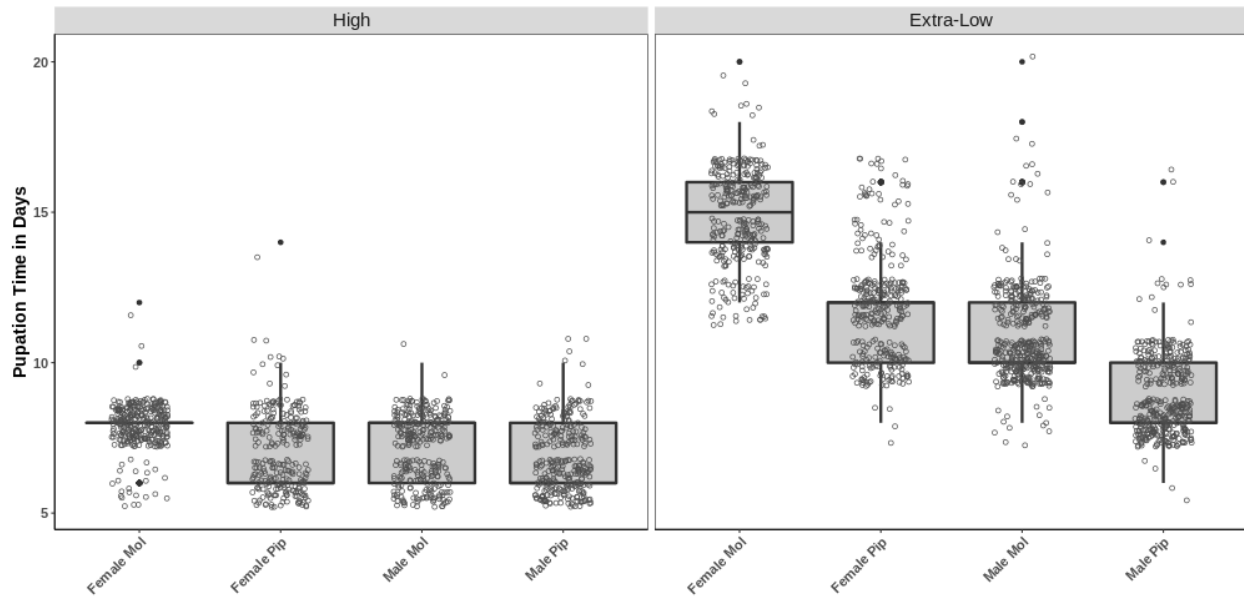
Figure 3: Days until pupation, per treatment, for *molestus* and *pipiens*. Males and females are shown separately. Unfilled points represent observations for individual larvae, while filled points represent outlier observations (1.5X greater or less than the interquartile range).
