Aging, Life Span, and Energetics under Adult Dietary Restriction in Lepidoptera

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Accepted 6/4/2014; Electronically Published 8/19/2014

ABSTRACT

Stressful conditions can affect resource allocation among different life-history traits. The effect of dietary restriction (DR) on longevity and reproduction has been studied in many species, but we know little about its effects on energetics, especially in flying animals that have high energy demand. We assessed the effects of DR on metabolic rate throughout the entire adult life span in two butterfly species, *Colias eurytheme* and *Speyeria mormonia*. We cut the food intake of adult females in half and measured resting metabolic rate (RMR) and flight metabolic rate (FMR) together with body mass repeatedly throughout life. In both species, DR reduced body mass, but mass-corrected FMR was not affected, indicating that flight capacity was retained. DR lowered RMR and reduced fecundity but had no effect on life span. FMR declined with age, but the rate of senescence was not affected by DR. In contrast, aging had a strong negative effect on RMR only in control females, whereas food-restricted females had more stable RMR throughout their lives. The results suggest that flight capacity is conserved during nutritional stress but that investment in flight and survival may negatively affect other important physiological processes when resources are limited.

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Physiological and Biochemical Zoology 87(5):684–694. 2014. 1522-2152/2014/8705-4057$15.00. DOI: 10.1086/677570

Introduction

How resources are allocated among different processes is a fundamental question in life-history theory (Zera and Harshman 2001; Roff 2002; Boggs 2009). In short, available resources may be allocated to growth, somatic maintenance, survival, acquisition of more resources, and reproduction. When the available resource pool is limited, as may happen in the wild, trade-offs may occur among processes that share the same resource pool (Zera and Harshman 2001). Under such conditions, individuals may adjust their resource allocation strategies.

Poor food availability may trigger investment in survival. Dietary restriction (DR) has been shown to increase life span in various organisms (Speakman and Mitchell 2011). Curiously, the effect appears to be strongest in well-established laboratory model species (Nakagawa et al. 2012). Increased life span is often accompanied by reduced reproduction, although these traits are not necessarily coupled (Piper et al. 2005). Recent work on *Drosophila melanogaster* and other insects with complete adult diets suggests that the quantity of food is not the factor driving extended life span but rather that the composition of the diet, particularly amino acid composition, is more important (Lee et al. 2008; Grandison et al. 2009). However, the effect of DR on life span appears to be different in butterflies that acquire most of the amino acids during the larval stage and consume primarily carbohydrates at the adult stage. Adult DR has no effect on life span in the nectar-feeding butterfly *Speyeria mormonia* (Boggs and Ross 1993), whereas DR shortens life span in the fruit-feeding butterfly *Bicyclus anynana* (Saastamoinen et al. 2010). The role of adult diet composition in determining life span and fecundity in butterflies is still unclear (Bauerfeind and Fischer 2005). Life span can in some cases be positively affected by amino acids in the diet (Beck 2007) or nitrogen-rich nuptial gifts (Karlsson 1998), but in other cases amino acids have had no effect on life span (Beck 2007) or on both life span and fecundity (Molleman et al. 2008).

DR may up- and downregulate various processes. However, we do not yet have a complete understanding of the effects of DR on energy use in animals (Speakman and Mitchell 2011). Resting metabolic rate (RMR) in invertebrates and other ectotherms and basal metabolic rate (BMR) in endothermic vertebrates represent the minimum energy consumption rate and therefore the maintenance cost of the metabolic machinery of a resting animal. In insects, starvation reduced RMR in some cases (Harshman et al. 1999; Nespolo et al. 2005; Roark and Bjorndal 2009), although in some cases reduced CO_2 emission rate reflects changes in energy substrate use rather than reduced oxygen consumption (Sinclair et al. 2011). However, artificial selection experiments suggest that reduced energy consumption

rate is not necessarily the key to starvation resistance (Pijpe et al. 2008). In *D. melanogaster*, starvation-resistant strains do have lower mass-specific RMR than controls, but the effect disappears when metabolically inactive lipids and carbohydrates are subtracted from the body mass (Djawdan et al. 1997).

Compared with RMR, maximum metabolic rate is a less-studied trait, especially in the context of DR. Most of the existing work has been performed on mammals, for example, by measuring the metabolic rate of starved rodents forced to swim or run. Rats that were fasted for 48 h showed lower RMR, increased fat oxidation, and decreased carbohydrate oxidation but no change in energy expenditure during forced swimming (Benthem et al. 1995). Similarly, rats that were fasted for 24 h showed no change in maximum oxygen consumption rate during running (Koubi et al. 1991). In humans, short-term fasting did not affect maximum oxygen consumption rate, but it did reduce endurance (Dohm et al. 1986; Zinker et al. 1990). One study examined the effect of long-term DR on mice and found that maximum metabolic rate during swimming was reduced by DR (Brzęk et al. 2012). The effects of DR on maximum metabolic rate in insects are virtually unexplored. This is surprising, as the mass-specific metabolic rates of flying insects are among the highest recorded among any organisms (Suarez 2000), and flight is fundamentally important for many insects. Apart from routine movements during foraging, mate discovery, courtship, and egg laying, flight enables dispersal to potentially more favorable areas (Zera and Denno 1997). Individual variation in flight metabolic rate (FMR) and peak metabolic rate (PMR) have fitness implications and have been shown to be tightly associated with dispersal rate in the Glanville fritillary butterfly (*Melitaea cinxia*, Nymphalidae; Haag et al. 2005; Niitepõld et al. 2009; Niitepõld et al. 2011). Insect flight energetics is also directly linked to the roles of insects as disease vectors, agricultural pests, and pollinators.

