



## Research

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# Nectar resource limitation affects butterfly flight performance and metabolism differently in intensive and extensive agricultural landscapes

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Flight is an essential biological ability of many insects, but is energetically costly. Environments under rapid human-induced change are characterized by habitat fragmentation and may impose constraints on the energy income budget of organisms. This may, in turn, affect locomotor performance and willingness to fly. We tested flight performance and metabolic rates in meadow brown butterflies (*Maniola jurtina*) of two contrasted agricultural landscapes: intensively managed, nectar-poor (IL) versus extensively managed, nectar-rich landscapes (EL). Young female adults were submitted to four nectar treatments (i.e. nectar quality and quantity) in outdoor flight cages. IL individuals had better flight capacities in a flight mill and had lower resting metabolic rates (RMR) than EL individuals, except under the severest treatment. Under this treatment, RMR increased in IL individuals, but decreased in EL individuals; flight performance was maintained by IL individuals, but dropped by a factor 2.5 in EL individuals. IL individuals had more canalized (i.e. less plastic) responses relative to the nectar treatments than EL individuals. Our results show significant intraspecific variation in the locomotor and metabolic response of a butterfly to different energy income regimes relative to the landscape of origin. Ecophysiological studies help to improve our mechanistic understanding of the eco-evolutionary impact of anthropogenic environments on rare and widespread species.

## 1. Introduction

Much of the success of insects relates to their ability to fly [1]. The power for flight comes from muscles operating against the rigid cuticular exoskeleton and is highly energy demanding [2]. Energy acquisition and adequate metabolic pathways for efficient energy expenditure are of primary importance to fuel the flight machinery. Flying insects like butterflies use flight for almost all adult activities (e.g. search of mates or host plants, predator escape and dispersal).

Adult butterflies use the energy acquired during the larval stage (stored as abdominal lipids) and also acquire carbohydrates from adult foraging [3]. The degree to which adults rely on newly acquired versus stored resources places the species along the income–capital breeding continuum [4]. Income breeders rely on nectar consumption (or alternative feeding resources). Nectar intake positively affects life-history traits in several species [5,6]. Nectar is composed of water and sugars (mainly sucrose, fructose and glucose), and also amino acids [7]. Nectar quantity, composition and concentration vary between flower species [7–9]. Foraging behaviour will affect the energy budget available for fuelling flight. The allocation of energy at the adult stage is usually characterized by trade-offs between somatic maintenance, reproduction, other activities and further energy storage [3]. Resting metabolic rate (RMR) measured on quiescent individuals at a standard temperature and corrected for body mass ('standard metabolic rate' in ectotherms; [10]) estimates the

compulsory energy cost of self-maintenance that is central to life-history theory [11]. Butterflies are popular study systems for dispersal, habitat fragmentation and meta-population dynamics, and the measurement of flight metabolic rate (FMR) and flight performance have recently attracted much attention (e.g. [12–14]).

Anthropogenic environments strongly affect the availability of ecological resources (*sensu* [15]). Finding sufficient amounts of high-quality resources may become challenging as resources are scattered across the landscape. Intensively managed agricultural landscapes offer only limited and highly fragmented semi-natural habitats which are important to several flower-visiting insects [16]. Insects may then be forced to cover long distances to complement their resource needs [17].

Therefore, fragmented, agricultural landscapes could select for different levels of mobility and energy metabolism for dealing with such a different energy income on the one hand and altered performance levels on the other. Butterfly studies have shown flight-related morphological differences relative to the degree of habitat fragmentation [18,19]. Responses to habitat fragmentation were either towards higher mobility (e.g. [20]) or lower mobility (e.g. [21]). Other factors may also play a role in this context (e.g. population age, sex and species; [22–24]).