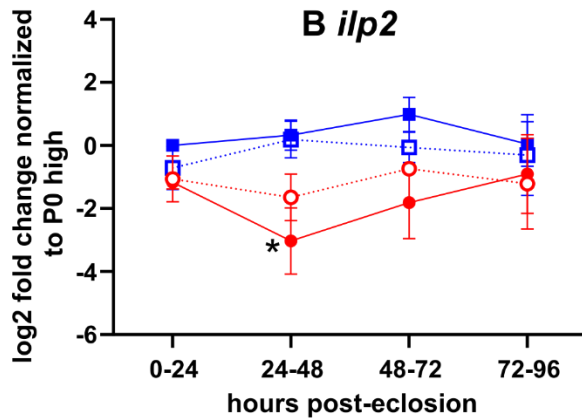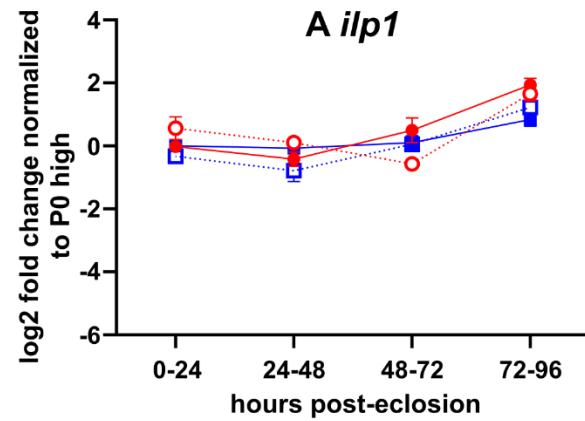
850
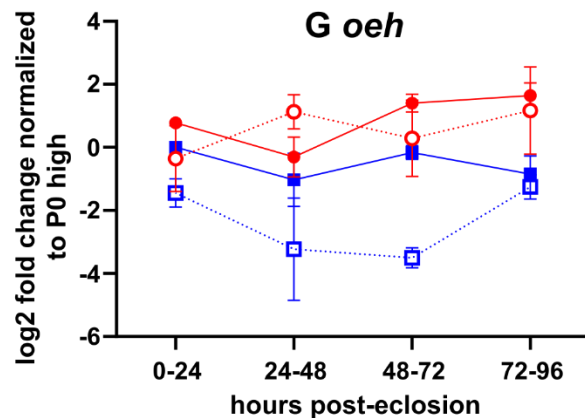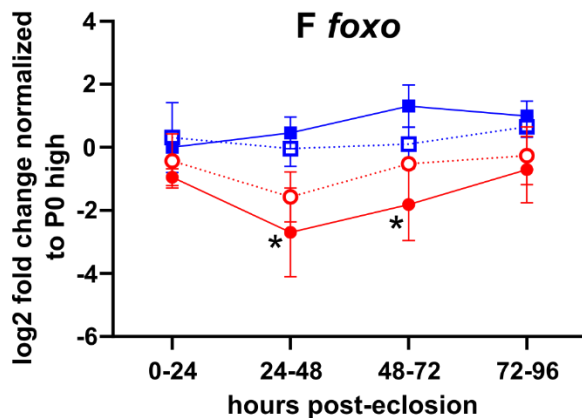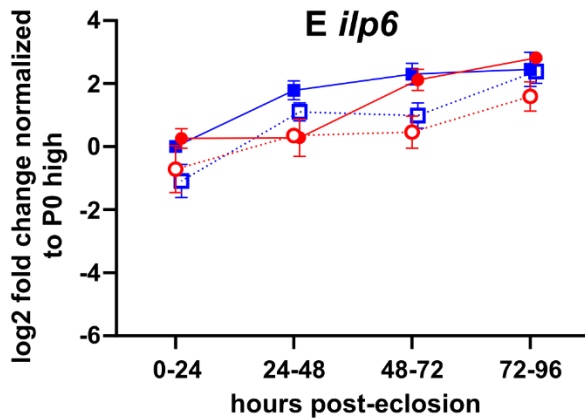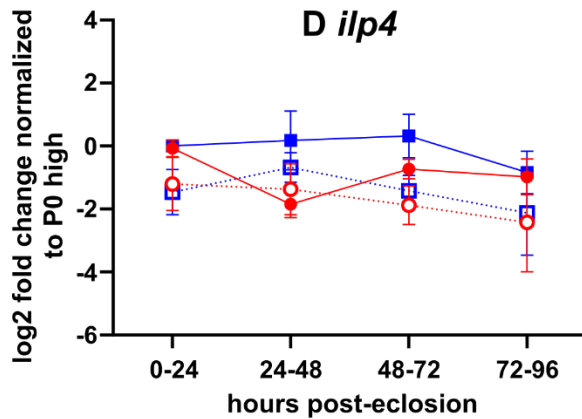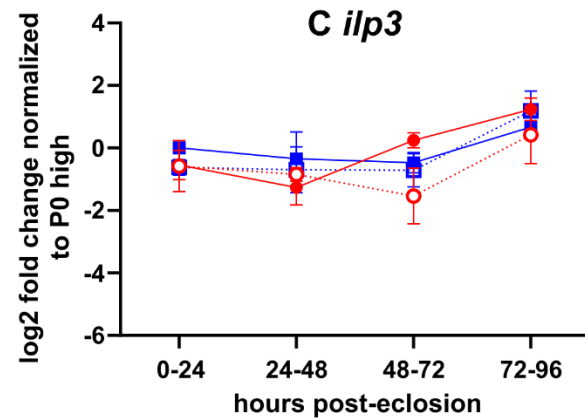
851 **Figure 4: Downregulation of *ilp2* and *foxo* in head tissues are correlated with autogeny**