We examined the effect of food shortage on energetics throughout the entire adult life span in two butterfly species, *Colias eurytheme* and *S. mormonia*. In nectar feeders, adult food intake is easily manipulated both qualitatively and quantitatively. Here, we performed the latter manipulation to mimic conditions where flower nectar is scarce. Laboratory-reared adult butterflies were subjected to a chronic food reduction treatment in which their sugar water intake was cut in half compared with that of similarly sized control individuals. Our study questions were as follows. Does DR affect RMR in our study species? Are flight metabolic capacity (PMR) and overall flight performance (FMR) affected by DR? Does aging affect metabolic rates, and, if so, do food-restricted butterflies senesce differently than those with an unlimited diet? Does DR affect life span and fecundity?

Material and Methods

Study Species

We used two butterfly species with contrasting life-history strategies: *Speyeria mormonia* (Nymphalidae) and *Colias eurytheme* (Pieridae). *Speyeria mormonia* is a montane species with one generation per year. Individuals overwinter as unfed first-instar larvae that initiate feeding with the spring snow melt. In contrast, the life cycle of lowland *C. eurytheme* does not include a diapause stage, and populations produce multiple generations annually. The two species also differ in their resource allocation strategies: *S. mormonia* has larger eggs and invests more adult-derived carbon in its eggs than does *C. eurytheme*, which relies more on larval resources (O'Brien et al. 2004).

Rearing

The *S. mormonia* were offspring of wild females captured in the vicinity of the Rocky Mountain Biological Laboratory, Gunnison County, Colorado (38°57′N, 106°58′W, 2900 m asl). The wild-caught parental generation laid eggs in individual cages in the laboratory. Hatched larvae were stored in family groups in 1.85-mL glass vials in a refrigerator for 5 mo. Diapause was broken and larvae were reared in a greenhouse in organdy bags tied over leaves of the rooted host plant *Viola soraria*. Pupae were placed in emergence cages. Females were mated in the afternoon of the day of emergence. The following day, females were marked with a felt-tip pen on the hind wings and weighed to the nearest 0.1 mg. We took a picture of the butterfly under standardized settings and used ImageJ (ver. 1.43; National Institutes of Health, Bethesda, MA) to measure forewing length to the nearest 0.01 cm. We kept the females in individual glass cylinder cages (described in O'Brien et al. 2004). One host plant leaf in a glass tube was provided to stimulate egg laying. We used a total of 49 females from 17 families.

The *C. eurytheme* were from a laboratory colony maintained by Ward Watt at Stanford University, founded from individuals collected near Tracy in the California Central Valley (37°44′N, 121°26′W, 16 m asl). The colony breeding protocol avoids sib or first-cousin crosses, and wild-caught individuals are introduced at least once a year. We reared newly hatched larvae as family groups on pots of hydroponically grown *Vicia villosa* in a greenhouse under a 16L : 8D cycle and a 27° : 15°C diurnal temperature cycle. Individuals were moved to an emergence cage on pupation. Females were mated on the day of eclosion. The next day, each female was weighed to the nearest 0.1 mg and numbered with a felt-tip pen on both hindwings. Females were kept in individual cages with fresh host plants. We used a total of 30 females from 9 families in the experiment.

Feeding Treatments

We assigned females to two treatments: adult feeding ad lib. or half of ad lib. In the latter treatment, we fed the individual half the volume of sugar water imbibed by its pair in the ad lib. treatment. The pairs were assigned on the basis of body mass in the experiment with *C. eurytheme* and forewing length with *S. mormonia*. When available, we preferred siblings as pairs. Members of the pair were randomly allocated to their respective treatments. If an individual could not be paired, we assigned it to the ad lib. treatment. When a matching individual emerged, we assigned it as the half ad lib. member of the pair.

We fed all females twice a day. We placed ad lib. females on a plastic plate and provided a 50-μL droplet of 20% v : v sugar water solution. We prepared a large batch of sugar water for each experiment and distributed it in 1.5-mL Eppendorf tubes that were stored in a freezer until use. Commercial granulated beet table sugar was used for *C. eurytheme*, and cane sugar was used for *S. mormonia*. We used sugar instead of nectar or honey as it allowed us to use carbon stable isotopes to differentiate whether carbon allocated to eggs was derived from the larval or adult diet. These results are reported elsewhere (Boggs and Niitepõld, forthcoming). We held the individual by its wings with forceps and used a needle to extend its proboscis and direct it into the sugar water droplet. After the individual finished feeding, we collected the unconsumed sugar water using a Hamilton syringe (Hamilton Company, Reno, NV) and calculated the imbibed volume to the nearest 0.5 μL. We gave the half ad lib. member of the pair half of the 2-d running average of the volume consumed by its pair in the ad lib. treatment. In virtually all cases, all of the available sugar water was consumed by the food-restricted individual. During the day, *S. mormonia* had access to water in a piece of moist paper towel in their individual cages.