Higher flight performance may require more energy allocated to muscle power output and flight endurance. Hence, less energy is available for other activities, unless the total energy budget would increase under such conditions. However, low flower diversity and abundance—i.e. reduced nectar supply—in intensively managed agricultural landscapes may jeopardize life strategies that rely on higher energy consumption for flight. Food stress has been shown to reduce RMRs in *Drosophila melanogaster* [25] and two butterfly species [5]. Under strong food stress, carbohydrate resources will be depleted and the insect has to switch from carbohydrates to reserve lipids as metabolic fuel [26]. Selection for stress resistance may lead to lower RMRs [27], or alternatively, to higher accumulation of lipids and carbohydrate (energy) rather than a reduction in RMR (e.g. [25]). FMR and flight capacities did not vary with food access at the adult stage in two butterflies [5]. The degree of plasticity for FMR may nevertheless vary significantly at the intraspecific level ( $G \times E$  interactions; [28]).

Here we used the meadow brown butterfly *Maniola jurtina* in a series of experiments to test the effect of nectar quality and quantity on flight performance, and both standard and FMRs relative to the landscape of origin. For the latter, we contrasted individuals of relatively intensively managed agricultural landscape (IL; nectar-poor environment) against individuals of extensively managed agricultural landscape (EL; nectar-rich environment).

Exposure to reduced nectar quantity is predicted to decrease RMR, but much less so for FMR and flight capacity. IL individuals are predicted to have stronger flight capacities and higher FMR, combined with slower RMR. In other words, we predict butterflies of IL origin to invest more in traits that relate to flight performance, and to be better able to increase energy expense for flight independently of the energy used for somatic maintenance. As metabolic rate can be a plastic trait [28], responses to nectar treatment may differ between butterflies of different landscape of origin. If nectar quantity reduction is strong, it will provoke depletion

of available carbohydrates and the butterfly has to switch from carbohydrates to lipid reserves to fuel flight. Effects of nectar quality are, however, harder to predict based on current knowledge. Adaptations to adult food stress in IL butterflies could result in higher levels of lipid storage, higher body mass and/or slower resting metabolism to save energy under such challenging conditions. Assuming local adaptation, we predict IL butterflies to be less affected by imposed food stress.

## 2. Material and methods

### (a) Study species

*Maniola jurtina* is a common, widespread European grassland butterfly. It is univoltine and overwinters as young larva. Caterpillars feed on a variety of grasses; adults use nectar and they feed on a variety of flower species, but show preference for species like *Centaurea jacea* [29]. In the absence of the preferred nectar source, they feed on alternative species such as *Trifolium pratense* [30]. Within a zone of suitable habitat, adults typically show resource-searching flights which are sinuous and relatively slow, but the species is also capable of making less frequent, longer dispersal movements (greater than 1 km) and is known to exhibit a different type of flight outside habitat [31,32]. We captured wild, recently emerged *M. jurtina* butterflies (i.e. no or non-significant wing wear) on 10 different meadows (i.e. different local populations) in central and south Belgium. Half of them were under extensive grassland management (i.e. low intensity late mowing that resulted in relative flower-rich meadows) surrounded by other such meadows, pastures and forest. The other half was under a more intensive grassland management (i.e. more frequent mowing and less flower-rich) surrounded by arable land with crop culture and other such grasslands. We will refer to the categories as extensive and intensive agricultural landscape (EL and IL, respectively).

### (b) Experimental set-up

We used an experimental set-up in outdoor flight cages separated in four compartments with each treatment representing a combination of nectar quality and quantity: 100 flowerhead units of the preferred *C. jacea* (C100), 10 of *C. jacea* (c10), 100 of the non-preferred *T. pratense* (T100) and 10 of *T. pratense* (t10). Individual flowers of *T. pratense* contain slightly more nectar than flowers of *C. jacea* (mean quantity of nectar in a single flower  $\pm$  s.e.m.:  $0.21 \pm 0.02 \mu\text{l}$  and  $0.18 \pm 0.02 \mu\text{l}$ , respectively; based on [8]), whereas they have similar numbers of simultaneously open flower on a flowerhead ( $23.2 \pm 1.1$  and  $21.1 \pm 0.8$ , respectively;  $N_{\text{flowerhead}} = 288$  for each species; Welch two sample *t*-test on  $\log(\text{number of florets} + 1)$ :  $t_{558,357} = -1.253$ ,  $p = 0.210$ ). Sugar composition (i.e. sucrose over glucose and fructose ratio), amino acid concentration and the larger corolla of *C. jacea* make the latter a preferred nectar source compared with *T. pratense* [8].

