Contact CEH NORA team at
noraceh@ceh.ac.uk

Flight-induced transgenerational maternal effects influence butterfly offspring performance during times of drought

3

4 MELANIE GIBBS[1*], HANS VAN DYCK[2] & CASPER J. BREUKER[3]

5

6 [1]NERC Centre for Ecology & Hydrology, Maclean Building, Crowmarsh Gifford,

7 Wallingford, OX10 8BB, UK

8 [2]Behavioural Ecology & Conservation Group, Biodiversity Research Centre,

9 Earth and Life Institute, Université catholique de Louvain (UCL), Louvain-la-

10 Neuve, Belgium

11 [3]Evolutionary Developmental Biology Research Group, Faculty of Health and

12 Life Sciences, Department of Biological and Medical Sciences, Oxford Brookes

13 University, Gipsy Lane, Headington, Oxford OX3 0BP, UK

14

15 * Corresponding author: mela1@ceh.ac.uk,

16

17 Running title: Maternal flight affects offspring performance during drought

18

22

Abstract

Maternal condition can generate resource-related maternal effects through differential egg provisioning that can negatively affect offspring performance especially when offspring growth occurs in stressful or sub-optimal environments. Using the Speckled Wood butterfly, *Pararge aegeria* (L.) we tested the hypothesis that repeated periods of intensive flight during female oviposition affects egg provisioning and reduces offspring performance when larval development occurs under stressful conditions on drought stressed host plants. We investigated whether (after controlling for egg size) maternal age and flight treatment resulted in changes in egg provisioning and whether this contributed to variation in offspring traits across life stages. Age-related changes in maternal condition were found to generate resource-related maternal effects that influenced offspring traits across all life stages. Flight-induced changes in maternal egg provisioning were found to have direct consequences for offspring development in the egg and larval stages.. There were significant interactive effects between maternal age and flight on larval development and growth. Compared to offspring from forced flight mothers, offspring from control (no forced flight) mothers that hatched from eggs laid early in the oviposition period (i.e. by younger mothers) had shorter larval development times and heavier pupal masses, suggesting that offspring from mothers in relatively good condition may be able to buffer some of the costs associated with growth on drought stressed host plants. Our multi-factor study demonstrates the importance of considering the various, and often interacting, mechanisms by which maternal effects may influence offspring performance in stressful environments.

Key-words: Egg quality, embryogenesis, life history, non-genetic effects, rainfall

Introduction

Maternal effects constitute a form of non-genetic transmission of environmental conditions across generations, and there is a growing realisation that maternal effects may have both short- and long-term consequences for population dynamics (Bitume et al. 2014; Uller 2008), particularly in rapidly changing environments, such as those found in fragmented landscapes (Gibbs and Van Dyck 2009). Maternal effects are not always adaptive (Marshall and Uller 2007; Uller 2008), and a key factor determining whether transgenerational maternal effects influence offspring performance positively or negatively is how well mothers are able to predict the environment of their offspring (Guillaume et al. 2016). Global climate change, particularly an increase in climatic extreme events such as drought (IPCC 2014), is likely to reduce the ability of mothers to accurately predict the environment of their offspring (Guillaume et al. 2016), and recent modelling work suggests that rapidly changing, unpredictable environments, actually selectively favour negative maternal effects (Ezard et al. 2014; Kuijper et al. 2014; Kuijper and Hoyle 2015). In nature, both mothers and offspring are likely to be subjected to multiple biotic and abiotic factors simultaneously (Carroll and Quiring 1993), and mothers and offspring may not always be subjected to the same factors, particularly in fast changing environments, further reducing the ability of mothers to accurately predict the environment of their offspring. Empirical studies focusing on the transmission of non-genetic developmental factors in response to ecologically relevant global change stressors are therefore essential to generate a better understanding of species responses to changing environments (Uller et al. 2013; Guillaume et al. 2016).

Ectotherms, like butterflies, are very sensitive to (changes in) their environment, but the influence of global change stressors such as temperature or

73 drought, on transgenerational effects in butterflies has been understudied to date

74 (Woestmann and Saastamoinen 2016). Consideration of the impacts of interactions

75 between multiple factors on transgenerational effects in butterflies is rare, while an

76 assessment of the impact of transgenerational effects on adult traits of the offspring

77 generation is also largely lacking (Woestmann and Saastamoinien 2016; but see

78 Cahenzli and Erhardt 2013).

79   Across various species (including butterflies), habitat fragmentation has been

80 shown to either increase or decrease mobility depending on both the spatial

81 configuration of key resources such as host plants for oviposition, as well as the costs

82 associated with dispersal in any given system (Bonte et al. 2012; Gibbs and Van Dyck

83 2009; Van Dyck and Baguette 2005). One classic example of a life-history

84 physiological trade-off that has attracted much attention for several decades (e.g.

85 Johnson 1969), and is of relevance when considering the costs of dispersal in

86 fragmented landscapes, is the relationship between flight and fecundity (i.e.

87 oogenesis-flight syndrome, Johnson 1969). Initially, there was a particular focus on

88 the quantitative aspects of fecundity (i.e. number of eggs and egg size), but there has

89 been a growing interest to expand this work to qualitative aspects as well (e.g. egg

90 composition), and hence, to the role of maternal effects in this context (Awmack and

91 Leather 2002). In our study species the Speckled Wood butterfly, *Pararge aegeria*

92 (L.), females in fragmented landscapes have a high fecundity strategy, laying larger

93 numbers of smaller-sized eggs; a strategy which is associated with increased flight

94 and a decrease in the time and resources a female allocates to searching for optimal

95 host plants and microsites for oviposition (Gibbs and Van Dyck 2009; Gibbs and Van

96 Dyck 2010).

4

97   *Pararge aegeria* is a drought-sensitive butterfly species, particularly in the

98   larval stage when growth occurs on drought stressed host plants (Talloen et al. 2004;

99   Gibbs et al. 2012; Vande Velde et al. 2013), and several studies have shown that

100   population recovery from drought events is significantly slower for populations of this

101   species in fragmented landscapes (Pollard 1988; Schweiger et al. 2006; Oliver et al.