Respirometry

We measured RMR and FMR using flowthrough respirometry every third day of an individual's life. RMR and FMR were measured in the same trial, starting with RMR. We obtained the measurements in the afternoon, so that the butterflies had had time to digest the sugar water consumed in the morning feeding trial. The experimental individual was placed in the 1-L respirometry chamber ca. 25 min before the measurement of metabolic rate. The chamber was covered with a black cloth and kept at a constant temperature. The measurements were carried out in a temperature-controlled room inside a plywood cabinet with an open front. The cabinet was equipped with one 26-W fluorescent light bulb and two 25-W fluorescent blacklights mounted to its ceiling. The temperature inside the respirometry chamber was measured with a negative temperature coefficient thermistor probe (Sable Systems International, Las Vegas, NV). Average temperature across measurements was 31.3° ± SD 0.6°C in the *S. mormonia* experiment and 33.3° ± 0.4°C in the *C. eurytheme* experiment. Dried and CO_2-free air was pushed through the respirometry chamber at the STP-corrected rate of 1.5 L min^{-1} using a SS-4 subsampler pump (Sable Systems International) and a mass flow controller (Sierra Instruments, Monterey, CA). From the chamber, air flowed through the LI-7000 differential infrared CO_2 analyzer (Li-Cor Biosciences, Lincoln, NE). We began the measurement of RMR when the individual rested motionless in the chamber and the CO_2 level had reached a stable baseline. Butterflies typically remain still when kept in the dark. If the individual began to move, this could be seen as a spike in the CO_2 level. In these rare cases, we waited until the movement ceased and the CO_2 level returned to baseline. RMR remained stable over tens of minutes. The large chamber volume used in the experiment further reduced short-term variation in the CO_2 signal, which produced an only slightly cyclical CO_2 emission pattern (fig. 1*A*). We determined visually that a recording of 1.5 min was long enough to represent an individual's RMR. To validate this statistically, we measured RMR over a 10-min period in a random subset of 20 *S. mormonia*. The Pearson correlation coefficient between 1.5-min and 10-min measurements was 0.999 (fig. 1*B*).

After measurement of RMR, the black cloth was removed, and the butterfly was exposed to light. When the butterfly had rested under the lights for 30 s, we shook the chamber sharply, which flung the butterfly in the air, after which it began to fly. When the butterfly alighted, we shook it up in the air again. If the butterfly clung to the side of the chamber and a shake was not enough to send it airborne, the chamber was tapped sharply. The butterfly was forced to fly as continuously as possible. Most individuals reached their PMR during the first minutes of the experiment (fig. 1*A*). As the experiment progressed, some individuals showed clear signs of fatigue and were unable to continue flying. The shaking was nevertheless continued, and the butterflies typically kept weakly flapping their wings without producing any significant lift. Some individuals were able to recover their flight ability after a period of rest while the shaking continued. Other individuals flew more or less continuously until the end of the experiment. After 7 min, we stopped the agitation and covered the chamber with the black cloth. When the CO_2 level reached baseline, we removed the butterfly from the chamber, recorded its identity and time of day, and wrote a description

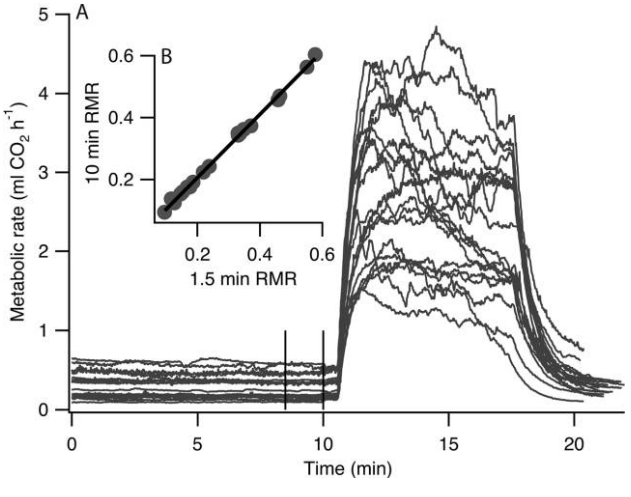

Figure 1. Metabolic rates of 20 female *Speyeria mormonia*. *A* depicts CO_2 emission rates against time. In this subset, resting metabolic rate (RMR) was measured for 10 min, followed by a 30-s adjustment period before 7 min of flight stimulation. After 7 min, agitation was stopped, and the CO_2 emission rate dropped to baseline levels. The two vertical bars mark the 1.5-min period that was used to represent RMR in the full data set. *B* shows 1.5-min RMR measurements plotted against 10-min measurements. The strong correlation ($r = 0.999$, $P < 0.0001$) indicates that the shorter measurement period captures most of the variation between individuals found in the 10-min measurements.

of each individual's flight behavior. We then weighed the butterfly and placed it back in its cage.

We used equations of Lighton (2008) to convert CO_2 concentration to milliliters of CO_2 per hour. As the flapping butterfly acts as a fan inside the chamber, the temporal resolution of the data was relatively good. We did not perform an instantaneous transformation (Bartholomew et al. 1981) in order to avoid artificial peaks affecting the results. This means that the actual peak CO_2 emission rate was somewhat higher than our results indicated. The instantaneous transformation does not affect the total volume of CO_2 emitted or RMR. We calculated the PMR as the highest rate of CO_2 production during sustained flight and the FMR as the total volume of CO_2 produced during the 7-min flight trial. PMR reflects maximum metabolic capacity and is typically reached during the first minutes of the experiment. FMR reflects a combination of flight capacity, endurance, and behavior. We removed the effect of body mass from metabolic rates statistically (see below).

Life Span

We recorded the date of adult eclosion and death for each individual. We collected dead butterflies twice a day and calculated life span in full days.

Fecundity

Eggs were collected and counted after 4 p.m. every day. The eggs were dried and assayed for stable isotopic composition (Boggs and Niitepõld, forthcoming) and dry mass, protein, glycogen, and triglyceride (results to be reported elsewhere: C. L. Boggs, K. Niitepõld, and A. Perez, unpublished manuscript).

