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

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

3

4 MELANIE GIBBS^{1*}, HANS VAN DYCK² & CASPER J. BREUKER³

5

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

7 Wallingford, OX10 8BB, UK

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

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

10 Neuve, Belgium

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

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

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

14

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

16

17 Running title: Maternal flight affects offspring performance during drought

18

19 Author Contributions: MG originally formulated the idea with input from HVD.

20 MG designed and executed the study. MG and CJB performed statistical analyses.

21 MG, HVD and CJB wrote the manuscript.

22

23 **Abstract**

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

46
47 **Key-words:** Egg quality, embryogenesis, life history, non-genetic effects, rainfall

48 **Introduction**

49 Maternal effects constitute a form of non-genetic transmission of environmental
50 conditions across generations, and there is a growing realisation that maternal effects
51 may have both short- and long-term consequences for population dynamics (Bitume
52 et al. 2014; Uller 2008), particularly in rapidly changing environments, such as those
53 found in fragmented landscapes (Gibbs and Van Dyck 2009). Maternal effects are not
54 always adaptive (Marshall and Uller 2007; Uller 2008), and a key factor determining
55 whether transgenerational maternal effects influence offspring performance positively
56 or negatively is how well mothers are able to predict the environment of their
57 offspring (Guillaume et al. 2016). Global climate change, particularly an increase in
58 climatic extreme events such as drought (IPCC 2014), is likely to reduce the ability of
59 mothers to accurately predict the environment of their offspring (Guillaume et al.
60 2016), and recent modelling work suggests that rapidly changing, unpredictable
61 environments, actually selectively favour negative maternal effects (Ezard et al. 2014;
62 Kuijper et al. 2014; Kuijper and Hoyle 2015). In nature, both mothers and offspring
63 are likely to be subjected to multiple biotic and abiotic factors simultaneously (Carroll
64 and Quiring 1993), and mothers and offspring may not always be subjected to the
65 same factors, particularly in fast changing environments, further reducing the ability
66 of mothers to accurately predict the environment of their offspring. Empirical studies
67 focusing on the transmission of non-genetic developmental factors in response to
68 ecologically relevant global change stressors are therefore essential to generate a
69 better understanding of species responses to changing environments (Uller et al. 2013;
70 Guillaume et al. 2016).

71 Ectotherms, like butterflies, are very sensitive to (changes in) their
72 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 Saastamoinen 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).

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 F₂ 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 °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 m³) 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 m³) 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

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

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 (mm²) 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, F₃ 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 \pm 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

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 mm², Forced
290 Flight = 0.78 ± 0.03 mm², $t_{31} = 0.82$ $P = 0.42$) 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 = $43.1 \pm$
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

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

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433 The authors declare that they have no conflict of interest.

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

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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 (\pm 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) |

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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)