Butterflies originating from IL and EL agricultural landscapes were introduced in an outdoor flight cage. Adult butterflies were randomly introduced in one of the four cage compartments. There were 24 individuals (12 males and 12 females) in each flight cage (half cylinder measuring 4.5 m long, 3.7 m wide and 1.8 m at maximal height) at the beginning of the treatment. A total of 960 butterflies were used for the whole experiment (five replicate series of both origins). After 48 h in the flight cage, surviving butterflies were collected and individually weighed to the nearest 0.1 mg (Ohaus Explorer balance). Three of the surviving females of each nectar treatment were placed in individual cages (30  $\times$  30  $\times$  30 cm) with access

to water (water imbibed cotton) in a climate room under standardized environmental conditions (16 L : 8 D cycle; 25°C during the day and 14°C at night).

### (c) Metabolism and flight capacities

For each repetition, the day after the nectar treatment, we tested the 12 females (three females from each nectar treatment) for RMR, flight capacities and FMR. We randomized the order in which females were tested. A total of 118 females were tested for RMR, FMR and flight performance across all repetitions (10 in total, 5 for each origins) of the treatment (58 from EL and 60 from IL).

Each individual was weighed to the nearest 0.1 mg (Ohaus Explorer balance; accuracy  $\pm 0.1$  mg), and next placed in a 1.5 l plastic cylindrical metabolic chamber. The chamber was connected to a flow-through respirometer (Sable Systems International) and placed in the dark (i.e. screened from the light source by a non-transparent cloth) to avoid butterfly movement under ambient temperatures close to optimal body temperatures for butterflies flight ( $30.0 \pm 0.1^\circ\text{C}$ ). We used a flow-through system with a dual-sensor oxygen analyser (Sable Systems International Oxzilla II) connected to a CO<sub>2</sub> analyser (Sable Systems CA-10a) and internal thermoprobe (Physitemp bimetal probe type MT-29/1B connected to Physitemp Model BAT-12 digital thermometer) to measure CO<sub>2</sub>. Data from the CO<sub>2</sub> analyser and from the thermoprobe were processed by a UI-2 analogue/digital converter (Sable Systems) and handled and stored on a computer (Sable System EXPEDATA software).

After 10 min, the chamber was emptied of water vapour and CO<sub>2</sub> by flushing with dry CO<sub>2</sub>-free air that had previously gone through three successive columns containing silicagel, drierite and ascarite, respectively. We closed the chamber, waited for another 10 min and then flushed it again (flow rate = 988 ml min<sup>-1</sup>; Sable Systems International subsample TR-SS3 pump) and the accumulated CO<sub>2</sub> (produced by the butterfly) during this period was measured, added to the quantity produced during measurement time. RMR was expressed in ml CO<sub>2</sub> h<sup>-1</sup> mg<sup>-1</sup>, based on the volume of CO<sub>2</sub> emitted for 12 min (10 min of measurement and 2 min of flushing).

Next, the individual was introduced in a flight mill within the metabolic chamber to measure simultaneously flight performance and FMR. The flight mill consisted of a light-weight carbon rod, threaded through a stainless pivot (magnets) to provide a near-frictionless rotation movement. In order to fix the butterfly to the flight mill, it was carefully glued (glue: Cyanoacrylate ZAP PT09) by the pterothoracic segment to a bent tip of a needle hanging to the flight rod. To do so, it was first anaesthetized with CO<sub>2</sub>. Then, we carefully removed thoracic hairs and cuticular lipids on the dorsal thorax with a fine cotton swab soaked with 90° ethanol. The butterfly was located at one extremity of the rod and an adapted counterweight was placed at the other extremity. Once connected, the butterfly could only perform tethered flight in a circle (rod length = 29 cm). The rotation of the metal rod was initially prevented by placing a magnet on top of the flight mill.