102   2015). Currently, few studies have explored the mechanisms underlying this drought

103   by habitat fragmentation interaction effect on population dynamics (but see Gibbs et

104   al. 2012), and currently it is unclear why populations in fragmented landscapes are

105   more vulnerable to drought. The role that flight-induced transgenerational maternal

106   effects may play in the response of offspring to drought has not been examined.

107   In this study we tested the hypothesis that repeated periods of intensive flight

108   during female oviposition affect egg provisioning and reduces offspring performance

109   when larval development occurs on drought stressed host plants. Our previous studies

110   have shown that in *P. aegeria*, changes in resource allocation to egg size and

111   composition in response to flight is dependent on maternal age (Gibbs et al. 2010a;

112   Gibbs et al. 2010b; Gibbs et al. 2010c). As such, in order to adequately assess the

113   impact of increased flight during oviposition on subsequent offspring performance it

114   is necessary to consider the interaction between these two intrinsic maternal factors

115   simultaneously. To do this we created two maternal flight treatment groups; i) females

116   that are forced to fly repeatedly during the oviposition period (referred to here as

117   forced flight females, and ii) females that are not forced to fly during the oviposition

118   period (here referred to as control females) (Gibbs et al. 2010a,c), and a sub-sample of

119   the eggs laid by mothers on days 2, 4, 6, 8 and 10 days of oviposition were collected

120   and used to assess the impact of both maternal age and flight treatment on subsequent

121   offspring performance. As has also been observed in other species (e.g. Bernardo

122   1996a; and for temperate butterflies in particular Karlsson and Wiklund 1984;

123   Karlsson and Wiklund 1985; Wickman et al. 1990), from previous work with *P.*

124   *aegeria* we know that benign laboratory test conditions can mask the maternal

125   nutritional effects of egg size and composition (Wiklund and Persson 1983; Wiklund

126   and Karlsson 1984). To ensure that our offspring larval tests conditions were not too

127   benign, we therefore reared our offspring on drought stressed host plants to create a

128   stressful, but ecologically relevant growth environment (after Talloen et al. 2004;

129   Gibbs et al. 2012; Vande Velde et al. 2013). This design enabled us to investigate

130   whether, after controlling for egg size, maternal age *per se*, and increased flight

131   during the oviposition period influences offspring performance across multiple life

132   stages; i) early in development during the egg stage, ii) during larval development on

133   drought-stressed host plants, and iii) in the adult stage.

134

135   **Materials and Methods**

136

137   **Study species**

138   *Pararge aegeria* is a temperate-zone species, primarily found in woodland, but in

139   north-west Europe this species has colonized agricultural landscapes containing small,

140   widely distributed 'woodland' fragments along hedgerows (Dover and Sparks 2000;

141   Merckx et al. 2003). Eggs are laid singly on grass species from the family Poaceae

142   (Shreeve 1986). Egg size and egg hatching success declines with female age due to a

143   decline in maternal resources over time (Gibbs et al. 2010a; Wiklund and Karlsson

144   1984; Wiklund and Persson 1983).

145

146   **Maternal treatment**

147   Our experimental females were derived from an outbred laboratory stock population,

148   which originated from a deciduous continuous woodland landscape in St. Hubert,

149   Belgium. Newly hatched F_2 generation larvae from this population were placed in

150   pairs on potted host plants of *Poa trivialis* (grown under standard conditions from

151   commercially produced seed provided by Limagrain Advanta Belgium) and reared in

152   a climate room under a direct development regime in common garden conditions (24

153   ^{o}C, LD 16:8, RH 60 ± 5 %) until eclosion. On the day of eclosion (between 9 and

154   12hr; from now on called day -1) 32 females were individually weighed (AMD

155   Instrument Ltd balance; accuracy: ± 0.1 mg) and each female was placed in a netted

156   cage (0.5 m^3) along with a potted *P. trivialis* for oviposition and an artificial flower

157   containing a 10% honey solution (for design see Cory and Goulson 1993). *Pararge*

158   *aegeria* females actively feed from artificial flowers and this feeding method has been

159   routinely adopted in laboratory experiments (e.g. Gibbs et al. 2010a,b; Gibbs et al.

160   2005). Later the same day (between 13 and 16hr) a one-day-old randomly chosen

161   non-sibling male (originating from the same laboratory source population) was

162   introduced to the cage and, after mating, the pair was left undisturbed for 24 hours.

163   *Forced flight treatment*: On the day after mating (from now on called day 0),

164   females were assigned to one of two treatment groups: control or forced flight.

165   Control females were left undisturbed in their cages until the first eggs were laid (i.e.

166   day 1 of oviposition). When the first eggs were laid, the male was removed from the

167   cage and the female was left to continue laying. On the day after mating (day 0),

168   forced flight females were removed from cages and forced to fly continuously for 5

169   min at 24°C. These forced flight females were placed individually into an empty

170   netted cage (0.5 m^3) and stimulated to fly by gently touching their legs with a fine-

171   bristled paintbrush each time they alighted (after Gibbs et al. 2010a,b,c; Gibbs and

7

172   Van Dyck 2010). After forced flight, the females were returned to their mating cages

173   until the first eggs were laid (i.e. day 1 of oviposition). When the first eggs were laid,

174   the male was removed from the cage and the female was left undisturbed in the cage

175   to continue egg laying. Forced flight treatment was repeated on days 4 and 8 of

176   oviposition, to give a total of 3 periods of intensive flight treatment during

177   oviposition. In total, 17 control females and 15 forced flight females were set-up. All

178   32 females mated successfully, began ovipositing 48hr after mating and laid viable

179   eggs.

180   *Egg collection:* Each morning, from day 1 of oviposition until female death,

181   between 9 am and 11 am all cages were inspected for eggs, the host plant was watered

182   and fresh honey solution was provided via artificial flowers to ensure a permanent

183   food source for the ovipositing females. The number of days between female eclosion

184   as an adult and death was used as a measure of longevity. Eggs laid by each female

185   were collected and counted each day. The total number of eggs laid during a female's

186   life was used as a measure of lifetime fecundity. All of the eggs laid by each female

187   were kept and used to monitor egg hatching success. The total lifetime egg hatching

188   success was recorded for each female, and this measure included all of the eggs laid

189   by each female, even the eggs used to measure offspring performance (see below).