852 **phenotype.** qPCR was used to determine whether expression of *ilp1* (A), *ilp2* (B), *ilp3* (C), *ilp4*

853 (D), *ilp6* (E), *foxo* (F), and *oeh* (G), differ between bioforms and feeding conditions over the first

854 four days of adult development, when autogenous egg production occurs in *molestus*. Only *ilp2*

855 (B) and *foxo* (F) are significantly differentially expressed, as determined by a two-way ANOVA

856 of log2 fold change values. p values are indicated by *s as presented in the key. Fold change

857 gene expression determined by ΔΔCt method, normalizing first to housekeeping gene *EF1a*,

858 then to *pipiens* high food 0-24 hr reference sample. Data are shown on a log2 scale such that

859 "0" means no change vs. the reference sample. "High" and "low" indicate diet types used in

860 larval rearing. Error bars indicate SEM. Each data point is the mean of 3-4 biological
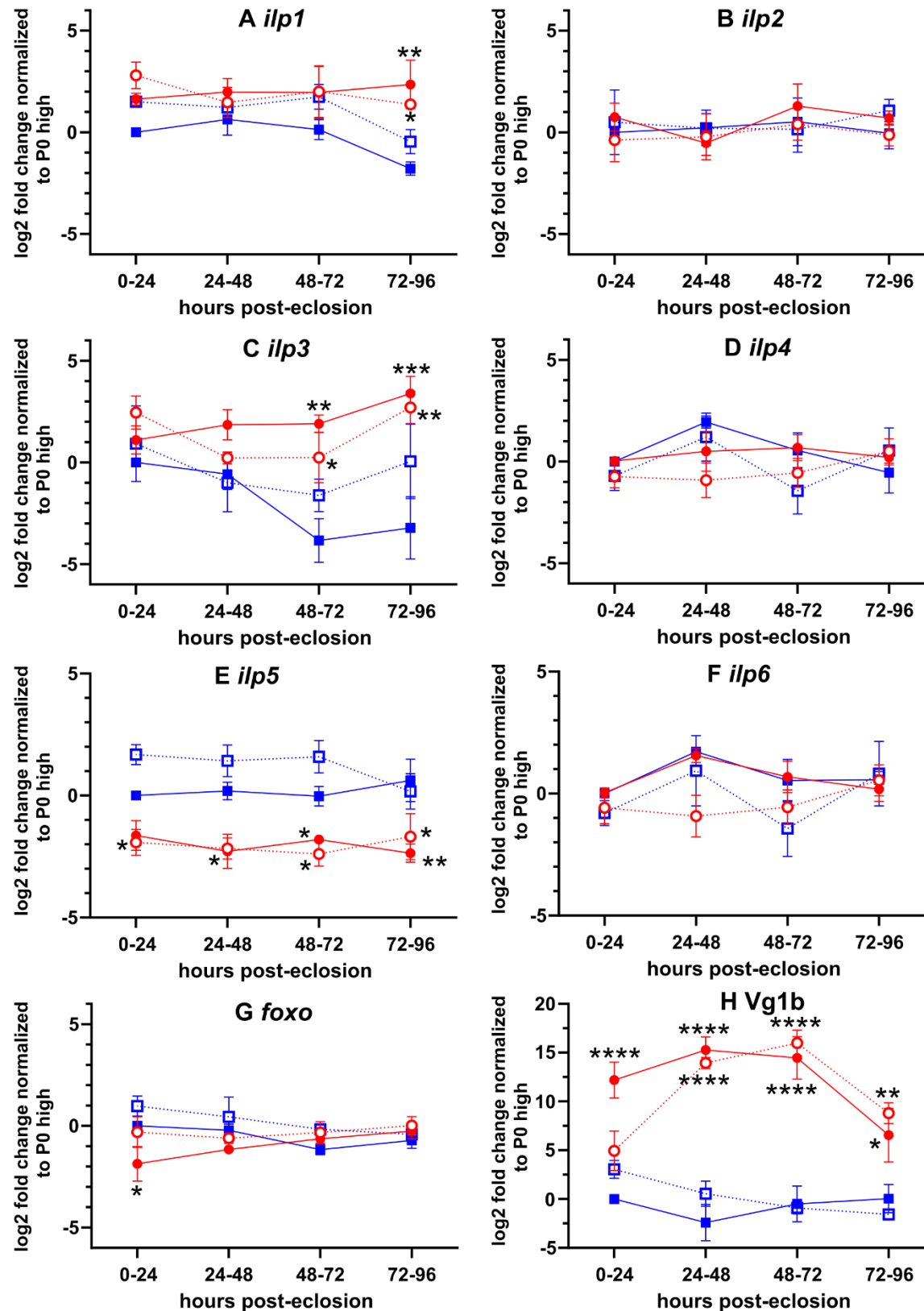
861 replicates.