The flight mill was placed in a plastic, cylindrical metabolic chamber (height = 8.5 cm, diameter = 33 cm). The chamber was then connected to the respirometer, but was still screened from the light source. During acclimation of the butterfly (temperature: approx. 30°C), we flushed the air in the chamber as described above (approx. 20 min). Once the chamber was CO<sub>2</sub>-free, we closed it and removed the magnet and the cloth that covered the metabolic chamber from the light source. In the chamber, there was a small piece of folded paper attached to the bottom on which the butterfly rested before flight and losing contact with the paper stimulated the butterfly to fly (i.e. tarsal reflex). The butterfly was allowed to fly for 10 min,

during which CO<sub>2</sub> accumulated. Ten minutes represents a considerable flight performance that exceeds short routine movements like between two consecutive flower visits and, therefore, we consider it to be a reasonable proxy for flight endurance [33,34]. The chamber was positioned under a light source that approaches the natural light spectrum (Philips HPL-R 400 W). The number of tours flown by the butterfly was recorded automatically by a Hall Effect sensor detecting two small magnets (LABVIEW v. 8.6 software).

If the butterflies stopped flying after the initial stimulus, they were further encouraged to continue flying by gently tapping on the chamber with a pen. We estimated the willingness to fly of each individual according to a three-level score: 0 (no tapping required) to 3 (frequent tapping required). The willingness to fly was also analysed relative to origin and nectar treatment. After 10 min, the experiment was stopped and the CO<sub>2</sub> in the chamber was measured as for RMR. The butterfly was detached from the needle, weighed and frozen ( $-20^\circ\text{C}$ ). FMR was expressed in ml CO<sub>2</sub> h<sup>-1</sup> mg<sup>-1</sup> and flight distance (metre) was calculated based on the number of rounds flown in the flight mill. We expressed the efficiency of fuel combustion for flight by calculating the distance covered while emitting 1 ml of CO<sub>2</sub> mg<sup>-1</sup>.

### (d) Statistical analyses

Means are given  $\pm$  standard error. All variables (except willingness to fly) were analysed using generalized linear mixed models (GLMM) with MIXED procedure in SAS v. 9.3 [35] and landscape of origin, nectar treatment and the interaction effect as fixed factors. Temperature in the respirometer and time of day were introduced as random covariates. Covariances were estimated by REML (residual maximum likelihood). Denominator degrees of freedom of fixed effects were estimated by the Satterthwaite method. Model selection was done by backward selection from the full model removing non-significant interaction terms.

For each butterfly ( $N_{\text{total}} = 102$  individuals), we obtained the total volume of CO<sub>2</sub> produced during 12 min. We corrected for body mass and unit of time (i.e. ml CO<sub>2</sub> h<sup>-1</sup> mg<sup>-1</sup>). RMR was log<sub>10</sub> transformed to obtain normal residuals. For FMR calculation, we subtracted from the FMR measurement (total volume of CO<sub>2</sub> produced during 20 min) in ml CO<sub>2</sub> h<sup>-1</sup> mg<sup>-1</sup> (correction for mass and time) the value of RMR of that individual. This is to correct FMR for initial variation in RMR. We first analysed the absolute values of FMR with the full model to compare between origins. Because FMR is not independent of the flight distance, we chose to work for each origin separately by replacing origin in the full model by flight distance, and performing a second analysis. Efficiency of fuel consumption was analysed with the full model. Flight distance was analysed with the full model, and flight distance was put at 0 m for individuals that did not fly at all ( $N = 16$ ). For the flying individuals, mean flight speed, as the ratio between flight distance and time spent in flight (m s<sup>-1</sup>), was analysed following the same procedure. Willingness to fly was analysed by fitting a cumulative logit mixed model (ordered regression model) considering origin, nectar treatment and the interaction term as factors of interest and time of day and body mass were introduced as random factors. We applied backward model selection with R software [36] using the clmm function of the ordinal package [37]. Linear regression equations were calculated with centred predictor variables.

