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

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Author Contributions**:** MG originally formulated the idea with input from HVD. MG designed and executed the study. MG and CJB performed statistical analyses. MG, HVD and CJB wrote the manuscript.

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 drought, on transgenerational effects in butterflies has been understudied to date (Woestmann and Saastamoinen 2016). Consideration of the impacts of interactions between multiple factors on transgenerational effects in butterflies is rare, while an assessment of the impact of transgenerational effects on adult traits of the offspring generation is also largely lacking (Woestmann and Saastamoinien 2016; but see Cahenzli and Erhardt 2013).

Across various species (including butterflies), habitat fragmentation has been shown to either increase or decrease mobility depending on both the spatial configuration of key resources such as host plants for oviposition, as well as the costs associated with dispersal in any given system (Bonte et al. 2012; Gibbs and Van Dyck 2009; Van Dyck and Baguette 2005). One classic example of a life-history physiological trade-off that has attracted much attention for several decades (e.g. Johnson 1969), and is of relevance when considering the costs of dispersal in fragmented landscapes, is the relationship between flight and fecundity (i.e. oogenesis-flight syndrome, Johnson 1969). Initially, there was a particular focus on the quantitative aspects of fecundity (i.e. number of eggs and egg size), but there has been a growing interest to expand this work to qualitative aspects as well (e.g. egg composition), and hence, to the role of maternal effects in this context (Awmack and Leather 2002). In our study species the Speckled Wood butterfly, *Pararge aegeria* (L.), females in fragmented landscapes have a high fecundity strategy, laying larger numbers of smaller-sized eggs; a strategy which is associated with increased flight and a decrease in the time and resources a female allocates to searching for optimal host plants and microsites for oviposition (Gibbs and Van Dyck 2009; Gibbs and Van Dyck 2010).

Pararge aegeria is a drought-sensitive butterfly species, particularly in the larval stage when growth occurs on drought stressed host plants (Talloen et al. 2004; Gibbs et al. 2012; Vande Velde et al. 2013), and several studies have shown that population recovery from drought events is significantly slower for populations of this species in fragmented landscapes (Pollard 1988; Schweiger et al. 2006; Oliver et al. 2015). Currently, few studies have explored the mechanisms underlying this drought by habitat fragmentation interaction effect on population dynamics (but see Gibbs et al. 2012), and currently it is unclear why populations in fragmented landscapes are more vulnerable to drought. The role that flight-induced transgenerational maternal effects may play in the response of offspring to drought has not been examined.

In this study we tested the hypothesis that repeated periods of intensive flight during female oviposition affect egg provisioning and reduces offspring performance when larval development occurs on drought stressed host plants. Our previous studies have shown that in *P. aegeria*, changes in resource allocation to egg size and composition in response to flight is dependent on maternal age (Gibbs et al. 2010a; Gibbs et al. 2010b; Gibbs et al. 2010c). As such, in order to adequately assess the impact of increased flight during oviposition on subsequent offspring performance it is necessary to consider the interaction between these two intrinsic maternal factors simultaneously. To do this we created two maternal flight treatment groups; i) females that are forced to fly repeatedly during the oviposition period (referred to here as forced flight females, and ii) females that are not forced to fly during the oviposition period (here referred to as control females) (Gibbs et al. 2010a,c), and a sub-sample of the eggs laid by mothers on days 2, 4, 6, 8 and 10 days of oviposition were collected and used to assess the impact of both maternal age and flight treatment on subsequent offspring performance. As has also been observed in other species (e.g. Bernardo

1996a; and for temperate butterflies in particular Karlsson and Wiklund 1984; Karlsson and Wiklund 1985; Wickman et al. 1990), from previous work with *P. aegeria* we know that benign laboratory test conditions can mask the maternal nutritional effects of egg size and composition (Wiklund and Persson 1983; Wiklund and Karlsson 1984). To ensure that our offspring larval tests conditions were not too benign, we therefore reared our offspring on drought stressed host plants to create a stressful, but ecologically relevant growth environment (after Talloen et al. 2004; Gibbs et al. 2012; Vande Velde et al. 2013). This design enabled us to investigate whether, after controlling for egg size, maternal age *per se*, and increased flight during the oviposition period influences offspring performance across multiple life stages; i) early in development during the egg stage, ii) during larval development on drought-stressed host plants, and iii) in the adult stage.

