Evol Ecol (2013) 27:221–234
DOI 10.1007/s10682-012-9580-4

ORIGINAL PAPER

Effect of larval food stress on male adult behaviour, morphology and reproductive investment in the butterfly *Pararge aegeria*

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Received: 5 February 2012 / Accepted: 11 May 2012 / Published online: 9 June 2012
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Abstract Males of several animals increase their reproductive success by territorial behaviour. In butterflies, males may defend a territory (i.e., territorial perching tactic), but this is assumed to be an energetically costly way to locate mates. Limitations of the energy budget may affect fight performance, and may, consequently, force males to adopt an alternative non-territorial searching behaviour (i.e., patrolling tactic) to maximize reproductive success. In this study, we tested to what extent behavioural tactics adopted by adult males of the butterfly *Pararge aegeria* (L.) were affected by the nutritional conditions during the larval stage. We compared the occurrence of territorial versus patrolling behaviour, lipid mass, flight muscle ratio, metabolic rate and spermatophore production of low quality males that were reared as a larva on drought-stressed host plants and control, high quality males. Low quality males were less likely to adopt the territorial perching tactic and emerged as adults with lower lipid mass than high quality males, but they were able to restore their lipid mass through adult feeding (and perhaps the breakdown of flight muscles). Host plant quality also affected spermatophore size. Independent of the larval food treatment, territorial perching males metabolised more lipids than non-territorial males, produced larger spermatophores and copulated for longer than males adopting non-territorial behaviour. We discuss the results relative to the co-existence of the behavioural tactics (perching and patrolling).

Keywords Nutrition · Host plant quality · Lipids · Spermatophore · Alternative tactics · Territoriality · Mate location · Perching · Patrolling

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Introduction

In territorial species, the ability of males to monopolize a suitable territory is usually linked to mating success (Andersson 1994). But not all individuals are equally capable of obtaining and holding a territory. Individuals may adopt alternative behavioural tactics to enhance mating opportunities (see Gross 1996 for review). Alternative behaviours may arise in response to frequency- and condition-dependent selection (Maynard-Smith 1979). Alternative behaviours may not be fixed phenotypes, but result from genotype x environment interactions (Gross 1996; Gross and Repka 1998a, b).

In holometabolous organisms, like butterflies, males can be affected by the environment at the adult stage, but also by the conditions experienced during larval development. Butterflies have access to sources of nutrients at the larval stage that are unavailable (or at least more limited) at the adult stage (e.g. Baker and Baker 1973; Boggs and Ross 1993; O'Brien et al. 2005). Hence, larval host plant quality is a key determinant of the adult's phenotype and performance (Moreau et al. 2007). Variation in larval and adult food availability and quality is likely to be common in nature, and may affect resource allocation to life-history traits (e.g. Delisle and Hardy 1997; Moreau et al. 2007). Plaistow and Tsubaki (2000) showed, for example, that flight muscles and body lipid content was much reduced in males and females of the damselfly *Mnais costalis* that were reared under poor nutritional conditions.

In this study, we are interested in the issue of the co-occurrence of alternative mate-locating tactics. In butterflies, alternatives may include territorial perching and non-territorial patrolling (Scott 1974). Few studies have addressed the issue of energy budgets and physiological limits to male flight performance in the context of mate locating behaviour (e.g. Gwynne 1990; Gwynne and Simmons 1990; Proctor 1992). Warburg and Yuval (1997) showed, for example, in Mediterranean fruit flies that nutritional status affected the reproductive tactic a male adopted.

Besides physiological measures, functional morphology may also be of significance to male mate-locating behaviour. One of the most studied morphological traits in insects with respect to acceleration and manoeuvrability is the ratio of thoracic mass to total body mass, i.e., flight muscle ratio (FMR: Marden 1989; Berwaerts et al. 2002). Males adopting alternative mate locating tactics may differ in functional morphology. Territorial perching requires a design that favours high acceleration and manoeuvrability, which corresponds to high trait values of wing loading, wing aspect ratio and relative thoracic mass, while patrolling requires traits related to different flight performance, such as those which maximise aspects of flight endurance (Wickman 1992; Van Dyck 2003).

A reduction in larval food quality or quantity generally results in reduced adult body size and lipid content with negative effects on female fecundity (for review, see: Awmack and Leather 2002; for example, see: Fischer and Fiedler 2001), and on male reproductive success (e.g., Otronen 1995; Delisle and Hardy 1997). Butterfly reproduction is a nutrient-limited process (Boggs 1997). Ejaculates transferred by male lepidopterans contain proteins and lipids and their production represents a significant energetic cost (Oberhauser 1992; Svärd and Wiklund 1989; Lauwers and Van Dyck 2006). Consequently, energy available for flight may restrict male mating success; this is particularly the case in species that do not replenish their nutritional and energetic resources at the adult stage (Kemp & Alcock 2003).