190

191   **Offspring performance**

192   *Egg stage*: To determine how female flight treatment, female age and egg size

193   influence embryonic performance, for each female, on days 2, 4, 6, 8 and 10 days of

194   oviposition a sub-sample of 5 eggs (from the total number of eggs available on that

195   day) were randomly selected and placed into separate Petri dishes to be photographed

8

196   for egg size measurements. If fewer than 5 eggs were laid on one day, then all of the

197   eggs that had been laid were kept for egg size measurements. In total 779 eggs were

198   collected and individually measured. *Pararge aegeria* eggs are spherical and the size

199   of each egg could therefore be measured as a cross-sectional projection (mm^2) using a

200   digital camera (Canon A720 IS). The resulting images were then analysed using

201   Image J (freely available at http://rsb.info.nih.gov/ij/)(Abramoff et al. 2004). This

202   method is a highly reliable measure of egg size in *P. aegeria* as there is a strong

203   correlation between egg area and egg mass in this species (cf. Bauerfeind and Fischer

204   2008). After the eggs were photographed, each egg was placed individually into a

205   labelled Eppendorf (sealed with a small piece of fine netting secured with an elastic

206   band) and monitored each day for hatching. For each egg we recorded whether it

207   survived to hatch or not, and these data were included in the lifetime hatching success

208   analyses described previously. For eggs that hatched successfully, the total number of

209   days between being laid and hatching was used as a measure of embryonic

210   development time, and used to examine how maternal age and flight treatment

211   influence early offspring development.

212   *Larval stage*: To determine how female flight treatment, female age and egg

213   size influence larval performance on drought stressed host plants, on the day of

214   hatching, F_3 generation larvae from each of our 32 females, from each day of

215   oviposition were individually reared on a drought stressed host plant. In total 187

216   larvae were set-up (2-10 individuals/female, mean = 5.8 ± 0.3). Due to fewer eggs

217   hatching from eggs laid on days 8 and 10 of oviposition, smaller numbers of larvae

218   were reared from these treatment groups; in the control maternal treatment 20, 25, 20,

219   15 and 12 larvae from days 2, 4, 6, 8 and 10 of oviposition respectively were reared,

220   and in the forced flight treatment group 21, 21, 24, 17 and 12 larvae from days 2, 4, 6,

221   8 and 10 of oviposition respectively were reared. Drought-stressed plants were

222   deprived of water for 20 days prior to the start of the experiment (i.e. 20 days before

223   larval hatching) and then were subsequently only provided with rain water once every

224   6 days throughout the experimental period. By only watering the plants every 6 days,

225   we ensured that the plants remained alive (and had leaves available for larval

226   consumption) but experienced moderate drought stress throughout the experimental

227   period (after Gibbs et al. 2012; Talloen et al. 2004). For each larva we recorded

228   development time from hatching to pupation, pupal mass, and whether it survived to

229   eclose as an adult or not.

230   *Adult stage*: On the day of eclosion each adult was sexed. Butterflies were

231   killed within 24 hours of emergence, after their wings had fully hardened, by placing

232   them in a -20°C freezer. Fore- and hindwings were carefully removed from the thorax.

233   The body of each individual was dried for 24 h at 60 °C, and then weighed (AMD

234   Instrument Ltd balance; accuracy: ± 0.1 mg). The thorax was then carefully removed

235   and weighed, and used as a measure of investment in flight muscle mass, which is

236   known to correlate with dispersal ability in *P. aegeria* (Hughes et al. 2003). These

237   data enabled us to examine the potential for flight-induced maternal effects to

238   generate long-lasting maternal effects that influence adult offspring dispersal capacity.

239

240   **Statistical analyses**

241   Linear and generalised linear mixed effect (lme or glme) models were fitted where

242   appropriate, by means of restricted maximum likelihood (REML), which produces

243   unbiased estimates of variance and covariance parameters, with female (i.e. mother)

244   being declared as a random factor. Likelihood ratio tests were conducted to compare

245   different models with each other. The final model only included significant

246   interactions. Residuals were examined for non-linearity in all cases and for non-

247   normality where appropriate. Analyses were performed in R 3.2.1 (packages nlme and

248   lme4; http://lib.stat.cmu.edu/R/CRAN/). Significances for REML constructed models

249   in R are estimated by means of t_{df} -values (lme) and z-values (glme). The sign of

250   either the t- or z-values is indicative of the relationship between the effect and the

251   dependent variable (i.e. positive or negative). All mean values are presented in the

252   text with ± Standard Error (SE).

253   *Female longevity, lifetime fecundity, mean lifetime egg size and mean lifetime*

254   *egg hatching success*: These data were analysed by means of a lme model. Fixed

255   factors were maternal age and flight treatment. Female body mass was used as a

256   covariate.

257   *Offspring development*: lme models were constructed to investigate how each

258   of the four offspring traits, embryonic development time (in days), post-hatching

259   development time (time between hatching from an egg to eclosion as an adult, in

260   days), pupal mass (mg) and dry thorax mass (mg) changed over the egg-laying period

261   (i.e. as a function of maternal age), and whether there were differences in these traits

262   between females that had been forced to fly and the controls (i.e. flight treatment was

263   a fixed effect). In these models, day of oviposition (fixed effect) was thus an indicator

264   of female age. Egg size (mm^2), which is known to affect offspring development

265   (Gibbs et al. 2010b), for each individually measured egg was used as a covariate, as

266   any relationship between offspring traits and maternal age may simply be due to the

267   fact that females lay differently sized eggs as they age. Larvae that developed to the

268   pupal stage could be sexed, and thus offspring sex was also added as fixed effect to

269   the models for post-hatching development time, pupal mass and thorax mass. To take

270   allometry into account, total dry mass was included as a covariate when analysing

11

271   investment in thorax mass (i.e. flight muscle mass), and a total dry body mass by

272   maternal interaction effect was also included to account for allometric changes due to

273   a decline in maternal egg provisioning as mothers age (i.e. offspring mass declines

274   with maternal age; Gibbs et al. 2010a).

275   *Survival to eclosion* (0 = dead, 1 = alive): was analysed using a lme model

276   with a logit link function (i.e. a logistic regression with random effects). Maternal

277   flight treatment was used as a fixed effect. Egg size and maternal age were used as

278   covariates. Offspring sex was not included in these models as offspring that did not

279   survive could not be sexed.