862

863 **Figure 5: Multiple gene expression differences in the abdomen correlate with autogeny**

864 **phenotype when biotypes and feeding conditions are compared.** qPCR was used to

865 determine whether expression of *ilp1* (A), *ilp2* (B), *ilp3* (C), *ilp4* (D), *ilp5* (E), *ilp6* (F), *foxo* (G),

866 and *Vg1b* (H) differ between bioforms and feeding conditions over the first four days of adult

867 development, when autogenous egg production occurs in *molestus. ilp1* is upregulated in

868 *molestus* at 72-96 hrs post-eclosion (A). *ilp2* expression is stable between bioforms, feeding

869 conditions and time points (B). *ilp3* is upregulated in molestus from 48-96 hrs post-eclosion (C).

870 *ilp4* expression is stable between bioforms, feeding conditions and time points (D). *ilp5* is

871 significantly downregulated at all timepoints in *molestus*, irrespective of feeding condition (E).

872 *ilp6* expression is stable between bioforms, feeding conditions and time points (F). *foxo* is

873 initially downregulated in well-fed *molestus* (G). *Vg1b* is enormously upregulated in molestus,

874 but with a slight delay in onset in low-food conditions (H). p values are indicated by *s as

875 presented in the key. Fold change gene expression determined by ΔΔCt method, normalizing

876 first to housekeeping gene *EF1a*, then to *pipiens* high food 0-24 hr reference sample. Data are

877 shown on a log2 scale such that "0" means no change vs. the reference sample. "High" and

878 "low" indicate diet types used in larval rearing. Error bars indicate SEM. Each data point is the

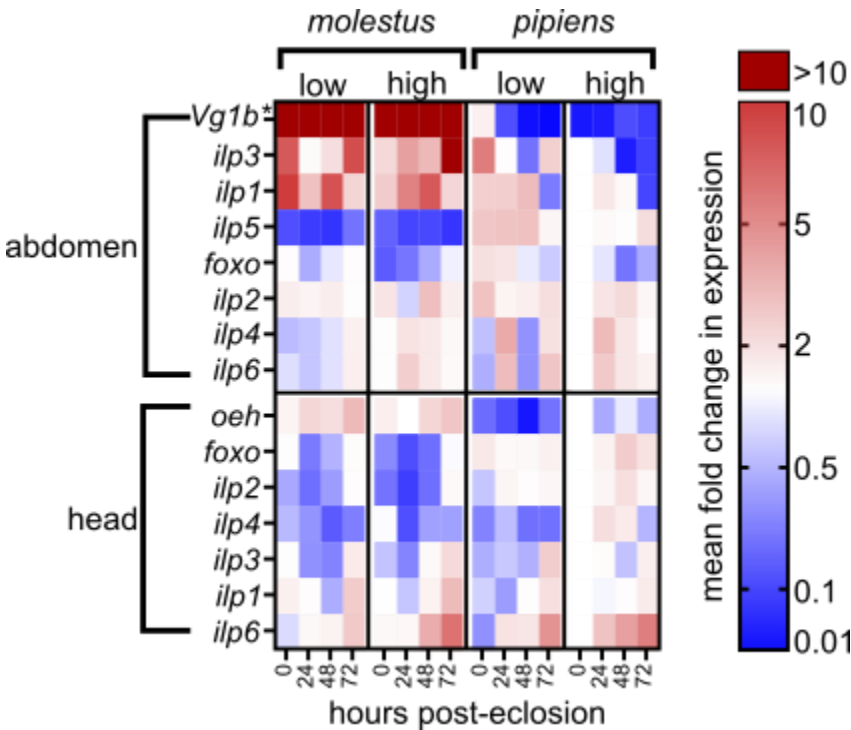879 mean of 3-4 biological replicates.

880

881

Figure 6: Heat Map Summary of Fold Change Gene Expression. Data from Figures 4 and 5 shown as fold changes in expression (as opposed to log2 scale in original figures) and are grouped to show genes that are up and downregulated during autogenic egg production (listed first in each grouping) vs. those that remain stable across forms (listed last in each grouping). *Vg1b* expression is shown as 1/100th of actual fold change values to allow for visualization on the same scale as other measured genes.