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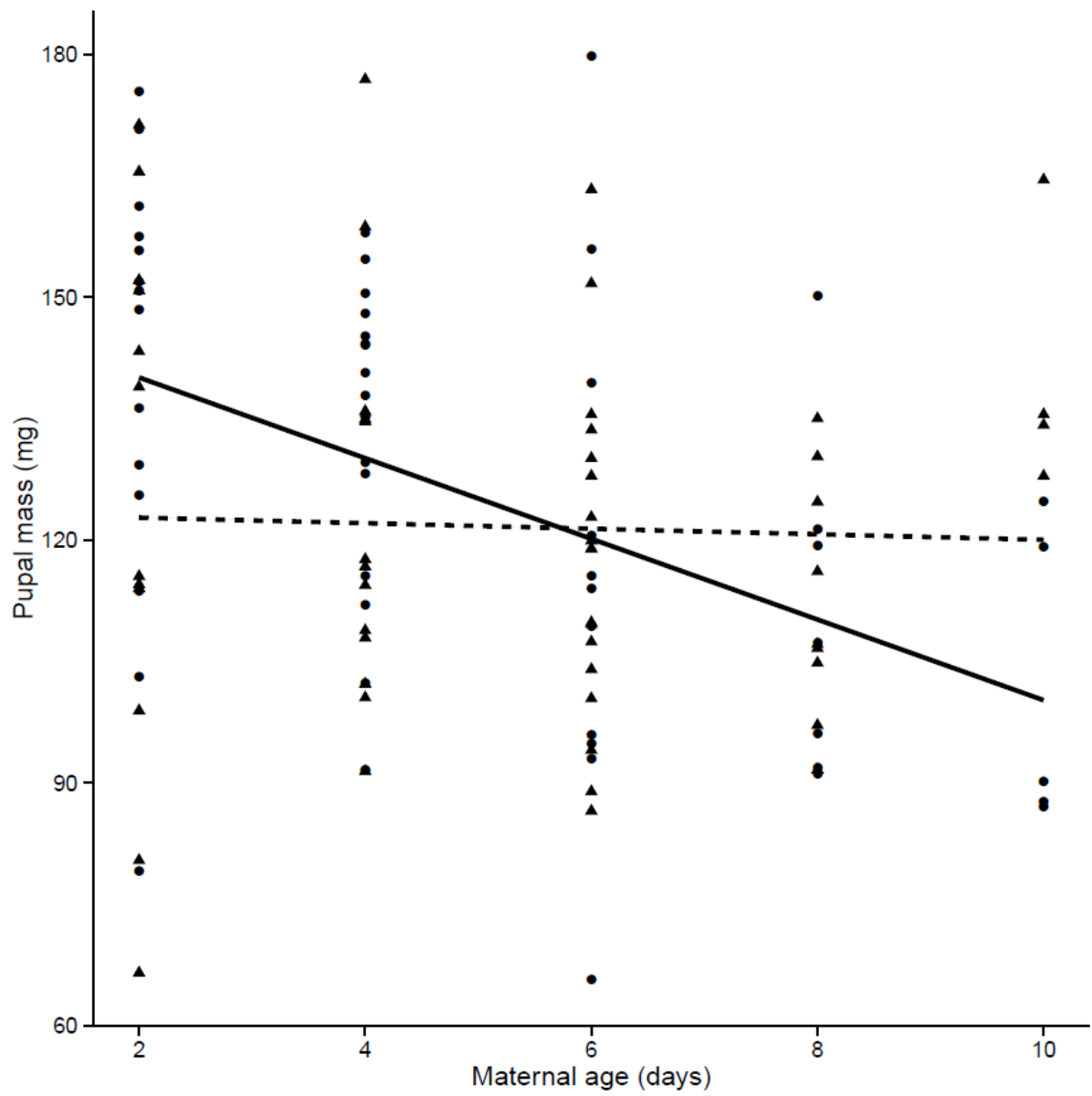
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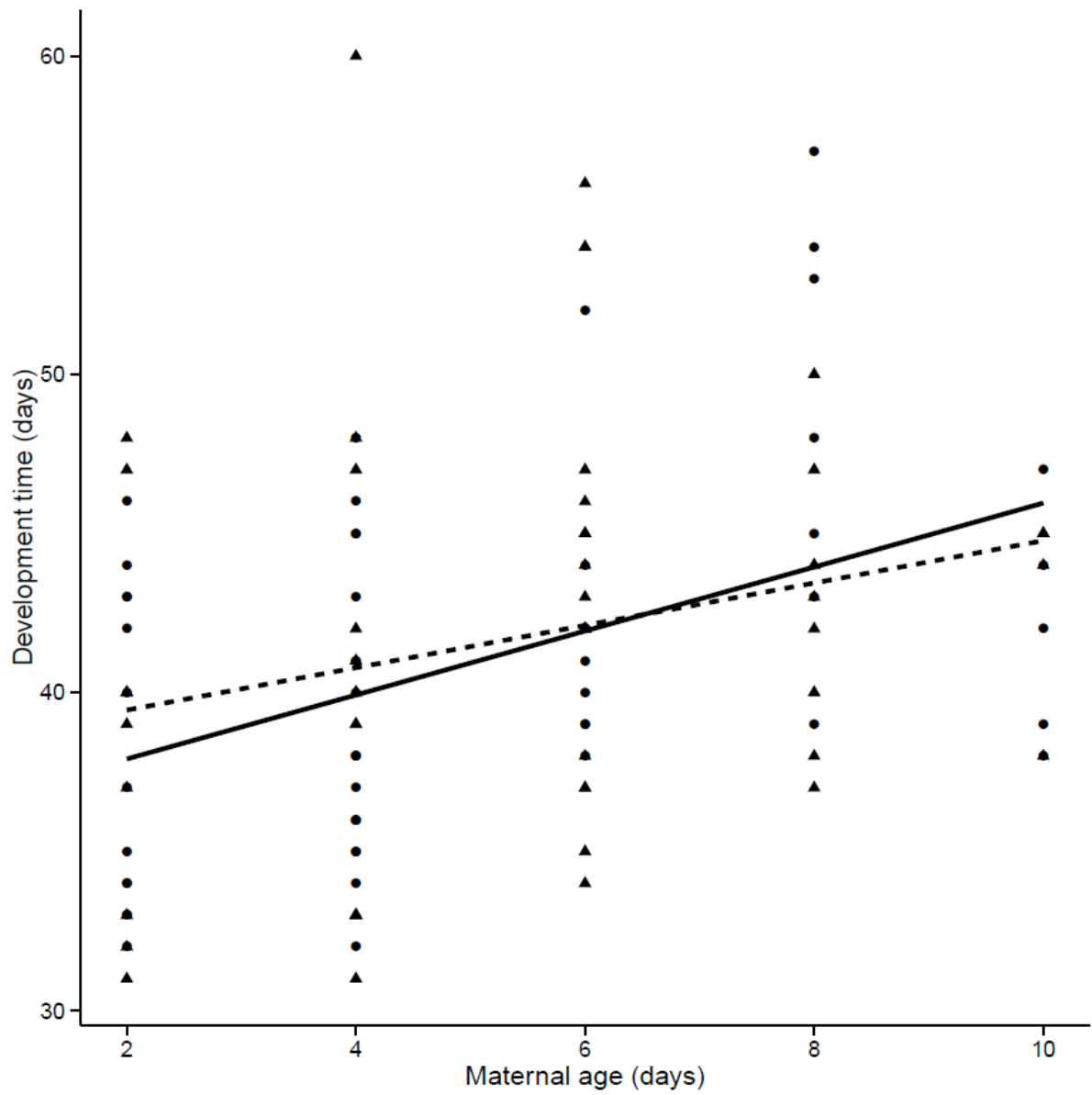
603 Figure 1



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606 Figure 2



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