280

281   **Results**

282

283   **Effects of flight treatment on female longevity and reproductive output**

284   *Longevity*: Females forced to fly had significantly shorter lifespans (19%) than control

285   females (Control 14.47 ± 0.68 days, Forced Flight = 11.73 ± 1.0 days, t_{31} = -2.40, P =

286   0.02).

287   *Reproductive output*: Forced flight females laid significantly fewer eggs than

288   control females (Control = 159.4 ± 9.8, Forced Flight = 125.0 ± 14.3, t_{31} = -2.40, P =

289   0.02). There was no difference in mean egg size (Control = 0.76 ± 0.02 mm^2, Forced

290   Flight = 0.78 ± 0.03 mm^2, t_{31} = 0.82 P = 0.42) or mean egg hatching success across

291   treatments (Control = 0.63 ± 0.03, Forced Flight = 0.68 ± 0.04, t_{31} = 1.24, P = 0.23).

292   Female body mass did not significantly contribute to variation in the number of eggs

293   laid, egg size or egg hatching success, and this covariate was therefore removed from

294   all models. Forced flight and control females did not differ in body mass (Control =

295   78.9 ± 3.1 mg, Forced Flight = 71.1 ± 4.5 mg, t_{30} = -1.71, P = 0.10). There were no

296   significant interaction effects.

297

298   **Effects of flight treatment on early offspring development**

299   Maternal flight treatment, maternal age and egg size each significantly contributed to

300   variation in embryonic development time. Offspring from eggs laid by forced flight

301   mothers had shorter embryonic development times (Control = 6.74 ± 0.05 days,

302   Forced Flight = 6.57 ± 0.05 days, t_{32} = -2.45, P = 0.02). There was a negative

303   relationship between embryonic development time and the day of oviposition on

304   which the egg was laid, such that offspring from eggs laid later in the oviposition

305   period (i.e. by older mothers) had shorter embryonic development times (t_{475} = -2.34,

306   P = 0.02). There was a negative relationship between embryonic development time

307   and egg size, such that offspring from larger eggs had shorter embryonic development

308   times (t_{367} = -3.13, P = 0.002).

309

310   **Effects of maternal flight treatment on offspring performance on drought**

311   **stressed host plants**

312   *Pupal mass:* Offspring from control mothers had heavier pupal masses (Table 1; t_{105} =

313   -3.28, P = 0.001). There was a significant effect of maternal age on pupal mass (Table

314   1; t_{105} = -2.76, P = 0.007), and a significant maternal age by flight treatment

315   interaction effect (t_{105} = 3.1, P = 0.003; Figure 1). Offspring from eggs laid by control

316   older mothers had lighter pupal masses, but this maternal age-specific decline in

317   offspring pupal mass was not apparent in offspring from forced flight mothers (Figure

318   1). Offspring that hatched from larger-sized eggs had heavier pupal masses (t_{109} =

319 3.54, P = 0.0006). Male offspring had lighter pupae than female offspring (Male =

320 117.2 ± 2.7 mg, Female = 128.7 ± 3.1 mg; t_{105} = -2.73, P = 0.007).

321   *Post-hatching development time*: Offspring from mothers forced to fly had

322 longer development times (Table 1; t_{98} = 2.05, P = 0.04). Offspring from eggs laid by

323 older mothers had longer development times (t_{106} = 4.60, P < 0.0001). There was a

324 significant maternal flight treatment by maternal age interaction effect (t_{101} = -2.109,

325 P = 0.04; Figure 2). Compared to offspring from control mothers, offspring from

326 mothers forced to fly had longer development times when they hatched from eggs laid

327 early in the oviposition period, but shorter development times when they hatched

328 from eggs laid later in the oviposition period (Figure 2). Male offspring had shorter

329 development times than female offspring (Male = 39.7 ± 0.9 days, Female = 43.1 ±

330 0.9 days; t_{104} = -3.38, P = 0.001).

331   *Thorax mass*: Offspring thorax mass did not differ across maternal flight

332 treatment groups (t_{26} = 0.95, P = 0.35). Offspring that hatched from large eggs had

333 heavier thorax masses (t_{83} = 2.32, P = 0.02). Body mass scaled significantly with

334 flight muscle mass (t_{104} = 7.78, P < 0.0001), such that adults with heavier body

335 masses had higher resource investment to flight muscle mass. However, the nature of

336 the (allometric) scaling between adult body mass and investment in flight muscle

337 mass is dependent on the age of the mother at the time of egg laying (i.e. a significant

338 adult body mass by maternal age interaction effect; t_{104} = 2.87, P = 0.005). That is,

339 although offspring that hatched from eggs laid by old mothers had lighter thorax

340 masses (t_{104} = -2.08 P = 0.04), the precise slope of the regression line between adult

341 body mass and investment in flight muscle mass depended on maternal age. Male

342 offspring allocated relatively more resources to thorax mass, and hence flight muscle

343   mass, than females (Male = 5.8 ± 0.1 mg, Female = 4.8 ± 0.1 mg; t_{104} = 7.03, P <

344   0.0001).

345   *Survival to eclosion*: There was no difference in survival across maternal flight

346   treatments (z = -0.40, P = 0.69). There was no effect of egg size (z = 1.40, P = 0.16)

347   or maternal age (z = -1.80, P = 0.07) on survival to eclosion.

348

349   **Discussion**

350   Intensive maternal flight during oviposition directly influenced the performance of

351   offspring on drought-stressed host plants. Offspring from forced flight mothers had

352   significantly longer development times and smaller pupal masses. Given that there

353   were no differences in egg size across flight treatment groups, these results strongly

354   indicate egg-provisioning differences between flight treatment groups that are not

355   related to egg size *per se* (cf. Gibbs et al. 2010a). As far as we are aware, this is the

356   first study to show that flight-induced changes in maternal egg provisioning can

357   influence the performance of offspring when growth occurs on drought stressed host

358   plants. These effects have the potential to manifest across generations because small-

359   sized adults have lower investment in reproduction, i.e. smaller spermatophore size in

360   males and lower egg quality in females (Gibbs et al. 2012; Vande Velde et al. 2013).