In this paper, we tested to what extent behavioural tactics adopted by adult males of the butterfly *Pararge aegeria* (L.) are constrained by the nutritional conditions during the larval stage. In this species, perching males defend sunlit spots on the forest floor where

they wait for females, while patrolling males actively search for females through larger areas in the habitat. Gibbs et al. (2004) suggested that males reared on stressed host plants are able to compensate for being small by adopting the perching tactic to maximize female encounter rate. However, the energetic cost of obtaining and maintaining a territory is typically high (e.g. Yuval et al. 1998; Plaistow and Siva-Jothy 1996; Plaistow and Tsubaki 2000). Therefore, males should only be able to defend a territory if they have a sufficient amount of resources to do so. Flight performance varies between males practising the perching and patrolling tactics; perching males show a high frequency of take-off flights and aerial combats with high levels of manoeuvrability, whereas patrolling males show longer flight periods. Powerful take-offs are known to be the most energy demanding type of locomotion (Dudley 2000). By varying host plant quality through a drought-stress treatment, we have manipulated male energy level to determine the impact on male mate location behaviour, functional morphology, metabolic rate and spermatophore production. Hence, we predict that low quality males, fed as larvae on drought-stress host plants of lower nutritional quality, will be less able to defend a territory compared to high quality males, and that they will therefore preferentially adopt less costly, non-territorial behaviour. As adult males strongly depend on their larval-derived resources for reproduction (i.e. mate location, courtship, spermatophore production), we also predict males reared on poor host plant quality to be constrained in their reproductive investment measured by assessing the spermatophore mass, fertile sperm number and copulation duration. Finally, we also explored effects of the treatment on the flight muscle ratio and resting metabolic rate.

Materials and methods

Laboratory breeding and larval food stress

All butterflies used in the experiments were reared from eggs laid by wild caught females of a woodland population in central Belgium (Bois de Lauzelle; 50°67′74″N, 4°59′78″E). Breeding took place under standardized environmental conditions in a climate room (Light–Dark: 16–8 h; 24 °C during the day and 16 °C at night). *P. aegeria* has different seasonal cohorts that experience different environmental conditions and that differ in flight morphology (Van Dyck and Wiklund 2002); spring males (first generation) have a more pronounced 'perching design' (i.e. larger relative thorax mass, wing loading and aspect ratio) than summer males (second generation). In our breeding stock, larvae were allowed to develop directly without diapause (Wiklund et al. 1983). We did a drought-stress experiment in the laboratory. The drought-stress treatment was similar to Talloen et al. (2004), with the grass *Poa trivialis* used as the host plant. Potted host plants of the control group had full access to water, whereas the drought-stressed host plants had been deprived of water for 10 days in the climate room before first instar caterpillars from the breeding program were transferred to them. During the period of larval feeding, the stressed host plants received a 1 day water/7 days drought regime. Talloen et al. (2004) showed that leaf nitrogen, carbon and water concentrations were lower in drought stressed-plants compared to the control group. Henceforth in the paper, we refer to the males of the control group as high quality males and to the males reared on the drought-stress treatment as low quality males. Hatched larvae were randomly transferred to similarly sized tufts of grass of one of the two treatments. Pupae were placed individually in transparent jars (125 ml) until adult eclosion. After 9 days of development, the pupae were weighed (Ohaus Explorer balance; accuracy: ±0.1 mg). Pupal mass correlated strongly with adult mass at eclosion ($R = 0.92$,

$P < 0.001$, N = 156), and was used as an index of adult body mass. Butterflies enclosed in the transparent jars and had access to a 10 % sugar-solution on cotton. Date of eclosion was recorded so that the adult age of each individual was known. Males were individually marked and kept in a cold room (Light–Dark: 10–14 h; 11 ± 2 °C) for the experiment.