Statistical Methods

Body mass, RMR, PMR, and FMR were analyzed using mixed models in the program SAS (ver. 9.2; SAS Institute, Cary, NC). We accounted for repeated measurements on the same individuals by using the "Repeated" statement in the "Mixed" procedure and chose the first-order heterogeneous autoregressive covariance structure, arh(1). The initial model for body mass contained treatment as a category variable, age and age squared as covariates, and the interactions between treatment and the covariates. The initial models for metabolic rate contained treatment as a category variable; body mass, time of day, time squared, measurement temperature, temperature squared, age, and age squared as covariates; and the interactions between treatment and the covariates. We used backward elimination

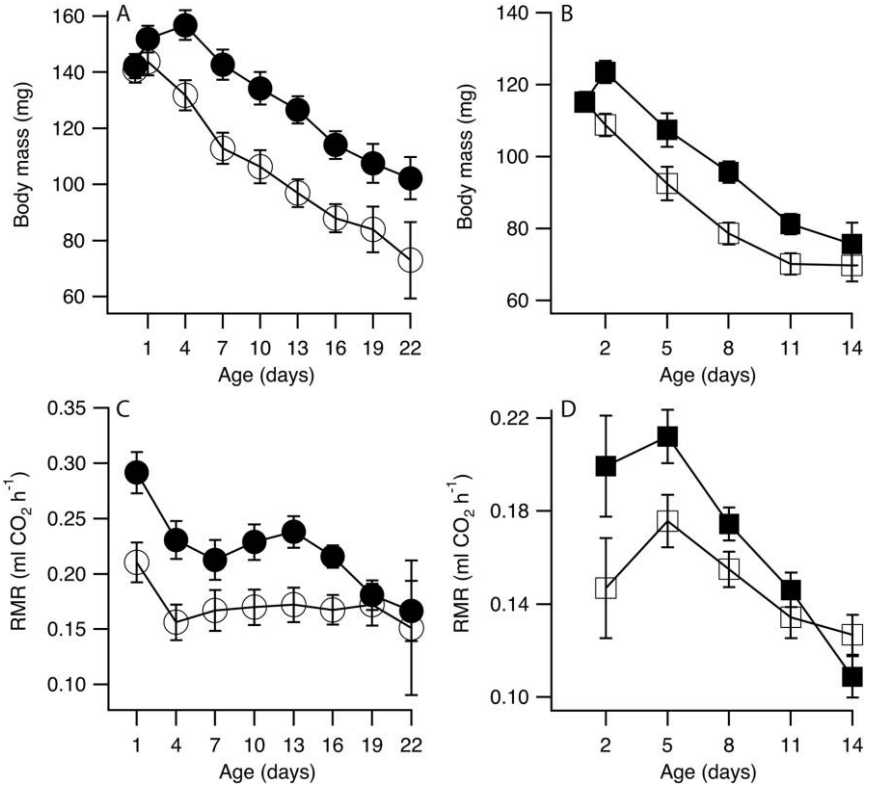

Figure 2. Body mass decreased with age in *Speyeria mormonia* (*A*) and *Colias eurytheme* (*B*). Black symbols represent controls, and white symbols represent food-restricted females. Control females gained mass during the first days, after which their body mass decreased. Food-restricted females lost mass since the beginning. Resting metabolic rate (RMR) decreased with age independent of body mass in *S. mormonia* (*C*) and *C. eurytheme* (*D*). In both species, control females had higher RMR than did food-restricted females, and the controls experienced a stronger decrease in RMR with age. In all panels, data are least square means with SEs as the error bars.

Table 1: Factors explaining variation in resting metabolic rate

	Speyeria mormonia		*Colias eurytheme*	
	F (df)	*P*	*F* (df)	*P*
Body mass	24.04 (1, 222)	<.0001	34.16 (1, 82)	<.0001
Age	8.51 (1, 222)	.0039	53.07 (1, 82)	<.0001
Treatment	12.27 (1, 47)	.001	16.92 (1, 28)	.0003
Time	63.30 (1, 222)	<.0001	5.52 (1, 82)	.021
Temperature	...	...	6.50 (1, 82)	.013
Time × treatment	5.47 (1, 222)	.02	...	...
Age × treatment	6.42 (1, 222)	.012	15.61 (1, 82)	.0002

Note. Data were modeled using a repeated-measures mixed model with heterogeneous autoregressive (1) covariance structure and type III sum of squares. Nonsignificant factors not included in the model are indicated by an ellipse. Treatment was included in all models.

and removed nonsignificant ($P > 0.05$) terms from the model. We used the corrected Akaike Information Criterion (AICc) to assess the model fit among possible models and generally chose the model with lowest AICc value, bearing in mind that adding one factor to the model is penalized by two AIC points.

The effects of DR treatment on life span and fecundity were modeled using ANOVA and the paired *t*-test, respectively. We omitted individuals that did not lay eggs or whose pair did not lay eggs from the paired *t*-test (C. L. Boggs, K. Niitepõld, and A. Perez, manuscript in preparation).

Results

Effect of DR on Body Mass

Speyeria mormonia female body mass (fig. 2*A*) decreased significantly with age ($F_{1, 274} = 129.85$, $P < 0.0001$). The treatment × age interaction was significant, indicating a steeper decrease in food-restricted females ($F_{1, 274} = 12.83$, $P = 0.0004$). With the strong interaction, the main effect of treatment was not significant ($F_{1, 47} = 0.01$, $P = 0.93$). We also constructed a more complicated model that contained a significant treatment × age squared interaction that captured the nonlinear relationship between mass and age in controls. Because the difference in AICc values was only 1.3 between the models, we present the simpler model here.

In *Colias eurytheme* (fig. 2*B*), female body mass decreased linearly with age ($F_{1, 116} = 232.07$, $P < 0.0001$). Individuals receiving the DR treatment had consistently lower body mass than did individuals fed ad lib., as only the treatment main effect was significant ($F_{1, 28} = 9.98$, $P = 0.004$).

Effect of DR on RMR

In female *S. mormonia*, RMR correlated positively with body mass and negatively with the time of day (see table 1 for statistics). RMR declined with age, and there was a significant interaction between age and treatment: the slope was steeper in individuals fed ad lib. than in food-restricted individuals (fig. 2*C*). Throughout most of their lives, individuals fed ad lib. had a higher RMR than did individuals receiving the DR treatment. There was a significant interaction between time of day and treatment, suggesting that control individuals had a higher RMR earlier in the day.