361   We did not find, however, any effect of maternal flight treatment on offspring

362   investment to adult flight muscle mass. Male offspring allocated relatively more

363   resources to flight muscle mass than females. These sex differences in resource

364   allocation patterns may reflect differences in life history (Van Dyck 2003). Males and

365   females differ in their resource allocation patterns to flight morphological traits (e.g.

366   thoracic mass, and hence flight muscle mass), with males investing in fast

367   acceleration take-off flights used during territorial fights (Berwaerts et al., 2002;

368   Berwaerts et al., 2008; Vande Velde and Van Dyck, 2013). Overall, our data suggest

369   that flight-induced maternal effects may not influence the ability of *P. aegeria*

370   offspring to move out of drought-stricken areas in fragmented landscapes.

371   Maternal flight influenced maternal reproductive output. In line with previous

372   studies on *P. aegeria* we found that increased flight during oviposition reduced

373   maternal lifespan, fecundity and decreased the quality or composition of resources

374   mothers put into their eggs, as evidenced by the longer larval development times and

375   lighter pupal masses observed for offspring from forced flight mothers (Gibbs et al.

376   2010a; Gibbs et al. 2010b; Gibbs and Van Dyck 2010; Gibbs et al. 2010c). Previous

377   studies on *P. aegeria* have demonstrated that offspring hatching from large eggs have

378   improved performance when growth occurs on old, lower quality host plants (Gibbs et

379   al. 2010b), when embryogenesis occurs under high-temperature low-humidity

380   conditions (Gibbs et al. 2010c) and when larvae are exposed to sub-lethal viral

381   infection (Gibbs et al. 2010a). In the present study we found that larvae hatching from

382   large eggs had heavier pupal masses and increased investment in flight muscle mass.

383   This suggests that maternal investment in larger egg size confers advantages to

384   offspring during growth on drought stressed host plants. We also observed age-related

385   maternal effects on embryonic development time, pupal mass, post-hatching

386   development time and thorax mass, suggesting that age-related changes in maternal

387   condition can generate resource-related maternal effects that influence offspring traits

388   across life stages in *P. aegeria* when development occurs on drought-stressed host

389   plants. We also found significant maternal age by flight treatment interaction effects

390   on pupal mass and larval development time. Our data suggest that control (i.e. no

391   forced flight) females may adjust their resource allocation patterns to eggs with age,

392   allocating different or higher quality resources to eggs laid early in oviposition. As a

16

393   result of this change in maternal investment, offspring from eggs laid on days 2 and 4

394   of oviposition had shorter larval development times and heavier pupal masses than

395   offspring from eggs laid on days 8 and 10 of oviposition (Figures 1 and 2), suggesting

396   that they were able to buffer some of the costs associated with developing on drought

397   stressed host plants. Higher investment in early reproduction may be advantageous in

398   nature if females have a low survival probability (Begon and Parker 1986), and our

399   data suggest that this reproductive strategy may not only benefit maternal fitness, but

400   also offspring fitness when offspring develop on drought stressed host plants. By

401   contrast, forced flight mothers do not appear to markedly change their investment to

402   offspring early in reproduction (Figures 1 and 2). It is possible that physiological

403   constraints caused by an overlap in resources used during flight and oviposition

404   results in fewer resources being available for oviposition, preventing forced flight

405   females from being able to differentially invest more (or higher quality) resources

406   early in reproduction. In addition, forced flight mothers also have significantly

407   reduced longevity and fecundity compared to control females. This suggests that

408   increased flight during oviposition may prevent females from adopting an optimal

409   reproductive strategy, which in nature, may decrease their fitness, and in times of

410   drought, also decrease their offspring's fitness. These data demonstrate the

411   importance of considering the various mechanisms by which the transmission of non-

412   genetic developmental factors may shape population responses to environmental

413   change.

414   The severity of drought impact has been shown to vary across *P. aegeria*

415   populations depending on the degree of habitat fragmentation (Pollard 1988;

416   Schweiger et al. 2006; Oliver et al. 2015), but currently it is unclear why populations

417   in fragmented landscapes are more vulnerable to drought. This study identifies one

418   potential mechanism; flight-induced changes in egg provisioning generate

419   transgenerational maternal effects that, when offspring growth occurs on drought-

420   stressed host plants, extends the time to adult maturation, and results in small sized

421   individuals. Potentially these maternal effects could influence population dynamics by

422   influencing recruitment rates and, because small-sized adults have lower investment

423   in reproduction (Gibbs et al. 2012; Vande Velde et al. 2013), by reducing population

424   growth rates.

425

426   **Acknowledgements**

434   **Data Accessibility**

435   Data from this study are available for download from the NERC Environmental

436   Information Data Centre: (unique DOI to be provided on publication).

437

438   **References**

439   Abramoff MD, Magalhaes PJ, Ram SJ (2004) Image Processing with ImageJ.

440   Biophotonics International 11:36-42

441 Awmack CS, Leather SR (2002) Host plant quality and fecundity in herbivorous

442   insects. Annu. Rev. Entomol. 47:817-844. doi:

443   10.1146/annurev.ento.47.091201.145300

444 Bauerfeind SS, Fischer K (2008) Maternal body size as a morphological constraint on

445   egg size and fecundity in butterflies. Basic Appl. Ecol. 9:443-451. doi:

446   10.1016/j.baae.2007.05.005

447 Begon M, Parker GA (1986) Should egg size and clutch size decrease with age?

448   Oikos 47:293–302.

449 Berwaerts K, Van Dyck H, Aerts P (2002) Does flight morphology relate to flight

450   performance? An experimental test with the butterfly *Pararge aegeria*. Funct.

451   Ecol. 16:484–491.

452 Berwaerts K, Matthysen E, Van Dyck H (2008) Take-off flight performance in the

453   butterfly *Pararge aegeria* relative to sex and morphology: A quantitative

454   genetic assessment. Evolution 62(10):2525-2533.

455 Bitume EV, Bonte D, Ronce O, Olivieri I, Nieberding CM (2014) Dispersal distance

456   is influenced by parental and grand-parental density. Proc. R. Soc. B-Biol. Sci.