## 3. Results

Prior to measurements, butterfly mass did not vary significantly with origin or between nectar treatments (table 1;



**Table 1.** Female butterflies' mass immediately prior to flight and metabolism measurements; means  $\pm$  s.e.m. EL, extensively managed landscape of origin. IL, intensively managed landscape of origin. (C100, 100 flowerheads of *C. jacea*; c10, 10 of *C. jacea*; T100, 100 of *T. pratense*; t10, 10 of *T. pratense*.)

origin	nectar treatment	N	mean body mass $\pm$ s.e.m. (mg)
EL	C100	15	96.31 $\pm$ 4.85
	c10	15	87.41 $\pm$ 4.60
	T100	15	87.90 $\pm$ 4.52
	t10	15	87.51 $\pm$ 2.53
IL	C100	15	99.14 $\pm$ 5.43
	c10	12	91.32 $\pm$ 3.52
	T100	12	103.69 $\pm$ 4.13
	t10	15	88.20 $\pm$ 5.36

linear model: origin:  $F_{1,114} = 2.38$ ,  $p = 0.134$ ; treatment:  $F_{3,114} = 2.49$ ,  $p = 0.060$ ).

### (a) Flight distance and speed

The IL females flew longer distances in the flight mill than did EL females (GLMM: origin:  $F_{1,112} = 5.78$ ,  $p = 0.018$ ; nectar treatment:  $F_{3,112} = 1.75$ ,  $p = 0.162$ ; figure 1a). Flight distance increased with butterfly mass for both populations of origin (linear regression of flight distance against body mass: EL females: distance =  $142.8 + 4.05 \times$  body mass,  $F_{1,56} = 12.36$ ,  $p < 0.001$ ; IL females: distance =  $215 + 2.7 \times$  body mass,  $F_{1,57} = 4.84$ ;  $p = 0.031$ ). Note that the effect was much more significant in the EL group. In the t10 treatment, EL females tended to fly over slightly shorter distances compared with the other treatments (contrast:  $F_{1,53.2} = 3.89$ ,  $p = 0.054$ ); the differences between the populations of origin were the clearest in this treatment. Flight speed did not differ significantly with landscape of origin or nectar treatment (GLMM: origin:  $F_{1,95} = 0.06$ ,  $p = 0.811$ ; nectar treatment:  $F_{3,95} = 2.16$ ,  $p = 0.222$ ) (mean flight speed ( $\text{m s}^{-1}$ )  $\pm$  s.e.m.:  $0.58 \pm 0.03$ ).

### (b) Willingness to fly

IL females were slightly more willing to fly than EL females; their willingness was not reduced under adult food stress. EL females showed lower willingness to fly when nectar quantity was reduced (i.e. treatment c10 and t10; ordinal regression: origin: likelihood ratio (LR)  $\chi^2_1 = 3.64$ ,  $p = 0.056$ ; nectar treatment: LR  $\chi^2_3 = 5.82$ , LR test  $p = 0.120$ ; figure 2).

### (c) Efficiency of fuel consumption

Butterflies did not differ in their energy consumption during flight (GLMM: origin:  $F_{1,82.1} = 2.31$ ,  $p = 0.132$ ; nectar treatment:  $F_{3,89.4} = 0.21$ ,  $p = 0.887$ ; figure 1b). Results were similar when data were uncorrected for body mass.

### (d) Flight metabolic rate

The FMR was not affected by landscape of origin and butterflies tended to reach the fastest rates under the C100 treatment compared with the other treatments (GLMM:

origin:  $F_{1,90.7} = 0.01$ ,  $p = 0.904$ ; nectar treatment:  $F_{3,90.1} = 2.16$ ,  $p = 0.098$ ; figure 1c). Results were similar when the analysis was performed on data not corrected for body mass. FMR (uncorrected for mass,  $\text{ml CO}_2 \text{ h}^{-1}$ ) was positively related to body mass, but in IL females this relationship was only a non-significant trend (linear regression of FMR against body mass: EL females:  $\text{FMR} = 0.728 + 0.009 \times$  centred mass,  $F_{1,48} = 4.94$ ,  $p = 0.031$ ; IL females:  $\text{FMR} = 0.667 + 0.007 \times$  mass,  $F_{1,53} = 3.11$ ;  $p = 0.083$ ). However, the relationship between FMR and distance flown differed between the origins. In IL females, longer distances were associated with higher FMR, whereas this relationship was only a non-significant trend among EL females (table 2; electronic supplementary material, figure S1).