Experimental design

Males of both treatment groups were released in two identical outdoor cages (9.0 × 3.7 × 1.8 m). 3 low quality males and 3 high quality males were introduced per cage (i.e. 12 males in total per session with two cages). The cages were covered by camouflage netting with cut holes of different size. This allowed the sun to enter the cage and hence to created the typical dappled light conditions of a forest with sunlit patches and shaded parts. There were wood chips on the floor of each cage and also small green artificial trees that served as perches for males. Outdoor cages have been used before to study male behaviour in *P. aegeria* (Leimar et al. 2003; Merckx et al. 2003; Kemp et al. 2006a). Males stayed for 2–3 days in the cage and had access to a 10 % sugar solution on cotton and in artificial flowers. We did repeated observation sessions to score the behaviour of each male (Between 9 a.m.–12 a.m and 1 p.m–4 p.m. during sunny days, >16 °C). This allowed assigning each male to one of two types: (1) a permanent percher (i.e., high fidelity to a sunlit patch, high level of aggression, high frequency of short inspection flights); and (2) a non-percher (i.e., males that were rarely or never observed to defend a sunlit patch and that regularly flew through the cage, eventually alternated by resting on the netting). Males that showed mixed behaviour (i.e., males that defended a sunlit patch during one period in their stay in the cage, but behaved as a non-percher for most of the time in the cage) were not considered, and hence excluded from the analyses. Indeed, in this study we were particularly interested in the impact of male nutritional status on the probability to adopt the territorial versus the non-territorial tactic. After their stay in the outdoor cage, males were transferred again to the climate room for mating with a virgin female.

Copulations and spermatophore measurements

Males were placed individually in a small cage (0.3 m^3) under standardized climate room conditions (see above). A virgin female of known age and pupal mass from the breeding stock was introduced in the cage. All females were also reared on poor quality host plants. Twelve cages (and thus pairs) were observed at the same time (i.e. 6 low quality males and 6 high quality males) in order to record copulation events and duration. Immediately after the copulation, females were killed by freezing (−20 °C) to recover the received spermatophore. Males were kept for 1 day in the cold room before measuring resting metabolic rate.

The bursa copulatrix was carefully dissected from the abdomen of the frozen females. We carefully removed the spermatophore from the bursa and measured its mass (Mettler Toledo-MT5 balance; accuracy: ±0.001 mg). Next, the spermatophore was placed in a drop of modified Barth Saline on a slide (Gurdon 1991). The spermatophore wall was ruptured by forceps to release the sperm that was gently stirred with a fine needle. Males transfer the eupyrene sperm in bundles which later break apart (Cook and Wedell 1996). In order to count the number of bundles, we fixed the drop with 70 % ethanol and coloured the DNA of the spermatozoids with 4′,6′-diamidino-2-phenylindole. After drying, the sperm bundles can be easily counted under microscope (100×; Epifluorescence Polyvar). In butterflies, each bundle contains 256 eupyrene sperm cells (Cook and Wedell 1996).

Metabolic rate

Resting metabolic rates (RMR) were measured by respirometry analysing the CO_2 production of a male at rest (Hack 1997). A flow-through system using a dual-sensor oxygen analyser (Sable Systems International Oxzilla II) connected to a CO_2 analyser (Sable Systems CA-10a) was used to measure the CO_2 emission. After flushing the metabolic chamber with CO_2-free and water-free room air, each butterfly was maintained in the metabolic chamber which consisted of hard plastic box with a total volume 25 cl at 30 °C. Water and CO_2 were removed of the chamber by air going through three successive columns containing silica gel, drierite and ascarite, respectively placed before the chamber. After 10 min, the chamber was flushed again at a flow rate of 630 ml/min (Sable Systems International subsample TR-SS3 pump) and CO_2 gas accumulated during this period in the chamber was measured. Chamber temperature varied less than 1 °C during any single trial (Physitemp Model BAT-12 digital thermocouple thermometer). Dry body mass was included as a covariate in the analyses of metabolic rate ($mlCO_2\ h^{-1}$), but in order to compare the results with the literature, we reported the metabolic rate corrected by the mean dry body mass (i.e. $mlCO_2\ g^{-1}\ h^{-1}$). At the end of the experiment, males were frozen at −20 °C for lipid extraction.

Lipid extraction and flight muscle ratio

Lipid reserves were compared between low quality males and high quality males that had flown in the cages. We included here two additional groups of individuals: (1) a sample of high and low quality males directly frozen at emergence to evaluate the impact of drought-stress on the accumulation of lipid reserves, and (2) a sample of males from both treatment groups that were maintained at rest under laboratory conditions during the same number of days to compare lipid consumption for somatic maintenance when males had access to sugar solution.

Lipids were extracted from the body using the method of Marden (1989). After removal of the wings, dry body mass was measured for each individual male. Body parts were dried to a constant weight at 70 °C. All individuals were individually placed into a paper filter and placed in refluxing diethyl ether in a Soxhlet apparatus for 8 h before being dried and weighed again (Mettler Toledo-MT5 balance; accuracy: ±1 μg). Lipid mass (mg) corresponds to the difference in weight before and after the extraction. Male body mass was included as a covariate in the analyses but, in order to compare the results with the literature, we also reported the results as the proportion of lipids on dry body mass (%).