457   281. doi: 10.1098/rspb.2014.1061

458 Bonte D et al. (2012) Costs of dispersal. Biological Reviews 87:290-312. doi:

459   10.1111/j.1469-185X.2011.00201.x

460 Carhenzli F, Erhardt A (2013) Transgenerational acclimatization in a herbivore-host

461   plant relationship. Proc. R. Soc. B-Biol. Sci 280:20122856. doi:

462   org/10.1098/rspb.2012.2856

463 Carroll AL, Quiring DT (1993) Interactions between size and temperature influence

464   fecundity and longevity of a torticid moth, *Zeiraphera canadiensis*. Oecologia

465   93:233–241.

466   Cory JS, Goulson D (1993) Flower constancy and learning in foraging preferences of

467   the green veined butterfly *Pieris napi*. Ecol. Entomol. 18:315-320. doi:

468   10.1111/j.1365-2311.1993.tb01107.x

469   Dover J, Sparks T (2000) A review of the ecology of butterflies in British hedgerows.

470   J. Environ. Manage. 60:51-63. doi: 10.1006/jema.2000.0361

471   Ezard THG, Prizak R, Hoyle RB (2014) The fitness costs of adaptation via

472   phenotypic plasticity and maternal effects. Func. Ecol. 28:693-701. doi:

473   10.1111/1365-2435.12207

474   Gibbs M, Breuker CJ, Hesketh H, Hails R, Van Dyck H (2010a) Maternal effects,

475   flight versus fecundity trade-offs, and offspring immune defence in the

476   Speckled Wood butterfly, *Pararge aegeria*. BMC Evol. Biol. 10:345. doi:

477   10.1186/1471-2148-10-345

478   Gibbs M, Breuker CJ, Van Dyck H (2010b) Flight during oviposition reduces

479   maternal egg provisioning and influences offspring development in *Pararge*

480   *aegeria* (L.). Physiol. Entomol. 35:29-39. doi: 10.1111/j.1365-

481   3032.2009.00706.x

482   Gibbs M, Lace LA, Jones BC, Moore AJ (2005) Egg size-number trade-off and a

483   decline in oviposition site choice quality: female *Pararge aegeria* butterflies

484   pay a cost of having males present at oviposition. J. Insect Sci. 5.39. doi:

485   10.1673/1536-2442(2005)5[1:ESTAAD]2.0.CO;2

486   Gibbs M, Van Dyck H (2009) Reproductive plasticity, oviposition site selection, and

487   maternal effects in fragmented landscapes. Behav. Ecol. Sociobiol. 64:1-11.

488   doi: 10.1007/s00265-009-0849-8

489   Gibbs M, Van Dyck H (2010) Butterfly flight activity affects reproductive

490   performance and longevity relative to landscape structure. Oecologia 163:341-

491   350. doi: 10.1007/s00442-010-1613-5

492   Gibbs M, Van Dyck H, Breuker CJ (2012) Development on drought-stressed host

493   plants affects life history, flight morphology and reproductive output relative

494   to landscape structure. Evol. Appl. 5:66-75. doi: 10.1111/j.1752-

495   4571.2011.00209.x

496   Gibbs M, Van Dyck H, Karlsson B (2010c) Reproductive plasticity, ovarian dynamics

497   and maternal effects in response to temperature and flight in *Pararge aegeria*.

498   J. Insect Physiol. 56:1275-1283. doi: 10.1016/j.jinsphys.2010.04.00

499   Guillaume AS, Monro K, Marshal DJ. 2016. Transgenerational plasticity and

500   environmental stress: do paternal effects act as a conduit or a buffer? Func.

501   Ecol. 30:1175-1184. doi: 10.1111/1365-2435.12604

502   Hughes CL, Hill JK, Dytham C (2003) Evolutionary trade-offs between reproduction

503   and dispersal in populations at expanding range boundaries. Proc. R. Soc.

504   Lond. Ser. B-Biol. Sci. 270:S147-S150. doi: 10.1098/rsbl.2003.0049

505   IPCC (2014) Climate Change 2014: Synthesis Report. Contribution of working

506   groups I, II and III to the Fifth Assessment Report of the Intergovernmental

507   Panel on climate change. In: Pachauri RK, Meyer LA (eds). IPCC, Geneva, p

508   151

509   Johnson CG (1969) Migration and dispersal of insects by flight. Methuen, London

510   Karlsson B, Wiklund C (1984) Egg weight variation and lack of correlation between

511   egg weight and offspring fitness in the wall brown butterfly *Lasiommata*

512   *megera*. Oikos 43:376–385.

513   Karlsson B, Wiklund C (1985) Egg weight variation in relation to egg mortality and

514   starvation endurance of newly hatched larvae in some satyrid butterflies. Ecol.

515   Entomol. 10:205–211.

516 Kuijper B, Johnstone RA, Townley S (2014) The Evolution of Multivariate Maternal

517   Effects. PLoS Comput Biol 10(4): e1003550. doi:10.1371/journal.pcbi.

518   1003550

519 Kuijper B, Hoyle RB (2015) When to rely on maternal effects and when on

520   phenotypic plasticity? Evolution 69-4:950-968. doi:10.1111/evo.12635

521 Marshall DJ, Uller T (2007) When is a maternal effect adaptive? Oikos 116:1957–

522   1963.

523 Merckx T, Van Dyck H, Karlsson B, Leimar O (2003) The evolution of movements

524   and behaviour at boundaries in different landscapes: a common arena

525   experiment with butterflies. Proc. R. Soc. Lond. Ser. B-Biol. Sci. 270:1815-

526   1821. doi: 10.1098/rspb.2003.2459

527 Oliver TH, Marshall HH, Morecroft MD, Brereton T, Prudhomme C, Huntingford C

528   (2015) Interacting effects of climate change and habitat fragmentation on

529   drought-sensitive butterflies. Nature Climate Change 5:941-945. doi:

530   10.1038/nclimate2746

531 Pollard E (1988) Temperature, rainfall and butterfly numbers. J. Appl. Ecol. 25:819–

532   828.

533 Schweiger O, Dormann CF, Bailey D, Frenzel M (2006) Occurrence pattern of

534   *Pararge aegeria* (Lepidoptera: Nymphalidae) with respect to local habitat

535   suitability, climate and landscape structure. Landscape Ecology 21:989–1001.