Materials and Methods

Study species

Pararge aegeria is a temperate-zone species, primarily found in woodland, but in north-west Europe this species has colonized agricultural landscapes containing small, widely distributed ‘woodland’ fragments along hedgerows (Dover and Sparks 2000; Merckx et al. 2003). Eggs are laid singly on grass species from the family Poaceae (Shreeve 1986). Egg size and egg hatching success declines with female age due to a decline in maternal resources over time (Gibbs et al. 2010a; Wiklund and Karlsson 1984; Wiklund and Persson 1983).

Maternal treatment

Our experimental females were derived from an outbred laboratory stock population, which originated from a deciduous continuous woodland landscape in St. Hubert, Belgium. Newly hatched F_2 generation larvae from this population were placed in pairs on potted host plants of *Poa trivialis* (grown under standard conditions from commercially produced seed provided by Limagrain Advanta Belgium) and reared in a climate room under a direct development regime in common garden conditions (24 °C, LD 16:8, RH 60 ± 5 %) until eclosion. On the day of eclosion (between 9 and 12hr; from now on called day -1) 32 females were individually weighed (AMD Instrument Ltd balance; accuracy: ± 0.1 mg) and each female was placed in a netted cage (0.5 m^3) along with a potted *P. trivialis* for oviposition and an artificial flower containing a 10% honey solution (for design see Cory and Goulson 1993). *Pararge aegeria* females actively feed from artificial flowers and this feeding method has been routinely adopted in laboratory experiments (e.g. Gibbs et al. 2010a,b; Gibbs et al. 2005). Later the same day (between 13 and 16hr) a one-day-old randomly chosen non-sibling male (originating from the same laboratory source population) was introduced to the cage and, after mating, the pair was left undisturbed for 24 hours.

Forced flight treatment: On the day after mating (from now on called day 0), females were assigned to one of two treatment groups: control or forced flight. Control females were left undisturbed in their cages until the first eggs were laid (i.e. day 1 of oviposition). When the first eggs were laid, the male was removed from the cage and the female was left to continue laying. On the day after mating (day 0), forced flight females were removed from cages and forced to fly continuously for 5 min at 24°C. These forced flight females were placed individually into an empty netted cage (0.5 m^3) and stimulated to fly by gently touching their legs with a fine-bristled paintbrush each time they alighted (after Gibbs et al. 2010a,b,c; Gibbs and

Van Dyck 2010). After forced flight, the females were returned to their mating cages until the first eggs were laid (i.e. day 1 of oviposition). When the first eggs were laid, the male was removed from the cage and the female was left undisturbed in the cage to continue egg laying. Forced flight treatment was repeated on days 4 and 8 of oviposition, to give a total of 3 periods of intensive flight treatment during oviposition. In total, 17 control females and 15 forced flight females were set-up. All 32 females mated successfully, began ovipositing 48hr after mating and laid viable eggs.

Egg collection: Each morning, from day 1 of oviposition until female death, between 9 am and 11 am all cages were inspected for eggs, the host plant was watered and fresh honey solution was provided via artificial flowers to ensure a permanent food source for the ovipositing females. The number of days between female eclosion as an adult and death was used as a measure of longevity. Eggs laid by each female were collected and counted each day. The total number of eggs laid during a female's life was used as a measure of lifetime fecundity. All of the eggs laid by each female were kept and used to monitor egg hatching success. The total lifetime egg hatching success was recorded for each female, and this measure included all of the eggs laid by each female, even the eggs used to measure offspring performance (see below).