Colias eurytheme RMR was positively affected by body mass and measurement temperature, and there was a negative association with time of day during the measurement (table 1). There was no significant interaction between time and treatment. Individuals receiving the ad lib. treatment had higher RMR than did food-restricted females. RMR decreased with age, but the decrease was steepest in individuals receiving the ad lib. treatment (fig. 2*D*).

Effect of DR on PMR and FMR

In *S. mormonia*, PMR was positively correlated with body mass and negatively correlated with time of day (table 2). Age had a nonlinear effect on PMR, peaking at 4–7 d of age (fig. 3*C*). Treatment had no significant effect on PMR, nor were any of the interactions significant. FMR was affected by body mass and time of day (table 3; fig. 3*C*). The quadratic age term was significant, as FMR was highest during the second measurement and decreased after that. Treatment did not have a statistically significant effect on FMR ($P = 0.14$).

In *C. eurytheme*, PMR decreased with age (table 2). The quadratic age term was significant, indicating a steep decline during the last days of life, as seen in figure 3*B*. The DR treatment had no significant effect on PMR. The results for FMR were qualitatively identical to PMR (table 3; fig. 3*D*).

Effect of DR on Life Span and Fecundity

Life span was not affected by DR in *S. mormonia* ($F_{1, 47} = 0.98$, $P = 0.33$) or *C. eurytheme* ($F_{1, 26} = 0.14$, $P = 0.71$; fig. 4). In *S. mormonia*, food restriction significantly lowered fecundity (paired *t*-test: $t_{17} = 5.85$, $P = 0.00002$). The mean numbers of eggs for the ad lib. treatment and the half ad lib. treatment were 398 ± 33 and 178 ± 18 eggs, respectively. In *C. eurytheme*, the total number of eggs laid was lower in the food-

Table 2: Factors explaining variation in peak metabolic rate

	Speyeria mormonia		*Colias eurytheme*	
	F (df)	*P*	*F* (df)	*P*
Body mass	47.38 (1, 223)	<.0001	82.59 (1, 84)	<.0001
Age	.52 (1, 47)	.47	47.92 (1, 84)	<.0001
Treatment	1.51 (1, 223)	.23	1.12 (1, 28)	.30
Time	18.45 (1, 223)	<.0001	...	...
Age × age	16.19 (1, 223)	<.0001	...	...

Note. Data were modeled using a repeated-measures mixed model with heterogeneous autoregressive (1) covariance structure. We used type I sum of squares, as the effect of *Colias* body mass was not significant when using type III sum of squares. The variables were entered in the model in the order presented here. Nonsignificant factors not included in the model are indicated by an ellipse. Treatment was included in all models.

restriction treatment group ($t_{14} = 3.53$, $P = 0.003$). Females receiving the DR treatment laid on average 488 ± 41 eggs, while control females laid 663 ± 49 eggs.

Discussion

Adult DR resulted in significant changes in some traits, while others were conserved. Body mass decreased dramatically with food restriction in both species, *Speyeria mormonia* and *Colias eurytheme*. Food-restricted females had lower mass-independent RMR, suggesting that they had lower levels of physiological activity than females fed ad lib. The reproductive output of food-restricted females was significantly reduced. On the other hand, DR did not alter maximum metabolic capacity or overall flight performance, as both PMR and FMR remained unaffected. In both treatments, flight metabolism showed clear senescence, suggesting impaired flight capacity in older butterflies. However, the rate of senescence in PMR and FMR did not differ between the treatments, and there were no differences in life span. Both species therefore followed a conservative survival strategy under food stress with no change in flight capacity and longevity, whereas investment in reproduction was reduced. This strategy may be advantageous under short-term environmental stress and when higher fitness can be achieved by dispersing to other areas.

Table 3: Factors explaining variation in flight metabolic rate

	Speyeria mormonia		*Colias eurytheme*	
	F (df)	*P*	*F* (df)	*P*
Body mass	58.66 (1, 223)	<.0001	55.48 (1, 84)	<.0001
Age	.54 (1, 223)	.46	19.99 (1, 84)	<.0001
Treatment	2.30 (1, 47)	.14	1.40 (1, 28)	.25
Time	24.49 (1, 223)	<.0001	...	...
Age × age	20.33 (1, 223)	<.0001	...	...

Note. Data were modeled using a repeated-measures mixed model with heterogeneous autoregressive (1) covariance structure and type I sum of squares. Nonsignificant factors not included in the model are indicated by an ellipse. Treatment was included in all models.

DR Did Not Affect Life Span but Decreased Body Mass, RMR, and Fecundity

DR reduced body mass in both species. Before the first feeding, control and DR-treatment individuals did not differ in body