536 Shreeve TG (1986) Egg laying by the speckled wood butterfly (*Pararge aegeria*) -

537   The role of female behavior, host plant abundance and temperature. Ecol.

538   Entomol. 11:229-236. doi: 10.1111/j.1365- 2311.1986.tb00298.

539   Talloen W, van Dyck H, Lens L (2004) The cost of melanization: butterfly wing

540   coloration under environmental stress. Evolution 58:360-366. doi: 10.1554/03-

541   250

542   Uller T (2008) Developmental plasticity and the evolution of parental effects. Trends

543   Ecol. Evol. 23:432-438. 10.1016/j.tree.2008.04.005

544   Uller T, Nakagawa S, English S (2013) Weak evidence for anticipatory parental

545   effects in plants and animals. J. Evol. Biol. 26:2161-2170. doi:

546   10.1111/jeb.12212

547   Van Dyck H (2003) Mate location, a matter of design? Adaptive morphological

548   variation in the speckled wood butterfly. In: Boggs CL, Watt WB, Ehrlich PR

549   (eds.), *Butterflies: Ecology and Evolution Taking Flight*. University of

550   Chicago Press, Chicago, pp. 353-366

551   Van Dyck H, Baguette M (2005) Dispersal behaviour in fragmented landscapes:

552   Routine or special movements? Basic Appl. Ecol. 6:535-545. doi:

553   10.1016/j.baae.2005.03.005

554   Vande Velde, L., Van Dyck, H., 2013. Lipid economy, flight activity and

555   reproductive behaviour in the speckled wood butterfly: on the energetic cost of

556   territory holding. Oikos 122, 555-562.

557   Vande Velde L, Schtickzelle N, Van Dyck H (2013) Effect of larval food stress on

558   male adult behaviour, morphology and reproductive investment in the

559   butterfly *Pararge aegeria*. Evol. Ecol. 27:221-234. doi: 10.1007/s10682-012-

560   9580-4

561   Wiklund C, Karlsson B (1984) Egg size variation in Satyrid butterflies - adaptive vs

562   historical, "Bauplan", and mechanistic explanations. Oikos 43:391-400

563   Wiklund C, Persson A (1983) Fecundity, and the relation of egg weight variation to

564   offspring fitness in the Speckled Wood butterfly, *Pararge aegeria*, or why

565   don't butterfly females lay more eggs? Oikos 40:53-63. doi: 10.2307/3544198

566   Wickman P-O, Wiklund C, Karlsson B (1990) Comparative phenology of four

567   satyrine butterflies inhabiting dry grasslands in Sweden. Holarctic Ecology

568   13:238–346.

569   Woestmann L, Saastamoinen M (2016) The importance of trans-generational effects

570   in Lepidoptera. Current Zoology 62(5): 489-499. doi: 10.1093/cz/zow029

571

572   Table 1. Post-hatching larval performance for offspring from eggs laid on days 2, 4, 6,

573   8 and 10 days of oviposition: mean offspring trait values (± SE) across maternal flight

574   treatments (where C = control, no forced flight, and F = forced flight).

575

	Mean offspring trait value across flight treatments					
Day of oviposition	**Larval development time (days)**		**Pupal mass (mg)**		**Dry thorax mass (mg)**	
	C	**F**	**C**	**F**	**C**	**F**
2	38.2 (7.3)	38.6 (2.0)	139.9 (7.3)	126.0 (9.6)	6.4 (0.3)	5.4 (0.4)
4	39.2 (1.0)	40.5 (2.2)	131.5 (4.7)	123.1 (6.8)	5.8 (0.3)	5.3 (0.3)
6	41.9 (1.3)	43.6 (1.6)	116.7 (9.6)	118.0 (112.6)	5.0 (0.5)	4.9 (0.3)
8	48.4 (2.5)	42.8 (1.4)	111.0 (8.0)	112.6 (5.0)	4.3 (0.6)	4.9 (0.4)
10	42.0 (1.6)	43.0 (1.7)	101.7 (8.3)	140.5 (8.2)	4.0 (0.5)	5.7 (0.4)

576

577

578   Figure 1. Relationship between pupal mass (mg) and the day on which the egg was

579   laid (i.e. maternal age), for offspring from mothers that were forced to fly (dashed

580   line) and control mothers (solid line)

581

582   Figure 2. Relationship between larval development time (days) and the day on which

583   the egg was laid (i.e. maternal age), for offspring from mothers that were forced to fly

584   (dashed line) and control mothers (solid line)