Offspring performance

Egg stage: To determine how female flight treatment, female age and egg size influence embryonic performance, for each female, on days 2, 4, 6, 8 and 10 days of oviposition a sub-sample of 5 eggs (from the total number of eggs available on that day) were randomly selected and placed into separate Petri dishes to be photographed for egg size measurements. If fewer than 5 eggs were laid on one day, then all of the eggs that had been laid were kept for egg size measurements. In total 779 eggs were collected and individually measured. *Pararge aegeria* eggs are spherical and the size of each egg could therefore be measured as a cross-sectional projection (mm^2) using a digital camera (Canon A720 IS). The resulting images were then analysed using Image J (freely available at http://rsb.info.nih.gov/ij/)(Abramoff et al. 2004). This method is a highly reliable measure of egg size in *P. aegeria* as there is a strong correlation between egg area and egg mass in this species (cf. Bauerfeind and Fischer 2008). After the eggs were photographed, each egg was placed individually into a labelled Eppendorf (sealed with a small piece of fine netting secured with an elastic band) and monitored each day for hatching. For each egg we recorded whether it survived to hatch or not, and these data were included in the lifetime hatching success analyses described previously. For eggs that hatched successfully, the total number of days between being laid and hatching was used as a measure of embryonic development time, and used to examine how maternal age and flight treatment influence early offspring development.

Larval stage: To determine how female flight treatment, female age and egg size influence larval performance on drought stressed host plants, on the day of hatching, F_3 generation larvae from each of our 32 females, from each day of oviposition were individually reared on a drought stressed host plant. In total 187 larvae were set-up (2-10 individuals/female, mean = 5.8 ± 0.3). Due to fewer eggs hatching from eggs laid on days 8 and 10 of oviposition, smaller numbers of larvae were reared from these treatment groups; in the control maternal treatment 20, 25, 20, 15 and 12 larvae from days 2, 4, 6, 8 and 10 of oviposition respectively were reared, and in the forced flight treatment group 21, 21, 24, 17 and 12 larvae from days 2, 4, 6, 8 and 10 of oviposition respectively were reared. Drought-stressed plants were deprived of water for 20 days prior to the start of the experiment (i.e. 20 days before larval hatching) and then were subsequently only provided with rain water once every 6 days throughout the experimental period. By only watering the plants every 6 days, we ensured that the plants remained alive (and had leaves available for larval consumption) but experienced moderate drought stress throughout the experimental period (after Gibbs et al. 2012; Talloen et al. 2004). For each larva we recorded development time from hatching to pupation, pupal mass, and whether it survived to eclose as an adult or not.

Adult stage: On the day of eclosion each adult was sexed. Butterflies were killed within 24 hours of emergence, after their wings had fully hardened, by placing them in a -20°C freezer. Fore- and hindwings were carefully removed from the thorax. The body of each individual was dried for 24 h at 60 ˚C, and then weighed (AMD Instrument Ltd balance; accuracy: ± 0.1 mg). The thorax was then carefully removed and weighed, and used as a measure of investment in flight muscle mass, which is known to correlate with dispersal ability in *P. aegeria* (Hughes et al. 2003). These data enabled us to examine the potential for flight-induced maternal effects to generate long-lasting maternal effects that influence adult offspring dispersal capacity.

Statistical analyses

Linear and generalised linear mixed effect (lme or glme) models were fitted where appropriate, by means of restricted maximum likelihood (REML), which produces unbiased estimates of variance and covariance parameters, with female (i.e. mother) being declared as a random factor. Likelihood ratio tests were conducted to compare different models with each other. The final model only included significant interactions. Residuals were examined for non-linearity in all cases and for non-normality where appropriate. Analyses were performed in R 3.2.1 (packages nlme and lme4; http://lib.stat.cmu.edu/R/CRAN/). Significances for REML constructed models in R are estimated by means of t_{df}-values (lme) and z-values (glme). The sign of either the t- or z-values is indicative of the relationship between the effect and the dependent variable (i.e. positive or negative). All mean values are presented in the text with ± Standard Error (SE).

Female longevity, lifetime fecundity, mean lifetime egg size and mean lifetime egg hatching success: These data were analysed by means of a lme model. Fixed factors were maternal age and flight treatment. Female body mass was used as a covariate.