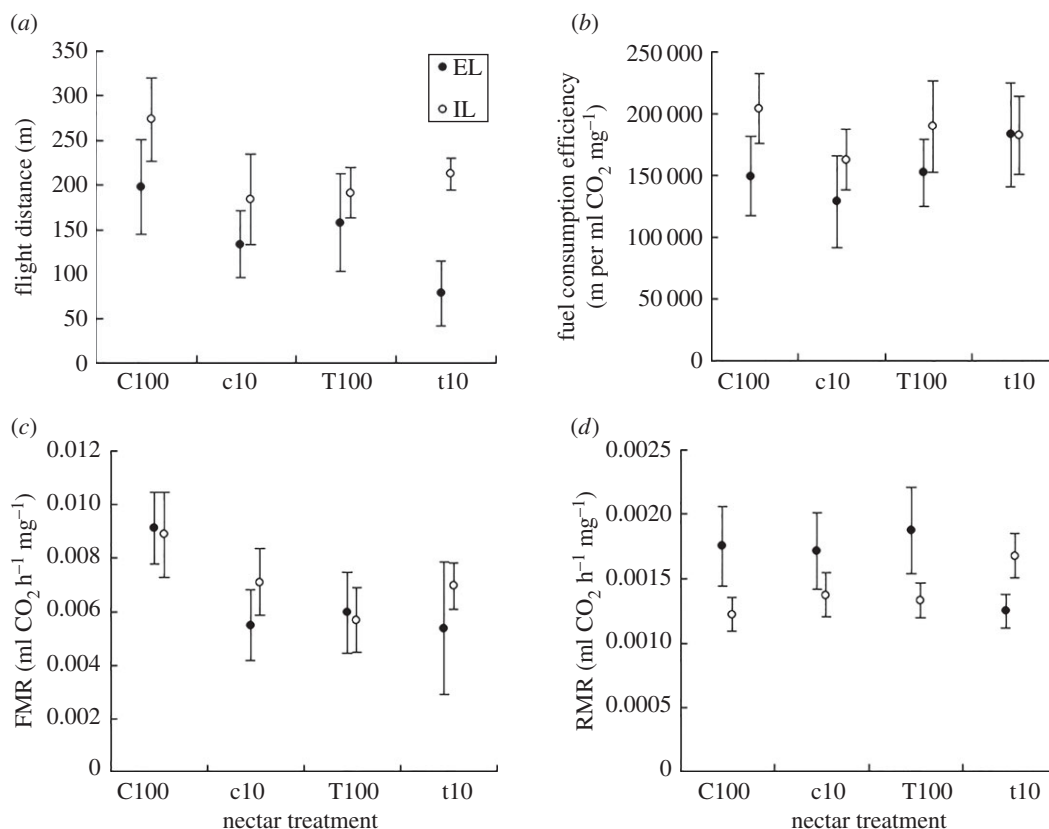
### (e) Resting metabolic rate

EL females had higher RMR than IL females, but this pattern was reversed in the most stressful treatment (i.e. t10 treatment) (GLMM: origin:  $F_{1,108} = 0.13$ ,  $p = 0.721$ ; nectar treatment:  $F_{3,108} = 0.27$ ,  $p = 0.850$ ; origin  $\times$  nectar treatment:  $F_{3,109} = 3.51$ ,  $p = 0.018$ ) (figure 1d; least squares mean comparison of IL and EL females in the t10 treatment:  $t_{107} = -2.54$ ,  $p = 0.012$ ). Hence, IL females reached their fastest standard metabolic rate under the high food stress treatment, whereas EL females showed the slowest one in this treatment. RMR did not vary with body mass (all  $p > 0.05$  for linear regression of RMR against body mass).