In order to evaluate the FMR, we measured the ratio of the dry thorax mass of males after lipid extraction divided by the total dry body mass after lipid extraction (Mettler Toledo balance). Note that FMR-measures are not directly comparable with values in the literature and in our earlier papers as the prior lipid extraction will affect the absolute values.

Statistical analyses

Generalised linear models with a normal error distribution were used to analyse the effect of the drought-stress treatment, the behavioural tactic and their interaction effect on physiological (i.e. lipid reserves and resting metabolic rates), reproductive and morphological traits of males. Male age and male body mass (i.e. pupal mass and dry male body mass) were included as explanatory variables; for reproductive traits, female age and body

mass were also included. Data on lipid reserves and metabolic rate were log transformed to improve normality.

We used a multi-model inference approach based on AICc model selection to evaluate whether effects were significant (Anderson 2008; Burnham and Anderson 2002; Garamszegi 2011 and references therein). We fitted the full model and chosen submodels (consisting of all combinations of the explanatory variables with main effects remaining in the model if they figured in interaction terms) and computed for each model AICc value and its AICc weight. The AICc weight of each explanatory variable was computed as the sum of the weights for each model in which the explanatory variable is present; it is an estimate of the relative importance of each explanatory variable in terms of predictive power. Because models with interactions, but not the corresponding main effects, were not considered, the interaction terms appeared in fewer models than the main effects. This is likely to bias the final AICc weight of the explanatory variables, the weight of a rarer variable being the sum of a smaller number of terms. We therefore used a permutation test to assess whether the observed weight of each explanatory variable was significantly higher than expected under the null hypothesis (i.e. no relation between the response and the explanatory variable). This test comprises randomly shuffling the response variable column, disrupting any relationship that could exist with the explanatory variables, and fitting the full set of models to compute the weight of all explanatory variables, 1,000 random permutations were done to give the distribution of weights of explanatory variables under the null hypothesis. Then, a P value was computed for each explanatory variable as the proportion of the 1,000 random permutations for which the variable weight was higher than its value in the observed dataset, representing, as a classical P value, the probability to obtain a value equivalent or higher to the observed AICc weight of the variable if it had no predictive value on the response. Variables with a P value <0.05 were considered as significant and interpreted as having an effect on the response variable. The parameter estimate (and associated SE) for each variable was computed as the average over all models, weighted by the AICc weight of each model, considering a value of 0 for the parameter estimate (and missing value for its SE) when the variable was not present in a model; this is the shrinkage approach to model averaging (Burnham and Anderson 2002; Anderson 2008). These analyses were implemented using a program written for SAS (v. 9.3, SAS Institute Inc.; unpublished program written by N. Schtickzelle, UCL Louvain-la-Neuve).

A one-tailed Fisher's exact test was used to test the effect of the larval food treatment on the behavioural tactics adopted by the males in the outdoor cages.

Two-way ANOVAs were used to compare lipid reserves between the groups of males that flew in the cages, males that rested in the laboratory in small recipients and males that were killed at adult eclosion relative to drought-stress treatment. Average values are reported $\pm$SE.

Results

We mainly focus on the direct effects of the larval food treatment on adult phenotypic traits. Independently of the larval feeding treatment, we also report indirect effects as several behavioural, morphological and life-history traits are interconnected. We also briefly report effects of covariates that were in the models, but which were not key factors of interest here (e.g. age).

Host-plant stress and behavioural tactics

High and low quality males were able to defend a territory, but in line with our prediction, low quality males adopted the more energetically costly territorial perching behaviour less frequently than high quality males: 49 % (17/35) for low quality males versus 72 % (26/36) for high quality males (one-tailed Fisher's exact test: $P = 0.036$).

Metabolic rate

There was no evidence of an effect of larval stress treatment on resting metabolic rate in adults (10.97 ± 0.83 ml CO_2 g^{-1} h^{-1} for high quality males vs. 9.97 ± 0.48 ml CO_2 g^{-1} h^{-1} for low quality males). Independently of the larval food treatment, the behavioural tactic did not affect the resting metabolic rate (11.16 ± 0.66 ml CO_2 g^{-1} h^{-1} for territorial males vs. 9.37 ± 0.61 ml CO_2 g^{-1} h^{-1} for non-territorial males) (Table 1). The resting metabolic rate increased with male body mass (Table 1).

Male reproductive investment

The larval stress treatment affected spermatophore production; low quality males produced smaller spermatophores compared to high quality males (0.332 ± 0.006 mg for high quality males vs. 0.309 ± 0.006 mg for low quality males). In addition, territorial males produced larger spermatophores than non-territorial males (0.328 ± 0.007 and 0.297 ± 0.008 mg, respectively; Table 2). Female body mass also tended to be positively correlated with spermatophore mass; larger females tended to receive on average larger spermatophores (Table 2).