Offspring development: lme models were constructed to investigate how each of the four offspring traits, embryonic development time (in days), post-hatching development time (time between hatching from an egg to eclosion as an adult, in days), pupal mass (mg) and dry thorax mass (mg) changed over the egg-laying period (i.e. as a function of maternal age), and whether there were differences in these traits between females that had been forced to fly and the controls (i.e. flight treatment was a fixed effect). In these models, day of oviposition (fixed effect) was thus an indicator of female age. Egg size (mm^2), which is known to affect offspring development (Gibbs et al. 2010b), for each individually measured egg was used as a covariate, as any relationship between offspring traits and maternal age may simply be due to the fact that females lay differently sized eggs as they age. Larvae that developed to the pupal stage could be sexed, and thus offspring sex was also added as fixed effect to the models for post-hatching development time, pupal mass and thorax mass. To take allometry into account, total dry mass was included as a covariate when analysing investment in thorax mass (i.e. flight muscle mass), and a total dry body mass by maternal interaction effect was also included to account for allometric changes due to a decline in maternal egg provisioning as mothers age (i.e. offspring mass declines with maternal age; Gibbs et al. 2010a).

Survival to eclosion (0 = dead, 1 = alive): was analysed using a lme model with a logit link function (i.e. a logistic regression with random effects). Maternal flight treatment was used as a fixed effect. Egg size and maternal age were used as covariates. Offspring sex was not included in these models as offspring that did not survive could not be sexed.

Results

Effects of flight treatment on female longevity and reproductive output

Longevity: Females forced to fly had significantly shorter lifespans (19%) than control females (Control 14.47 ± 0.68 days, Forced Flight = 11.73 ± 1.0 days, $t_{31} = -2.40$, P = 0.02).

Reproductive output: Forced flight females laid significantly fewer eggs than control females (Control = 159.4 ± 9.8, Forced Flight = 125.0 ± 14.3, $t_{31} = -2.40$, P = 0.02). There was no difference in mean egg size (Control = 0.76 ± 0.02 mm^2, Forced Flight = 0.78 ± 0.03 mm^2, $t_{31} = 0.82$ P = 0.42) or mean egg hatching success across treatments (Control = 0.63 ± 0.03, Forced Flight = 0.68 ± 0.04, $t_{31} = 1.24$, P = 0.23). Female body mass did not significantly contribute to variation in the number of eggs laid, egg size or egg hatching success, and this covariate was therefore removed from all models. Forced flight and control females did not differ in body mass (Control =

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

Effects of flight treatment on early offspring development

Maternal flight treatment, maternal age and egg size each significantly contributed to variation in embryonic development time. Offspring from eggs laid by forced flight mothers had shorter embryonic development times (Control = 6.74 ± 0.05 days, Forced Flight = 6.57 ± 0.05 days, $t_{32} = -2.45$, $P = 0.02$). There was a negative relationship between embryonic development time and the day of oviposition on which the egg was laid, such that offspring from eggs laid later in the oviposition period (i.e. by older mothers) had shorter embryonic development times ($t_{475} = -2.34$, $P = 0.02$). There was a negative relationship between embryonic development time and egg size, such that offspring from larger eggs had shorter embryonic development times ($t_{367} = -3.13$, $P = 0.002$).

Effects of maternal flight treatment on offspring performance on drought stressed host plants

Pupal mass: Offspring from control mothers had heavier pupal masses (Table 1; $t_{105} = -3.28$, $P = 0.001$). There was a significant effect of maternal age on pupal mass (Table 1; $t_{105} = -2.76$, $P = 0.007$), and a significant maternal age by flight treatment interaction effect ($t_{105} = 3.1$, $P = 0.003$; Figure 1). Offspring from eggs laid by control older mothers had lighter pupal masses, but this maternal age-specific decline in offspring pupal mass was not apparent in offspring from forced flight mothers (Figure 1). Offspring that hatched from larger-sized eggs had heavier pupal masses ($t_{109} =$

3.54, $P = 0.0006$). Male offspring had lighter pupae than female offspring (Male = 117.2 ± 2.7 mg, Female = 128.7 ± 3.1 mg; $t_{105} = -2.73$, $P = 0.007$).