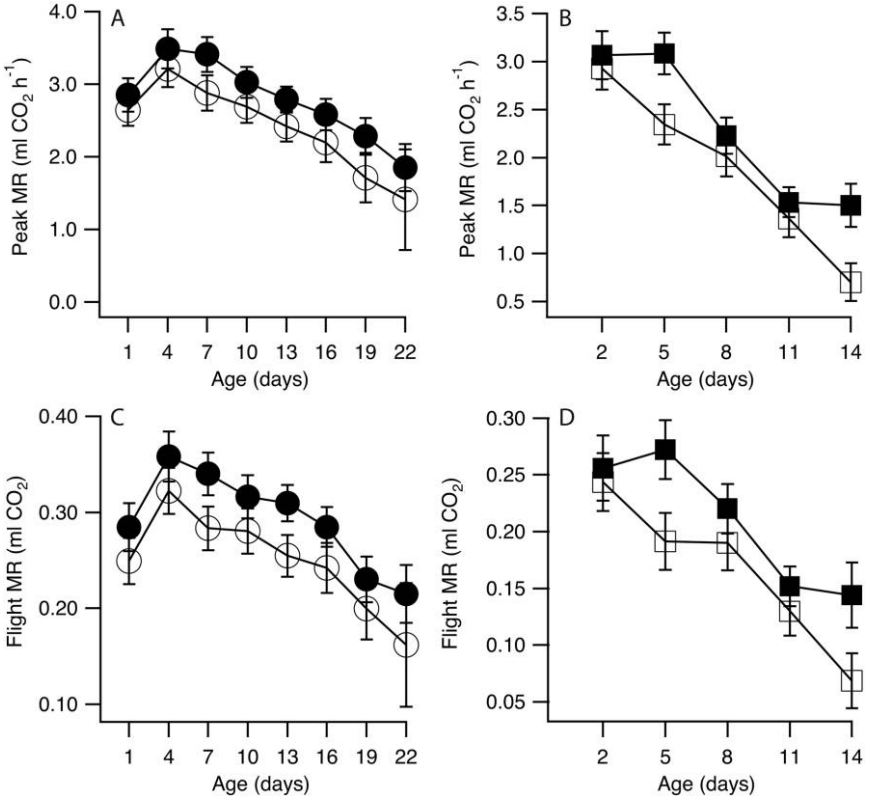

Figure 3. Peak metabolic rate with age in *Speyeria mormonia* (*A*) and *Colias eurytheme* (*B*). There was no statistically significant difference between control females (black symbols) and food-restricted females (white symbols). Flight metabolic rate—that is, the total volume of CO_2 emitted during the 7-min measurement—showed a similar relationship with age, as did peak metabolic rate. In *S. mormonia* (*C*), there was a nonlinear age effect, while the decrease with age was linear in *C. eurytheme* (*D*). Dietary restriction had no significant effect on flight metabolic rate. Data are least square means and SEs and have been adjusted for the effect of body mass.

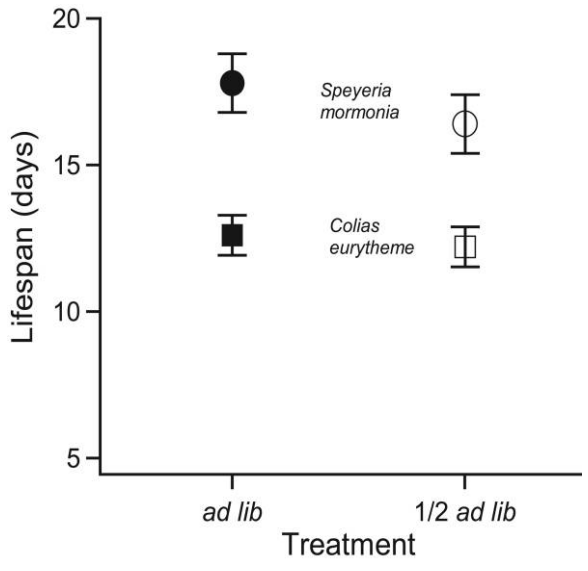

Figure 4. Life span in *Speyeria mormonia* and *Colias eurytheme*. In both species, dietary restriction had no significant effect on life span. Symbols represent arithmetic means with SEs.

mass. Control individuals, however, gained weight during the first few days, after which their body mass began to decrease. Food-restricted individuals began to lose weight from the beginning of life, which led to a consistent difference in body mass between the two groups throughout their adult lives. Egg laying is likely to be an important reason for the mass loss of females in our experiments. The average *S. mormonia* egg weighs 0.22 mg (Boggs 1986). The combined mass of eggs laid by control females would therefore have been on average 87.6 mg, while the approximate average total mass of eggs laid by food-restricted females was 39.2 mg. In control females, the difference between the highest and lowest body mass of an individual was on average 54.6 mg. Since this is less than the combined mass of the laid eggs, it is clear that control females did not rely on stored resources as much as resources acquired through adult feeding. In food-restricted females, however, the average body mass loss was 70.8 mg, more than the combined mass of eggs. This indicates that these females used stored resources for purposes other than egg production. Insects store resources in the fat body, and the size of fat body has been shown to decrease with age in *S. mormonia* females (Boggs 1986). Interestingly, butterflies also reallocate resources from flight muscles during their life, and flight muscle mass decreases with age (Stjernholm et al. 2005; Stjernholm and Karlsson 2008). Previous work has shown that *S. mormonia* females resorb unlaid eggs under DR and use those resources for other functions, such as survival (Boggs and Ross 1993).

DR reduced fecundity in both species, but the reduction was greater in *S. mormonia*, probably reflecting differences in egg composition. Eggs of *S. mormonia* contain more carbon than those of *C. eurytheme*, and a larger proportion of the carbon is derived from adult feeding (O'Brien et al. 2004). *Speyeria mormonia* is thus more likely to be susceptible to adult food stress. The diet provided in the experiments consisted of pure sugar water, while flower nectar is known to contain small amounts of amino acids (Baker and Baker 1973). The role of adult-derived amino acids in determining reproductive success is still under examination. Lepidopteran eggs contain significant amounts of protein, and essential amino acids are known to originate solely from larval food sources, while nonessential amino acids are synthesized using carbon derived from adult feeding (O'Brien et al. 2002) in the absence of amino acids in the adult diet. In the fruit-feeder *Bicyclus anynana*, fecundity is higher on a banana diet compared with sugar even when amino acids are added to the diet (Bauerfeind and Fischer 2005; Molleman et al. 2008). However, amino acids in the adult diet of nectar-feeding butterflies have been shown to improve fecundity or offspring quality, in particular when larval host plants have been poor in nitrogen (Mevi-Schütz and Erhardt 2005; Cahenzli and Erhardt 2012). It is possible that sugar feeding reduced fecundity slightly in our experiments, but we do not expect it to affect the magnitude of the DR effect. In *S. mormonia*, the absolute number of eggs and the reduction in fecundity due to DR matched well with the results of Boggs and Ross (1993), who fed females with honey water solution.