The larval food stress treatment did not affect sperm production. Also, sperm production did not differ between the behavioural tactics. Sperm number was only affected by male age, with older males transferring more sperm bundles (Table 2).

The larval food stress treatment had no effect on the copulation duration. Independently, territorial males copulated for longer than did non-territorial males (22.34 ± 0.66 and

Table 1 Analysis of resting metabolic rate

Parameter	Freq (%)	AICc weight (%)	A priori weight	*P* value	Parameter estimate	SE
Ln(resting metabolic rate) (n = 63)						
Intercept	100	1.00	1.00		−2.6822	0.0202
Male age	50	0.57	0.29	0.1	0.0045	0.0032
Male dry body mass	**50**	**0.97**	**0.29**	**0.004**	**0.0285**	**0.0090**
Behaviour non-percher	60	0.43	0.34	0.311	0.0115	0.0115
Treatment food stress	60	0.28	0.34	0.791	0.0002	0.0062
Behav × TR	20	0.05	0.04	0.408	−0.0012	0.0016

Multi-model inference based on AICc weight of each factor expressing the probability of importance in explaining the variation in the response variable (Y = ln-transformed RMR). The *P* value, evaluating the significance of the difference between the AICc weight and the a priori weight, helps to interpret AICc weight value despite differences in the frequency of the variable in the models (Freq) (see text for details on the methodology). Factors with high support are indicated in bold. Model-averaged parameter estimate and standard error is given for each factor. For the categorical trait 'behavioural tactic', the value of territorial males was set as a reference. For the categorical trait 'food stress treatment', the value of the high quality males was set as a reference. The complete model was: Ln(RMR) = Male age (MA) + Male dry body mass (MDB) + Behavioural tactic (Behav) + Food stress treatment (TR) + Behav × TR

Table 2 Analysis of reproductive investment

Parameter	Freq (%)	AICc weight (%)	A priori weight	*P* value	Parameter estimate	SE
Spermatophore mass (n = 60)						
Intercept	100	1.00	1.00	–	0.2483	0.0549
Male age	50	0.30	0.28	0.443	0.0014	0.0021
Male body mass	50	0.25	0.28	0.656	−0.0007	0.0014
Female age	50	0.62	0.29	0.082	0.0015	0.0010
Female body mass	**50**	**0.65**	**0.29**	**0.048**	**0.0004**	**0.0002**
Behav non-percher	**60**	**0.84**	**0.33**	**0.026**	**−0.0110**	**0.0049**
TR food stress	**60**	**0.79**	**0.33**	**0.033**	**−0.0085**	**0.0044**
Behav × TR	20	0.17	0.04	0.073	0.0005	0.0010
Bundles number (n = 60)						
Intercept	100	1.00	1.00	–	49.7768	6.99092
Male age	**50**	**0.97**	**0.28**	**0.003**	**6.6684**	**2.05932**
Male body mass	50	0.23	0.28	0.97	−0.0319	0.47819
Female age	50	0.29	0.29	0.476	0.1144	0.16671
Female body mass	50	0.24	0.28	0.935	0.0057	0.02763
Behaviour non-percher	60	0.26	0.33	0.956	−0.1411	0.61609
Treatment Food stress	60	0.35	0.33	0.441	−0.7215	0.85658
Behav × TR	20	0.02	0.04	0.864	0.0041	0.04806
Copulation duration (n = 60)						
Intercept	100	1.00	1.00	–	25.6704	3.14186
Male age	50	0.27	0.29	0.594	0.0787	0.15504
Male body mass	50	0.43	0.28	0.188	−0.2686	0.24646
Female age	**50**	**0.99**	**0.29**	**0.001**	**−0.5425**	**0.11645**
Female body mass	50	0.37	0.28	0.274	0.0109	0.01149
Behaviour non-percher	**60**	**0.81**	**0.33**	**0.036**	**−0.9149**	**0.43035**
Treatment food stress	60	0.34	0.32	0.423	0.1295	0.18087
Behav × TR	20	0.08	0.04	0.206	0.0331	0.05159

Multi-model inference based on AICc weight of each factor expressing the probability of importance in explaining the variation in the response variable (Y = Spermatophore mass, bundles number and copulation duration, respectively). The *P* value, evaluating the significance of the difference between the AICc weight and the a priori weight, helps to interpret AICc weight value despite differences in the frequency of the variable in the models (Freq) (see text for details on the methodology). Factors with high support are indicated in bold. Model-averaged parameter estimate and standard error is given for each factor. For the categorical trait 'behavioural tactic', the value of territorial males was set as a reference. For the categorical trait 'food stress treatment', the value of the high quality males was set as a reference. The complete model was: Y = Male age (MA) + Male body mass (MB) + Female age (FA) + Female body mass (FB) + Food stress treatment (TR) + Behavioural tactic (Behav) + Behav × TR

21.14 ± 0.94 min, respectively; Table 2). In addition, copulations with young females lasted for longer than with older females (Table 2).