Post-hatching development time: Offspring from mothers forced to fly had longer development times (Table 1; $t_{98} = 2.05$, $P = 0.04$). Offspring from eggs laid by older mothers had longer development times ($t_{106} = 4.60$, $P < 0.0001$). There was a significant maternal flight treatment by maternal age interaction effect ($t_{101} = -2.109$, $P = 0.04$; Figure 2). Compared to offspring from control mothers, offspring from mothers forced to fly had longer development times when they hatched from eggs laid early in the oviposition period, but shorter development times when they hatched from eggs laid later in the oviposition period (Figure 2). Male offspring had shorter development times than female offspring (Male = 39.7 ± 0.9 days, Female = 43.1 ± 0.9 days; $t_{104} = -3.38$, $P = 0.001$).

Thorax mass: Offspring thorax mass did not differ across maternal flight treatment groups ($t_{26} = 0.95$, $P = 0.35$). Offspring that hatched from large eggs had heavier thorax masses ($t_{83} = 2.32$, $P = 0.02$). Body mass scaled significantly with flight muscle mass ($t_{104} = 7.78$, $P < 0.0001$), such that adults with heavier body masses had higher resource investment to flight muscle mass. However, the nature of the (allometric) scaling between adult body mass and investment in flight muscle mass is dependent on the age of the mother at the time of egg laying (i.e. a significant adult body mass by maternal age interaction effect; $t_{104} = 2.87$, $P = 0.005$). That is, although offspring that hatched from eggs laid by old mothers had lighter thorax masses ($t_{104} = -2.08$ $P = 0.04$), the precise slope of the regression line between adult body mass and investment in flight muscle mass depended on maternal age. Male offspring allocated relatively more resources to thorax mass, and hence flight muscle mass, than females (Male = 5.8 ± 0.1 mg, Female = 4.8 ± 0.1 mg; $t_{104} = 7.03$, $P < 0.0001$).

Survival to eclosion: There was no difference in survival across maternal flight treatments ($z = -0.40$, $P = 0.69$). There was no effect of egg size ($z = 1.40$, $P = 0.16$) or maternal age ($z = -1.80$, $P = 0.07$) on survival to eclosion.

Discussion

Intensive maternal flight during oviposition directly influenced the performance of offspring on drought-stressed host plants. Offspring from forced flight mothers had significantly longer development times and smaller pupal masses. Given that there were no differences in egg size across flight treatment groups, these results strongly indicate egg-provisioning differences between flight treatment groups that are not related to egg size *per se* (cf. Gibbs et al. 2010a). As far as we are aware, this is the first study to show that flight-induced changes in maternal egg provisioning can influence the performance of offspring when growth occurs on drought stressed host plants. These effects have the potential to manifest across generations because small-sized adults have lower investment in reproduction, i.e. smaller spermatophore size in males and lower egg quality in females (Gibbs et al. 2012; Vande Velde et al. 2013). We did not find, however, any effect of maternal flight treatment on offspring investment to adult flight muscle mass. Male offspring allocated relatively more resources to flight muscle mass than females. These sex differences in resource allocation patterns may reflect differences in life history (Van Dyck 2003). Males and females differ in their resource allocation patterns to flight morphological traits (e.g. thoracic mass, and hence flight muscle mass), with males investing in fast acceleration take-off flights used during territorial fights (Berwaerts et al., 2002;

Berwaerts et al., 2008; Vande Velde and Van Dyck, 2013). Overall, our data suggest that flight-induced maternal effects may not influence the ability of *P. aegeria* offspring to move out of drought-stricken areas in fragmented landscapes.