585

586

587

588

589

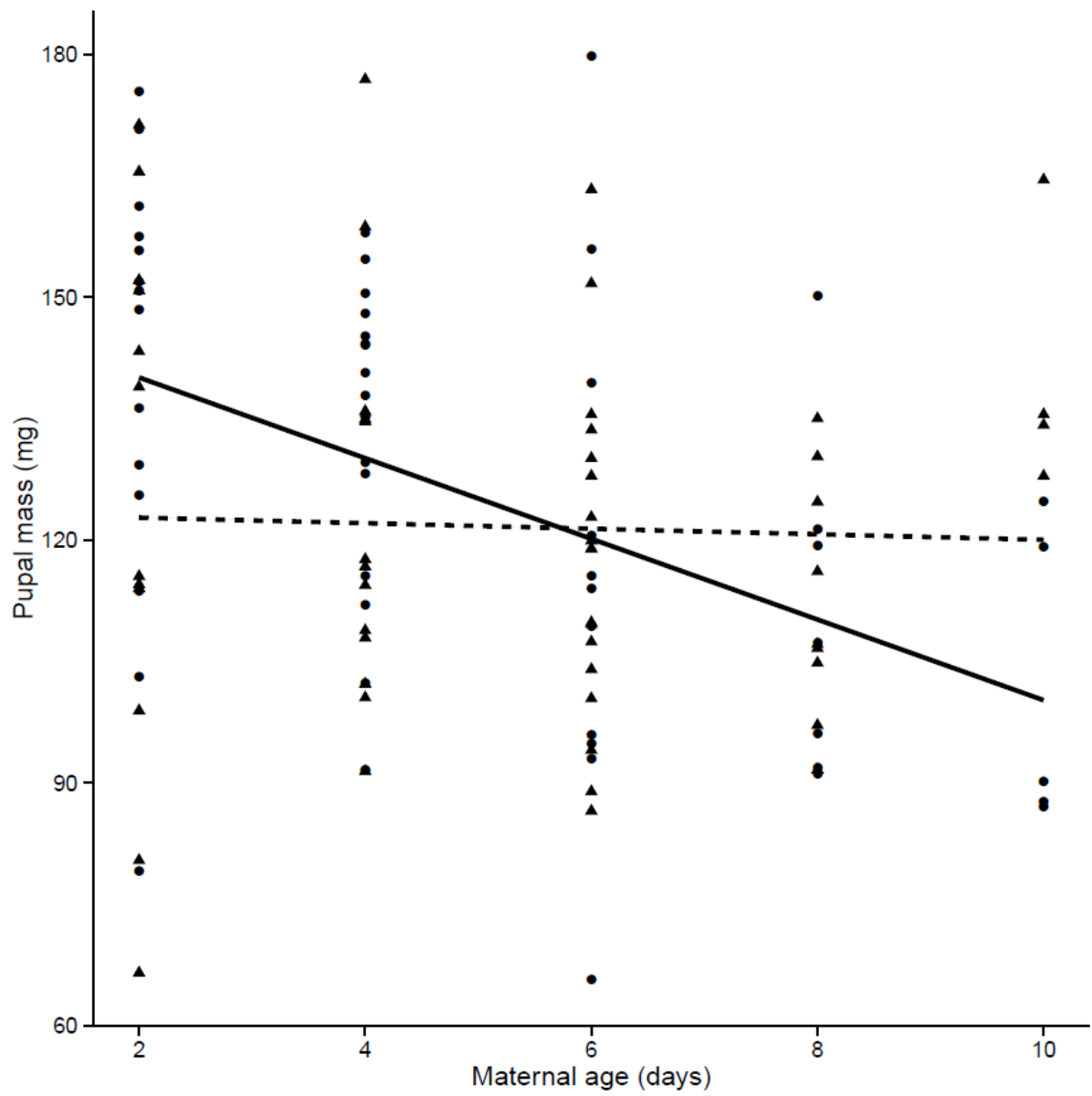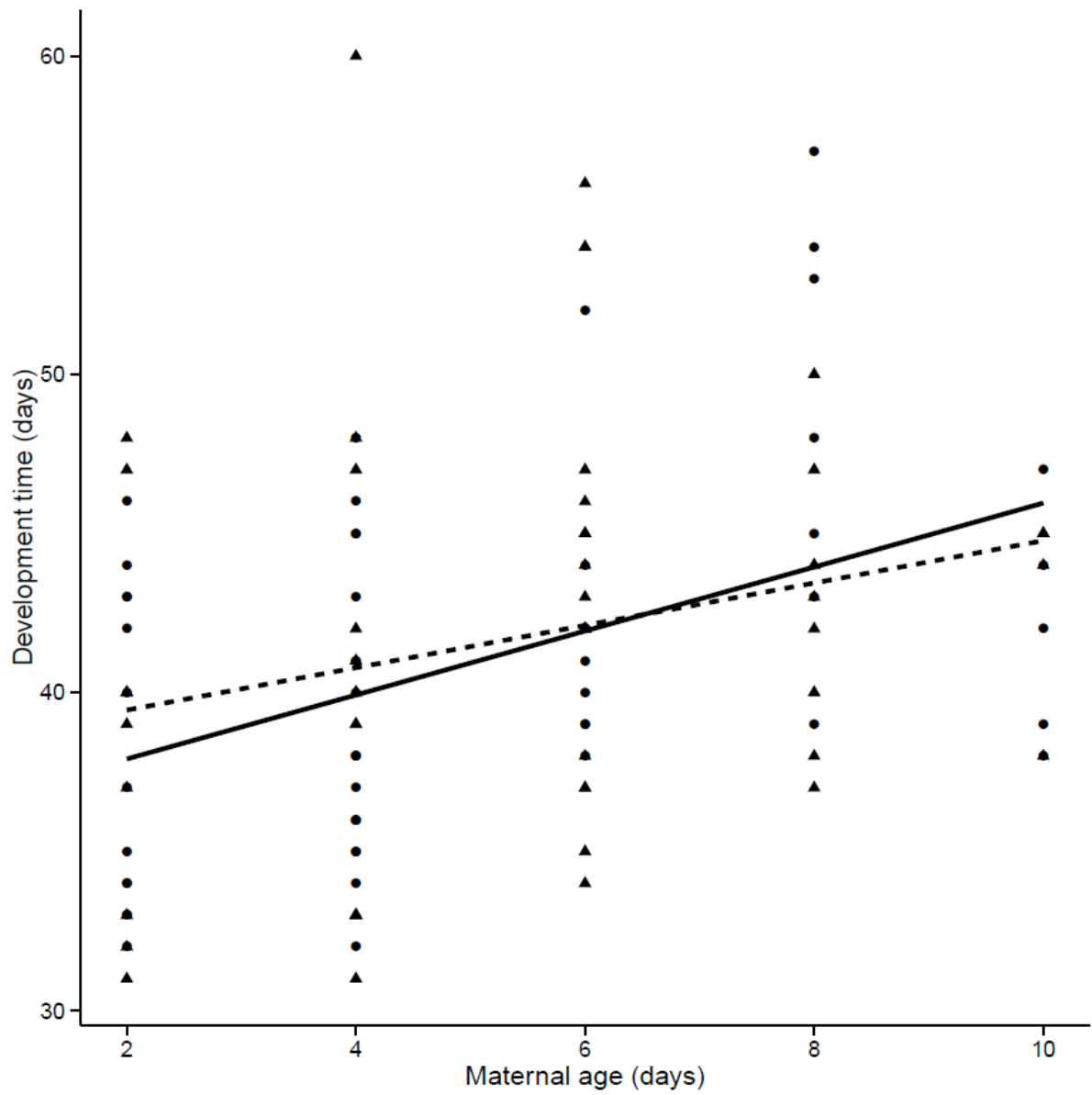590

591

592

593

594
595

596

597

598

599

600

601

602

603   Figure 1

604

605

606   Figure 2

607
608
609
610
611
612

28