We found that DR did not affect life span in either species. This result agrees with previous findings that adult DR does not prolong life span in *S. mormonia* (Boggs and Ross 1993). It appears that resources critical for survival are acquired at the larval stage of *S. mormonia*, as larval feeding has an effect on life span (Boggs and Freeman 2005). Our results also suggest that metabolic rate does not play a causal role in determining life span under DR. We found that DR reduced RMR, yet life span was not affected. The result adds to previous findings that challenge a simple link between DR, energy expenditure, and life span. For example, DR does extend life span in *Drosophila melanogaster* but RMR is not affected (Hulbert et al. 2004), and DR does shorten male housefly life span but metabolic rate is not affected (Cooper et al. 2004).

Starvation can lead to changes in fuel use when carbohydrate stores become depleted and animals shift to using lipids and proteins. This is turn affects the respiratory quotient, that is, the ratio between produced CO_2 molecules and consumed O_2 molecules. If only CO_2 emission rate is measured, reduced CO_2 production rate may simply be an artifact of increased lipid or protein use, while oxygen consumption is not affected (Sinclair et al. 2011). While food-restricted females utilized more stored resources than did control females, we do not think that switching to burning lipids would explain the observed reduction in CO_2 emission rate under DR. This is because DR reduced RMR beginning from the very youngest ages, and the effect disappeared at the oldest ages. Starvation-induced energy substrate shifts would likely show the opposite pattern. Instead, the lower RMR of food-restricted females is likely to reflect lower physiological activity due to less available energy and carbohydrates. As DR reduced fecundity in both species, we suggest that high RMR in control females reflects turning food into eggs. Gamete development is an energetically costly process, especially in the

female sex. The result may therefore reflect the energetic cost of egg production, which food-restricted individuals were not able to fully pay.

In addition to the age × treatment interaction, we also detected a significant interaction between time of day and treatment in *S. mormonia*. Declining RMR with time is likely to reflect digestion. It is well known that a short-term effect of feeding is an elevated metabolic rate and increased heat production, known as "specific dynamic action" (Secor 2009). The magnitude and duration of this effect probably depend on the amount and composition of the diet and the individual's feeding frequency. Woods et al. (2010) reported that the CO_2 emission rate returned to prefeeding levels in 2 h, whereas several butterfly species studied by Zebe (1954) showed elevated metabolic rates until 16–24 h after feeding. However, the butterflies measured by Zebe consumed large volumes of sugar water, often corresponding to 75% of their body mass. In both our experiments, individuals were fed in the morning and measured several hours later in the afternoon. Control females imbibed volumes corresponding to approximately 15% of their body mass. Although most of the digesting was probably over at the time of measurement, it is possible that the effects of digestion and nutrient processing lasted longer in control females that ingested larger volumes of sugar water than DR-treated females.

Flight Metabolism Not Affected by DR

Insect flight is an energetically expensive trait (Suarez 2000). Nonetheless, restricting food intake in adult butterflies had no significant effect on PMR in either species studied here. FMR, the total volume of CO_2 emitted during the 7-min measurement, also showed no statistically significant change due to food restriction. FMR reflects overall flight performance and tends to be high in individuals capable of continuous flight. Because flying inside the respirometry chamber requires repeated takeoffs and ascending flight, FMR is likely to overestimate the cost of free flight that also contains periods of gliding and soaring. Considering this, our interpretation of DR not affecting butterfly flight performance should be viewed as a conservative one. Our results resemble those of Nespolo et al. (2005), who found that RMR was affected by short-term fasting in a cricket, while the activity-induced maximum CO_2 production rate was not. The conservation of flight capacity under stressful conditions may be advantageous, first because adult fitness depends on flight, as mating, reproduction, and feeding all require flight. Second, flight enables dispersal to more favorable areas. In another butterfly, *Melitaea cinxia*, FMR has been shown to be positively associated with dispersal rate in the field (Niitepõld et al. 2009). Conserved FMR under food stress suggests that the capacity to disperse is not impaired under nutritionally adverse conditions. Dispersal has indeed been shown to be condition dependent in many systems (Clobert et al. 2009), and environmental changes may result in a proportional increase of dispersive phenotypes (Zera and Denno 1997). In some animals, DR has been shown to change activity patterns and in some cases produce a biphasic pattern where activity is first increased, then decreased (Speakman and Mitchell 2011). Contrary to our study, a clear negative effect of starvation on flight endurance has been found in experiments on two mosquito species (Kaufmann and Briegel 2004). Mosquito flight and butterfly flight differ, however, as fed mosquitoes could fly up to hours at a time, while nonmigratory butterflies typically fly in short bouts.

Absolute PMR and FMR were lower in food-restricted females. However, the reduction was accompanied by a reduction in body mass, so that mass-independent metabolic rates did not differ between the groups. Therefore, the lower body masses of chronically food-restricted females help to maintain flight capacity. Because of lower wing loading, less energy is needed to power flight. This allows food-restricted butterflies to invest less energy in flight while still retaining similar mass-independent PMR than heavier control butterflies. As food-restricted females also had lower mass-independent RMR than controls, this raises the interesting interpretation that food-restricted females had higher metabolic scope, that is, a larger proportion of all available energy could be allocated to flight. This too suggests that butterflies maintain investment in flight when resource availability is low.