Maternal flight influenced maternal reproductive output. In line with previous studies on *P. aegeria* we found that increased flight during oviposition reduced maternal lifespan, fecundity and decreased the quality or composition of resources mothers put into their eggs, as evidenced by the longer larval development times and lighter pupal masses observed for offspring from forced flight mothers (Gibbs et al. 2010a; Gibbs et al. 2010b; Gibbs and Van Dyck 2010; Gibbs et al. 2010c). Previous studies on *P. aegeria* have demonstrated that offspring hatching from large eggs have improved performance when growth occurs on old, lower quality host plants (Gibbs et al. 2010b), when embryogenesis occurs under high-temperature low-humidity conditions (Gibbs et al. 2010c) and when larvae are exposed to sub-lethal viral infection (Gibbs et al. 2010a). In the present study we found that larvae hatching from large eggs had heavier pupal masses and increased investment in flight muscle mass. This suggests that maternal investment in larger egg size confers advantages to offspring during growth on drought stressed host plants. We also observed age-related maternal effects on embryonic development time, pupal mass, post-hatching development time and thorax mass, suggesting that age-related changes in maternal condition can generate resource-related maternal effects that influence offspring traits across life stages in *P. aegeria* when development occurs on drought-stressed host plants. We also found significant maternal age by flight treatment interaction effects on pupal mass and larval development time. Our data suggest that control (i.e. no forced flight) females may adjust their resource allocation patterns to eggs with age, allocating different or higher quality resources to eggs laid early in oviposition. As a result of this change in maternal investment, offspring from eggs laid on days 2 and 4 of oviposition had shorter larval development times and heavier pupal masses than offspring from eggs laid on days 8 and 10 of oviposition (Figures 1 and 2), suggesting that they were able to buffer some of the costs associated with developing on drought stressed host plants. Higher investment in early reproduction may be advantageous in nature if females have a low survival probability (Begon and Parker 1986), and our data suggest that this reproductive strategy may not only benefit maternal fitness, but also offspring fitness when offspring develop on drought stressed host plants. By contrast, forced flight mothers do not appear to markedly change their investment to offspring early in reproduction (Figures 1 and 2). It is possible that physiological constraints caused by an overlap in resources used during flight and oviposition results in fewer resources being available for oviposition, preventing forced flight females from being able to differentially invest more (or higher quality) resources early in reproduction. In addition, forced flight mothers also have significantly reduced longevity and fecundity compared to control females. This suggests that increased flight during oviposition may prevent females from adopting an optimal reproductive strategy, which in nature, may decrease their fitness, and in times of drought, also decrease their offspring's fitness. These data demonstrate the importance of considering the various mechanisms by which the transmission of non-genetic developmental factors may shape population responses to environmental change.

The severity of drought impact has been shown to vary across *P. aegeria* populations depending on the degree of habitat fragmentation (Pollard 1988; Schweiger et al. 2006; Oliver et al. 2015), but currently it is unclear why populations in fragmented landscapes are more vulnerable to drought. This study identifies one potential mechanism; flight-induced changes in egg provisioning generate transgenerational maternal effects that, when offspring growth occurs on drought-stressed host plants, extends the time to adult maturation, and results in small sized individuals. Potentially these maternal effects could influence population dynamics by influencing recruitment rates and, because small-sized adults have lower investment in reproduction (Gibbs et al. 2012; Vande Velde et al. 2013), by reducing population growth rates.

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Data Accessibility

Data from this study are available for download from the NERC Environmental Information Data Centre: (unique DOI to be provided on publication).

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Table 1. Post-hatching larval performance for offspring from eggs laid on days 2, 4, 6, 8 and 10 days of oviposition: mean offspring trait values (± SE) across maternal flight treatments (where C = control, no forced flight, and F = forced flight).