Lipid reserves

Low quality males emerged as adults with a significantly lower ($t_{93} = -3.44$, $P = 0.0009$) relative lipid mass (lipid mass/total dry body mass ratio: 26.8 ± 4.8 %) than high quality

males (30.1 ± 3.8 %), while total dry body mass did not differ significantly ($t_{93} = 0.84$, $P = 0.41$) between low quality males (15.88 ± 0.27 mg) and high quality males (15.51 ± 0.35 mg) (Fig. 1). There was no longer such a difference between the two treatments when males were kept for 2–3 days in the laboratory in small jars (i.e. a low activity treatment) or when they had flown in the outdoor flight cages (Fig. 1). Both in the jars and the outdoor cage, males had access to honey water. Lipid reserves were larger in freshly emerged males compared to males that were kept in jars and in the outdoor cages (Two-way ANOVA: ln(Lipid mass) = Groups + Treatment + Groups × Treatment; $F_{5,333} = 26.24$, $P < 0.0001$; Fig. 1).

After flight in the outdoor cage, lipid mass did not differ between low quality males and high quality males (mean lipid mass for low quality males vs. high quality males: 2.76 ± 0.13 and 2.40 ± 0.10 mg, respectively; mean dry body mass for low quality males vs. high quality males: 13.24 ± 0.28 and 12.57 ± 0.23 mg; Table 3). However, behavioural tactic affected lipid mass; males adopting the territorial perching tactic had a lower lipid mass than males adopting a non-territorial behaviour (Proportion of lipids on dry body mass: 19.00 ± 0.05 and 23.00 ± 0.05 %, respectively; Table 3). There was also an interaction effect between larval food treatment and behavioural tactic; territorial males showed a much larger difference in lipid mass between the two larval food treatments than non-territorial males (Table 3).

Flight muscle ratio

Males of the larval food stress group had slightly lower FMR ratios than high quality males (Table 4; FMR: 56.7 ± 1.3 and 60.0 ± 0.9 %, respectively). Independently, FMR ratios were not significantly different between the behavioural tactic (Table 4; 58.9 ± 1.1 and 57.6 ± 1.1 % for territorial and non-territorial males, respectively). One potential pitfall when comparing flight muscle allocation is that the higher FMR may reflect greater thorax mass or alternatively lower abdomen mass. Therefore, we additionally analyzed thorax

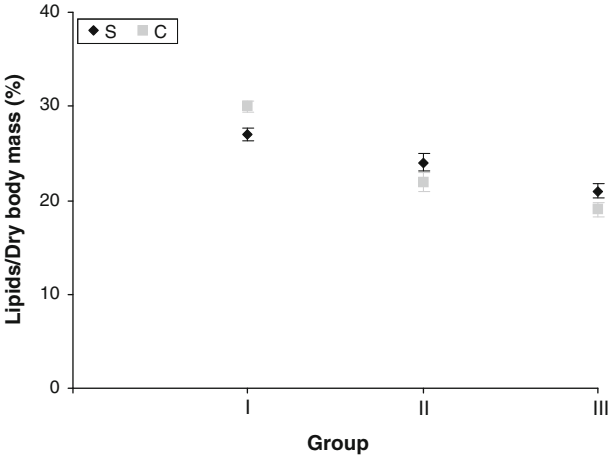

Fig. 1 Mean (±SE) relative lipid mass (i.e., ratio of lipids/ dry body mass) of adult males that were reared on drought-stressed host plants (S) or on control plants of high quality (C) when they enclosed as adult butterfly (I), after 2–3 days under laboratory conditions with only low flight activity and access to honey water (II), and after 2–3 days in an outdoor flight cage with access to honey water (III)

Table 3 Analysis of lipids reserves

Parameter	Freq (%)	AICc weight (%)	A priori weight	*P* value	Parameter estimate	SE
Ln(lipid mass) (n = 71)						
Intercept	100	1.00	1.00	–	0.9355	0.0391
Male age	50	0.37	0.29	0.305	−0.0171	0.0189
Male dry body mass	50	0.24	0.29	0.992	−0.0012	0.0090
Behaviour non-percher	**60**	**0.92**	**0.35**	**0.016**	**0.0967**	**0.0381**
Treatment food stress	60	0.62	0.35	0.115	0.0289	0.0266
Behav × TR	**20**	**0.30**	**0.05**	**0.035**	**−0.0182**	**0.0171**