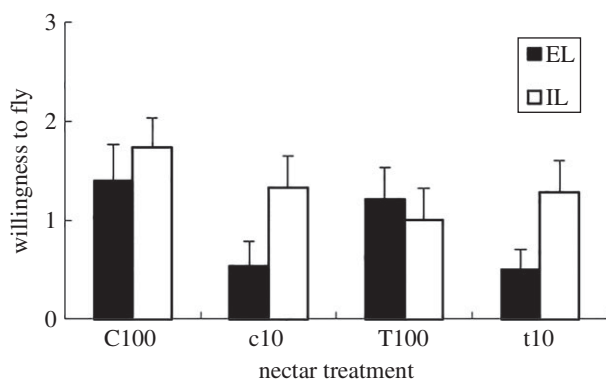
## 4. Discussion

Butterflies from landscapes that differ in nectar supply showed significant differences in flight performance and energy metabolism under experimental, standardized conditions. As predicted, *M. jurtina* females that originated from intensively managed, nectar-poor agricultural landscape showed stronger flight performance than conspecifics of extensively managed, nectar-rich landscape. RMRs also differed relative to the landscape of origin. Several of the effects of the nectar quality and quantity treatment on organismal and physiological performance had a signature of the landscape of origin. Hence, our results suggest significant differences in the lifestyle of this butterfly—and in the underlying metabolic mechanisms—for dealing with anthropogenic environments of different adult resource supply and spatial configuration. First, we discuss the differences in metabolic rate, and next, the results on flight capacity through a lens of different levels of phenotypic plasticity for flight-related traits in different anthropogenic landscapes.

The RMR is assumed to reflect the cost of self-maintenance [11]. An organism can only start to use energy for growth, reproduction and activity (flight in particular) when energy intake is above maintenance requirements. The context-dependence hypothesis—which attempts to reconcile earlier models including increased-intake and allocation or compensation models—predicts that high RMR individuals will have higher fitness when environmental conditions are productive, whereas low RMR individuals will have higher fitness under opposite conditions [10,11]. Accordingly, IL butterflies that came from hostile landscapes with little nectar production, had slower RMR than EL butterflies, except in the t10 treatment. Slower RMR would spare energy for other life-history traits in a nectar-poor



**Figure 1.** Flight and physiological performances (mean values  $\pm$  s.e.m.) of *M. jurtina* females by population origin (filled symbols, extensively managed landscape (EL); open symbols, intensively managed landscape (IL)) and by nectar treatment (C100, 100 flowerheads of *C. jacea*; c10, 10 of *C. jacea*; T100, 100 of *T. pratense*; t10, 10 of *T. pratense*): (a) Mean flight distance; (b) fuel consumption efficiency (distance covered by ml of CO<sub>2</sub> emitted) corrected for body mass; (c) flight metabolic rate and (d) resting (or standard) metabolic rate. Both metabolic rates are mass corrected.



**Figure 2.** Willingness to fly of *M. jurtina* females by population origin nectar treatment for both origins (black, extensively managed landscape (EL); white, intensively managed landscape (IL)) and nectar treatment (C100, 100 flowerheads of *C. jacea*; c10, 10 of *C. jacea*; T100, 100 of *T. pratense*; t10, 10 of *T. pratense*).

environment. But variation in RMR may also relate to other factors (e.g. immune system; [11]). Lower butterfly abundance in the IL landscape could result in lower density of pathogens, which in turn would less frequently trigger the immune system. However, there is currently little known about butterfly disease dynamics in different landscapes. Unlike IL females, EL females can afford higher RMR as they have no issues to fuel their metabolic machinery with nectar. Butterflies of different origin did not react in the same way to the simultaneous reduction of nectar quality

and quantity (i.e. the t10 treatment). EL butterflies reduced their RMR under food stress, which would save energy that could be subsequently used for reproduction. Food stress reduced RMR in a few other flying insects [5,25]. Butterflies used in our experiments were wild-caught, young adults, which all had briefly experienced nectar conditions in the landscape of origin. Hence, EL butterflies might have foraged on preferred nectar sources in the field prior to capture, and accumulated energy and nutrients from nectar that allowed them to better resist two days of food stress. IL females, on the other hand, were less responsive to nectar quantity/quality; food stress may have acted as an extension of the stressful conditions experienced in their landscape of origin.