Aging and Metabolic Rate

Senescence represents loss of function that is associated with advanced age. Both butterfly species studied here showed signs of metabolic senescence. Our study establishes that nutritional status affects the relationship between RMR and aging: only control females showed a decrease in RMR with age, while food-restricted females appeared to enter a state of stable, chronically lowered RMR. This is interesting because aging does not appear to have a uniform effect on RMR across different study systems. Metabolic senescence has been described in humans (Manini 2010); in laboratory organisms, such as rats (Even et al. 2001) and zebra finches (Moe et al. 2009); and in some wild animals (Broggi et al. 2010). However, several studies have not found effects of aging on metabolic rate. For example, a longitudinal study of BMR in a wild seabird did not reveal any effect of aging (Moe et al. 2007). Fruit flies are model organisms in aging research, but in five species of *Drosophila* no negative associations were found between RMR and age (Promislow and Haselkorn 2002; Hulbert et al. 2004). We suggest that nutritional status must be considered when studying aging patterns within and across different taxa, especially when comparing laboratory populations to natural populations.

The relationship between RMR and age differed in our two species: RMR was highest during the first measurement in *S. mormonia*, while in *C. eurytheme* RMR increased during the first days and reached a peak during the second measurement at the age of 5 d. We do not yet know whether the observed difference is consistent between the two species or whether it reflects methodological differences between the experiments. However, both shapes of the RMR-age relationship have been documented in other insects. An initially high RMR that subsequently decreases has been reported in *D. melanogaster* (Kha-

zaeli et al. 2005) and several butterflies (Zebe 1954; Woods et al. 2010; Niitepõld and Hanski 2013). The convex-shaped relationship occurs, for example, in the Colorado potato beetle (Piiroinen et al. 2010). Young, mated individuals experience significant physiological change, such as flight muscle maturation and production of gametes, which is likely to elevate RMR at a young age. Energetic investment in physiological maturation and egg production is likely to decrease after the first days. Decreasing RMR may therefore reflect a combination of development during early life and senescence during later life. Unlike this study, some studies in insects have reported increased RMR in old individuals, which is likely to reflect increased maintenance cost due to investment in somatic repair mechanisms (Melvin et al. 2007; Niitepõld and Hanski 2013). Since RMR reflects a combination of all processes that require energy in a resting animal, aging signals will not always be straightforward.

Functional senescence, when measured as a decrease in survival or reproduction, is widespread even in the wild (Jones et al. 2008). Even though *D. melanogaster* does not show a decrease in RMR, aging has been shown to reduce its wing-beat frequency (Petrosyan et al. 2007), flight performance (Miller et al. 2008), and crawling performance (Leffelaar and Grigliatti 1984). These kinds of negative effects on performance are in line with our result of decreasing PMR and FMR with age in both our study species. In *S. mormonia* and to a lesser degree in *C. eurytheme*, PMR and FMR were affected by age in a nonlinear fashion: metabolic rate initially increased and then decreased. The initial increase may reflect poor flight ability in very young female butterflies. Newly emerged females have high wing loading (mass–to–wing area ratio) because of traces of pupal meconium; a large fat body; and a large number of unlaid eggs in their abdomen. At the same time, flight muscles may not be fully matured. Work in other flying insects has shown age-related changes in structural flight muscle proteins (Marden et al. 2008; Schippers et al. 2010). Indeed, FMR increases in the honeybee in a similar fashion as in *S. mormonia* during the first 4 d of adult life (Schippers et al. 2010). In a bumblebee, young workers initiate flight before reaching maturity and experience elevation of metabolic enzyme activity, flight muscle respiration rate, and wing-beat frequency (Skandalis et al. 2011). Energetic trade-offs may also explain changes in flight metabolism in young butterflies. There was an inverse relationship between PMR and RMR during the first two measurements in *S. mormonia*, which could be due to flight metabolism being traded off with egg development and other expensive maturation processes. An initially low FMR may therefore be a consequence of several factors, including behavioral choices and physical inability to produce enough power and lift.

Decreasing mass-independent PMR and FMR suggests that flight ability decreased with age. Work on flight endurance in the butterfly *Pieris napi* indicates that old individuals are unable to fly for periods as long as those of younger individuals when forced to fly (Ahman and Karlsson 2009). Decreasing PMR contradicts findings in the butterfly *Melitaea cinxia*, where PMR showed a decrease only immediately before death (Niitepõld and Hanski 2013). In our study, butterflies experienced significant wing wear under the experimental conditions. This was mainly due to repeated handling when feeding the butterflies, coupled with butterflies flying inside small oviposition cages and the respirometer. In bumblebees, a 10% reduction in wing area has been shown not to affect FMR (Hedenström et al. 2001), whereas experimentally induced wing asymmetry did increase FMR (Skandalis and Darveau 2012). More work is needed to understand the potential effects of wing wear in butterflies.

Conclusions

Our study is among the first to look at the effects of DR on energetics throughout the entire adult life span. Environmental stress in the form of restricted food availability resulted in a lower level of maintenance metabolism but did not affect longevity. Investment in flight was conserved, which highlights the importance of flight for butterflies. Flight is especially important as it allows dispersal to more favorable areas. Even though flight metabolism showed marked senescence, the rate of metabolic aging did not differ between the treatments. RMR showed a different pattern, as RMR decreased with age in control females but not in food-restricted females. This result reflects the fundamental importance of adult nutrition on the physiological activity of nectar-feeding butterflies. Under DR conditions, physiological activity at rest was reduced during the important first days of life, when investment in reproduction is at its highest. Indeed, DR caused a strong decrease in fecundity in both species. DR can therefore have fundamental effects on life-history and population dynamics, even though some traits appear unaffected.

Acknowledgments

We thank S. Crawford, G. Delgadillo, C. Fernandez, A. Kindel, L. Murray, M. Pereyda, E. Sataua, S. Scarpetta, M. Sijstermans, T. Uluwehi, and C. Zabel for help during the experiments. We wish to thank three anonymous reviewers for their helpful comments. Funding was provided by the National Science Foundation (grants IOS 0923411 and 134367).

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