Multi-model inference based on AICc weight of each factor expressing the probability of importance in explaining the variation in the response variable (Y = ln-transformed lipid mass). The *P* value, evaluating the significance of the difference between the AICc weight and the a priori weight, helps to interpret AICc weight value despite differences in the frequency of the variable in the models (Freq) (see text for details on the methodology). Factors with high support are indicated in bold. Model-averaged parameter estimate and standard error is given for each factor. For the categorical trait 'behavioural tactic', the value of territorial males was set as a reference. For the categorical trait 'food stress treatment', the value of the high quality males was set at zero as a reference. The complete model was: Ln(Lipid mass) = Male age (MA) + Male dry body mass (MDB) + Behavioural tactic (Behav) + Food stress treatment (TR) + Behav × TR

mass and dry body mass as dependent variables. Lower FMR of low quality males tended to result from a higher total dry body mass (Final model: AICc weight of the variable larval food treatment = 0.65, $P = 0.098$, N = 71; Parameter estimates with control males fixed to zero: 0.23 ± 0.15) rather than a difference in the thorax mass per se (Final model: AICc weight of the variable larval food treatment = 0.28, $P = 0.829$, N = 71).

Discussion

Larval host plant stress has the potential to affect several behavioural, morphological and life history traits in adult *P. aegeria* butterflies (this study and see also Talloen et al. 2004; Gibbs et al. 2012). Here, we showed that low quality males were less likely to adopt territorial perching behaviour as an adult. Lower nutrient levels also resulted into the production of smaller spermatophores, and lower adult initial lipid mass as well as smaller investment in the flight muscle ratio. Independent of the larval feeding treatment, we found several physiological differences relative to the mate locating tactic adopted by a male; perchers used more of their lipids, produced larger spermatophores and copulated for longer than males adopting non-territorial behaviour.

The host plant stress treatment reduced the relative lipid mass of emerging males. But things were different after several days of activity as there was no longer such a treatment-dependent difference in males that were kept in small jars in the laboratory or in males that had flown in outdoor cages. Hence, males were able to compensate for their lower lipid content, probably by compensatory feeding. Males (both in the laboratory and the outdoor cages) had full access to honey water allowing them to synthesize lipids from adult feeding sources (Canavoso et al. 2001; Vande Velde and Van Dyck 2012). Further work using cameras at feeding sites is needed to confirm whether the low quality males fed more or more frequently than high quality males. The larval feeding treatment may have shifted males from being largely capital breeders to partly income breeders (e.g. May 1992). High-nutritional

Table 4 Analysis of flight muscles ratio (FMR)

Parameter	Freq (%)	AICc weight (%)	A priori weight	*P* value	Parameter estimate	SE
FMR (n = 71)						
Intercept	100	1.00	1.00	–	0.5835	0.0082
Male age	50	0.57	0.29	0.084	−0.0086	0.0063
Male dry body mass	50	0.24	0.29	0.981	−0.0002	0.0020
Behaviour non-percher	60	0.39	0.35	0.362	−0.0035	0.0043
Treatment food stress	**60**	**0.76**	**0.34**	**0.047**	**−0.0135**	**0.0071**
Behav × TR	20	0.071	0.05	0.297	−0.0001	0.0006

Multi-model inference based on AICc weight of each factor expressing the probability of importance in explaining the variation in the response variable (Y = FMR). The *P* value, evaluating the significance of the difference between the AICc weight and the a priori weight, helps to interpret AICc weight value despite differences in the frequency of the variable in the models (Freq) (see text for details on the methodology). Factors with high support are indicated in bold. Model-averaged parameter estimate and standard error is given for each factor. For the categorical trait 'behavioural tactic', the value of territorial males was set as a reference. For the categorical trait 'food stress treatment', the value of the high quality males was set as a reference. The complete model was: FMR = Male age (MA) + Male dry body mass (MDB) + Behavioural tactic (Behav) + Food stress treatment (TR) + Behav × TR

status may be a prerequisite to adopt energy-demanding behaviours like territorial perching (e.g. Warburg and Yuval 1997). Energy-depleted males may first invest in foraging to complete their reserves instead of starting immediately to locate mates (Proctor 1992). Males that defend non-resource-based territories like *P. aegeria* have little opportunity to forage (e.g. Kemp and Wiklund 2001; Kemp and Alcock 2003). Depletion of energy reserves can exclude males from costly territory holding (Otronen 1995; May 1992) making males more likely to adopt less costly, non-territorial tactics to find mates. *P. aegeria* are rarely seen on flowers but feed on honey dew and rotten berries (Shreeve 1986), but little is known about food intake in typical perching and patrolling males. Adult food intake in butterflies is largely limited to carbohydrates, and much less to other components like proteins for which they depend on larval intake (Boggs 2003).