Our results showed evidence of better flight capacity in IL compared with EL butterflies; they flew longer distances in all treatments. Whereas flight distance of IL females was not affected by the nectar treatment, EL females suffered much more from the simultaneous reduction of nectar quality and quantity. Butterfly mobility depends on the scale of habitat fragmentation and on the intrinsic willingness and capacity to fly [38]. Several behavioural responses can be mediated by boundaries and barriers in the landscape, and the perceptual distance to detect resources across the landscape [39,40]. As for other grassland butterfly species, habitat patches of *M. jurtina* are scattered and often small in the intensively managed agricultural landscape (e.g. field boundaries, road verges) [41]. Hence, under such conditions, butterflies have to deal with a coarser functional grain at the landscape level and require increased flight performance [38,42]. Female butterflies originating from a fragmented,

**Table 2.** Analysis (GLMM) of flight metabolic rate ( $\text{ml CO}_2 \text{ h}^{-1} \text{ mg}^{-1}$ ) during tethered flight in a flight mill relative to distance flown and nectar treatment analysed separately by population. (Origin EL, extensively managed landscape; IL, intensively managed landscape.)

origin	effect	estimate	s.e.	N.D.F.	D.D.F.	F	p-value
EL	flight distance	$4.79 \times 10^{-6}$	$4.68 \times 10^{-6}$	1	38.1	1.05	0.31
	nectar treatment			3	37.4	0.97	0.41
IL	flight distance	$1.30 \times 10^{-5}$	$3.09 \times 10^{-6}$	1	45.9	16.5	0.0002
	nectar treatment			3	45.1	0.24	0.87

nectar-poor landscape were indeed able to fly longer distances than conspecifics from less fragmented, nectar-rich landscape, when tested under standardized conditions in a flight mill. Such a tethered flight does not perfectly imitate free flight in the wild, in experimental cages or in a flight tunnel [39,43]. However, the construction of the flight mill within a metabolic chamber provides a novel way for these organisms to measure simultaneously flight capacity (endurance and speed) and FMR.

IL females were more willing to fly than EL females, which could relate to behavioural differences between landscapes. In insects, and butterflies in particular, little is known about personality-related traits relative to dispersal and flight performance (but see [44,45]). Bold individuals may spend more time in flight than would do shy individuals [46]. Our study was not designed for testing personality-related differences, but our results open interesting perspectives for such work [47]. With EL individuals being less prone to fly, there is the possibility that EL females have similar physical flight capacities than IL females, but rather differ in their behavioural propensity. Willingness to fly was similar between the populations of origin for the treatments offering high nectar quantity (i.e. C100 and T100); under these conditions, flight distances were indeed not longer for IL butterflies. Anyway, our results are in line with the view that IL butterflies represent a non-random selection of phenotypes/genotypes of EL landscapes that are able to colonize and to survive under nectar-poor environmental conditions and habitat fragmentation.

EL females showed higher levels of plasticity in flight performance in response to nectar treatment, whereas IL females did not. IL females were less responsive to this environmental variation and can be characterized by canalization (i.e. less plastic response) of flight performance. However, other life-history traits (e.g. survival and fecundity) have been reported to be affected by reduced nectar income in this butterfly species [48]. Trade-offs in the (re)allocation pathways of resources between the different life-history traits can be

identified under limited resources conditions [15]. It is often suggested that investment in higher mobility can be traded-off against life-history traits like fecundity or longevity [49,50], but for logistic reasons we could not test this on the very same individuals of the current study. But combining our current results with effects on reduced fecundity and longevity in IL butterflies under limited energy acquisition [48] suggests that these butterflies maintain flight performance at the cost of reduced fecundity and longevity.

## 5. Conclusion

We showed that butterflies from intensively managed, nectar-poor agricultural landscapes had better flight abilities (i.e. distance covered), were more willing to fly, and had lower RMR compared with conspecifics from extensively managed, nectar-rich agricultural landscapes. IL butterflies had more canalized (i.e. less plastic) responses relative to the different nectar treatments than EL butterflies. Under high nectar stress (i.e. low quantity and low quality combined), EL butterflies flew distances that were on average 2.5 times shorter than IL butterflies. Combining organismal research on flight and ecophysiology of natural populations improves our mechanistic understanding of the eco-evolutionary impact of anthropogenic environments [51].

**Data accessibility.** Data are available on the Dryad digital repository: doi:10.5061/dryad.dc440.

**Authors' contributions.** Study design: J.L., R.W. and H.V.D.; field/laboratory work: J.L.; data analysis: J.L. (with feedback by R.W. and H.V.D.); interpretation and writing: J.L., R.W. and H.V.D.

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