	Mean offspring trait value across flight treatments					
	Larval development time (days)		**Pupal mass (mg)**		**Dry thorax mass (mg)**	
Day of oviposition	**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)

Figure 1. Relationship between pupal mass (mg) and the day on which the egg was laid (i.e. maternal age), for offspring from mothers that were forced to fly (dashed line) and control mothers (solid line)

Figure 2. Relationship between larval development time (days) and the day on which the egg was laid (i.e. maternal age), for offspring from mothers that were forced to fly (dashed line) and control mothers (solid line)

Figure 1

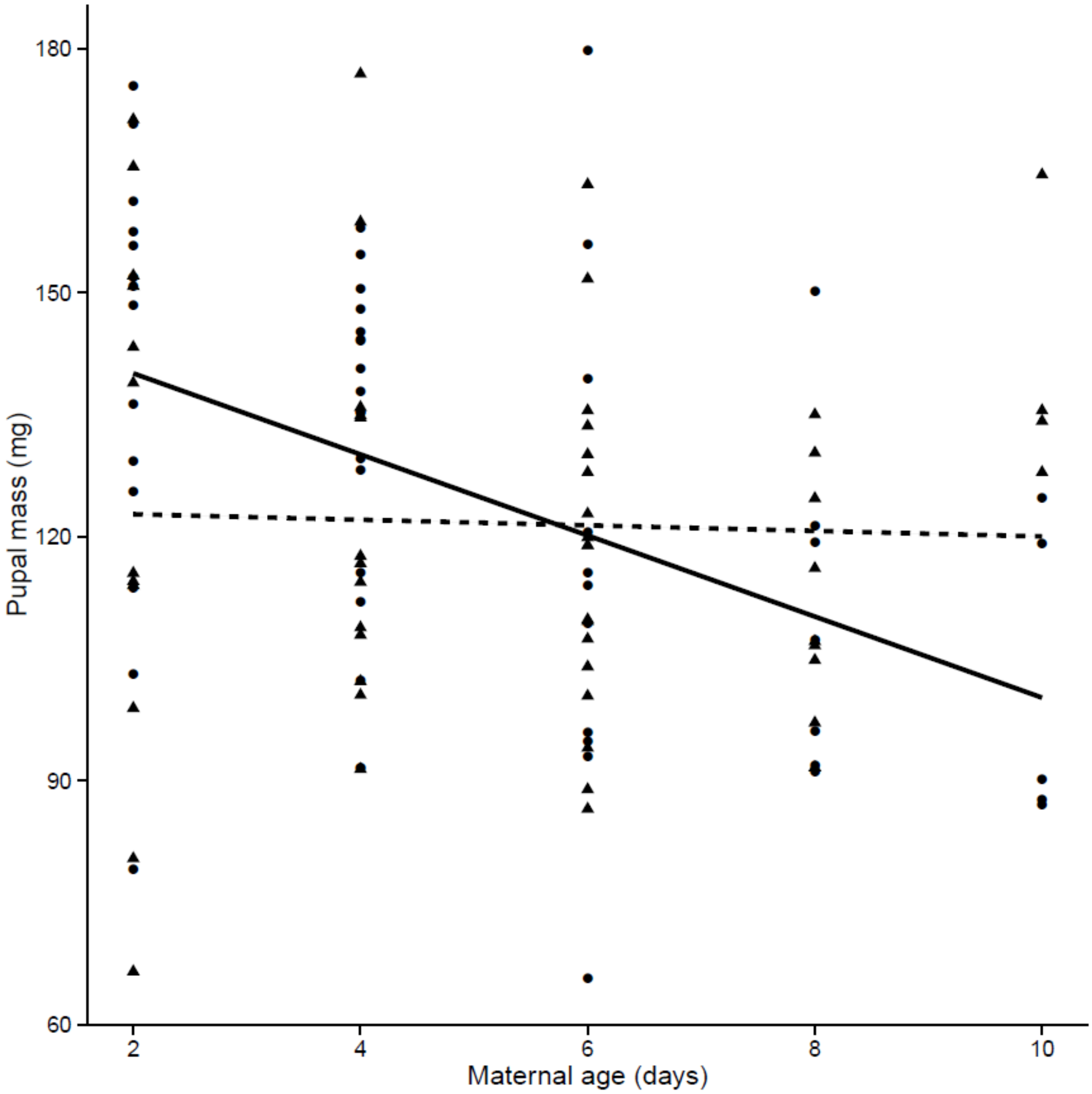

Figure 2