Low quality males tended to have, after a few days of activity, lower FMR than high quality males. Our results suggested that this lower FMR in males of the larval stress group resulted from higher abdomen mass rather than lower thorax mass. Nevertheless, we cannot exclude initial differences in thorax mass (i.e. at emergence) between the groups. In butterflies (including *P. aegeria*), there is evidence for compensating nutrient depletion via the breakdown of thoracic flight muscles (Karlsson 1994; Stjernholm et al. 2005 and references therein). Low quality males could have reallocated nutrients from their flight muscles into their reproductive system. Indeed, low quality males produced smaller spermatophores, but there was no significant difference for the number of sperm bundles.

While low quality males adopted preferentially the non-territorial tactic, some individuals of the poor larval food treatment group became successful territory holders. It is possible that some genotypes are better able to deal with poor quality food than others in building suitable phenotypes that allow aggressive perching. Moreover, this is in line with the significant interaction effect that we observed for lipid mass between behavioural tactic and larval food treatment. Perching males that experienced a development under stress had more lipid contents after the flight experiment than did perching males from the high quality group. For non-perching males, on the other hand, there was hardly any difference

in lipids between the treatment groups. This non-intuitive observation may suggest that genotypes may differ in their propensity to adopt perching tactic relative to lipid mass, but their propensity for doing so may be altered under stress. So, different threshold levels could exist for the relationship between lipid content and behavioural tactic relative to stress. In other words, the threshold for lipids at which males reared under stress adopt the perching tactic may have been higher that for males reared on good quality plants. One could argue that only a subsample of the genotypes better able to deal with the stress conditions (e.g. males with high lipid content) would be able to adopt a perching tactic. This scenario may explain why territorial males reared on stressed plants have higher mean lipid contents than territorial males from the high quality group. However, an alternative interpretation is that perching males of both treatment groups differed in the expression of the behavioural tactics, either by differences in amount of feeding or in the intensity of flight performance, which would also affect lipid mass at the end of the flight experiment. Further work with a full quantitative genetic approach would be necessary to test such G x E interaction effects on the adoption of alternative mate locating tactics.

Due to the high physiological cost of spermatophore production in Lepidoptera (e.g. Oberhauser 1992; Svärd and Wiklund 1989; Lauwers and Van Dyck 2006), the size of the spermatophore has often been used as a measure of male quality (reviewed by Marshall and McNeil 1989). We showed that *P. aegeria* males reared on low-quality plants produced 7 % smaller spermatophores than high quality males. Females receiving a small spermatophore (from a recently mated male) produce fewer offspring than females that received a normally-sized spermatophore (Lauwers and Van Dyck 2006). Females that received a small spermatophore showed a greater propensity to remate with another male (e.g. Vande Velde et al. 2011; Torres-Vila et al. 1997). Hence, larval diet may affect male reproductive success. The number of sperm bundles did not differ between the treatment groups, but other qualitative aspects could differ among spermatophores of differently treated males. For example, Delisle and Bouchard (1995) observed in *Choristoneura rosaceana* that whereas spermatophore mass of males reared on low quality food was only 33 % smaller than of males reared on high quality food, the fecundity of females that mated with males reared on low quality food was 40 % lower. In the same vein, Delisle and Hardy (1997) showed that males reared on an artificial diet produced larger spermatophores than males reared on high quality natural food, but their reproductive success was actually lower. Therefore, the quality of the spermatophore can be reduced independently of its mass (Marshall and McNeil 1989).

Given the significant cost to produce spermatophores, one may predict males to modulate their reproductive investment relative to female quality. In line with previous studies (e.g. Rutowski et al. 1983), males tended to transfer larger spermatophores to large females. Nevertheless, sperm quantity was not affected by female traits. It again suggests that the spermatophore mass may vary independently of its quality (see above). Moreover, larval food quality is clearly able to affect various life history traits (Boggs and Ross 1993; Talloen et al. 2004; Pellegroms et al. 2009; Gibbs et al. 2012) and may have consequences for male attractiveness (Kemp et al. 2006b). That is another interesting aspect that warrants further experimental work.

Acknowledgments Thanks are due to Christophe Pels and Gaëlle Rigaux for practical assistance, to Augustin Joiris for help with respirometry, to Gilles San Martin for statistical advice and to Katherine Mitchell for language editing. L. V. V. was supported by a FRIA PhD grant from the Fund for Scientific Research-FNRS (F.R.S.-FNRS). This research was also supported by a ARC-Research grant of the Académie Louvain/UCL (ARC Grant 10/15-031) to HVD and NS. This is publication no BRC 262 of the Biodiversity Research Centre (UCL).

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