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Adult diet affects lifespan and reproduction of the fruit-feeding butterfly *Charaxes fulvescens*

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Abstract

Fruit-feeding butterflies are among the longest lived Lepidoptera. While the use of pollen-derived amino acids by *Heliconius* butterflies has been interpreted as important for the evolution of extended lifespans, very little is known about the life-history consequences of frugivory. This issue is addressed by investigating effects of four adult diets (sugar, sugar with amino acids, banana, and moistened banana) on lifespan and reproduction in the fruit-feeding butterfly *Charaxes fulvescens* Aurivillius (Lepidoptera: Nymphalidae). Female butterflies were collected from Kibale National Park, Uganda, and kept individually in cages near their natural habitat and data were collected on lifespan, oviposition, and hatching of eggs. Lifespan in captivity was longer for the sugar and the amino acid cohort, than for the banana cohorts. The longitudinal pattern of oviposition was erratic, with many days without oviposition and few periods with high numbers of eggs laid. Butterflies typically did not lay eggs during their 1st week in captivity and the length of the period between capture and first reproduction was significantly shorter for butterflies fed moistened banana. The length of the reproduction period (first reproduction–last reproduction in captivity) and the reproduction rate (total number of eggs/length of the reproduction period) did not differ significantly between the diet treatments. Those fed with amino acid and moistened banana had significantly higher egg hatchability than those fed with sugar and banana. We found no evidence for a lifespan cost of reproduction. Our results show that (1) female *C. fulvescens* can use amino acids in their diet for laying fertile eggs, (2) more wing-wear does correlate with lower survival in captivity (indicating aging in the wild), but not with intensity of reproduction (providing no evidence for reproductive aging), and (3) fruit-feeding butterflies may be dietary restricted in the field.

Keywords

Lepidoptera; Nymphalidae; seasonality; weather; wild caught females; wing-wear

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Introduction

Because of the long lifespans recorded for several species of fruit-feeding butterflies and the potential for amino acid enriched diets for this guild, it is suspected that fruit-feeding butterflies are able to use amino acids in the adult diet for reproduction, and that their diet is partly responsible for the evolution of extended lifespans (Brakefield & Kesbeke, 1995) in a similar fashion to the pollen feeding *Heliconius* butterflies (Dunlap-Pianka et al., 1977). However, little evidence is available for a significant effect of adult diet amino acids on female reproduction in this guild. In Australian fruit-feeding *Mycalesis terminus* Fabricius butterflies, the conservation of egg weight on a fruit diet has been documented (Braby & Jones, 1995). Experiments with *Bicyclus anynana* Butler from Africa showed no response in reproductive output to enriched diets compared to a combination of sugars mimicking sugars in banana (Molleman, 2004; Molleman et al., 2008), while Bauerfeind & Fischer (2005) and Bauerfeind et al. (2007) found that this species laid significantly more eggs on a banana than on a sucrose diet, but they were unable to identify a single pivotal substance. Both these experiments on *B. anynana* showed that yeast or processes associated with decay of fruit do not affect reproduction or lifespan in this species. As both *M. terminus* and *B. anynana* are small satyrines, we here test the hypothesis that fruit-feeding butterflies use adult diet derived amino acids for female reproduction using a large and long-lived *Charaxes* species.

Among nectivorous butterflies, there appears to be a wide range in the effects of adult diet on survival and reproduction (Boggs, 1986, 1987; Mevi-Schutz & Erhardt, 2005) that may vary within species and with larval food quality (Mevi-Schutz & Erhardt, 2003). Sugars are often considered the most important resource in the nutritional ecology of butterflies, because they fuel the animal and can be incorporated into eggs (Moore & Singer, 1987; Watanabe, 1992; Boggs, 1997b; Fischer & Fiedler, 2001). However, amino acids derived from the adult diet also play a role in some species, because amino acids from nectar can be incorporated into eggs (Boggs, 1997a,b; Mevi-Schutz & Erhardt, 2005). However, stored protein from the larval stage is the primary nitrogen source in nectivorous butterflies (Hill & Pierce, 1989; Hill, 1989). Adding more detail, O'Brien et al. (2002, 2005) demonstrated that in a nectar-feeding hawkmoth and four butterfly species, females synthesize a large fraction of egg amino acids from nectar sugars using endogenous sources of nitrogen. However, essential amino acids were derived only from the larval diet, placing an upper limit on the use of adult dietary resources to enhance reproductive success.

In contrast to most butterfly species in temperate regions, in tropical forests many butterflies are specialized in feeding on fallen fruits and other alternative substrates, and do not normally visit flowers (Norris, 1936). This fruit-feeding guild is composed of members of certain subfamilies of the Nymphalidae. In tropical Africa, it consists of species of the subfamilies Charaxinae (including *Charaxes fulvescens* Aurivillius), Satyrinae, and Nymphalinae (Larsen, 1991; Molleman et al., 2006). Nectar feeding is considered the primitive feeding behaviour in butterflies (Barth, 1991), and therefore, phylogenetic considerations indicate that multiple diet shifts from nectar-feeding to fruit-feeding have occurred. Although the use of fruit baits for trapping butterflies has provided much information on tropical biodiversity, we know very little about the consequences of frugivory for life histories. Nectar and rotting fruit differ from each other in protein content, sugars, minerals, secondary plant components, and in microbial life and are often (but not always) reported to be more rich in nitrogen or amino acids than flower nectar (Fischer et al., 2004; Molleman et al., 2005b). The actual diet composition of this guild is, however, poorly known, both at the level of the substrates used and their nutritional composition.

Extraordinarily long lifespan records have been documented for fruit-feeding butterflies with lifespans exceeding 4 months not being uncommon (Beck & Schulze, 2000; Carey, 2001;

Molleman et al., 2007). *Charaxes fulvescens* is a fruit-feeding butterfly that occurs in forests of tropical Africa. The lifespan record of this species in the field is 230 days ($n = 474$ recaptured individuals; Molleman et al., 2007). *Charaxes* are unusually powerful, large, and fast flying butterflies, but *C. fulvescens* has a relatively small thorax compared to its wings for a *Charaxes* (Molleman et al., 2005a). In the wild, a few eggs can be laid on a single leaf, but caterpillars are usually found singly on understory shrubs of the genus *Allophylus* (F Molleman, unpubl.).

In this article, we address the consequences of the adult diet on (1) lifespan, (2) longitudinal patterns in reproduction, and (3) hatching of the eggs. In addition, we explored season and weather effects on reproduction, and a possible trade-off between intensity of reproduction and lifespan (Descamps et al., 2006). Because we were using field-collected individuals, this study also provided insights into life-history trajectories in the field and made a contribution to the development of the use of individuals of unknown age to extract life-history information (Müller et al., 2004, 2007; Crespon et al., 2006; Carey et al., 2008).

Materials and methods

Female butterflies were collected from Kibale National Park in Uganda (from 0°13' to 0°41' N and 30°19' to 30°32'E) using traps baited with fermented banana. Butterfly age was rated using a rough index of wing-wear (no = 0, little = 1, intermediate = 2, and severe damage = 3) and mainly butterflies with little or no damage of the wings were taken for this experiment as young individuals. For our analyses, the wing-wear categories 0 and 1 were pooled because their remaining lifespan in captivity did not differ significantly, and 2 and 3 were pooled, because few individuals with severe damage were included in the study (see Results). This left us with 'young' and 'old' butterflies. The butterflies were allocated to individual cages with the experimental diets. We had 28 cages in a room with an open window in a clearing near the forest at Makerere University Biological Field Station in Kibale National Park. Dead butterflies were replaced by freshly collected individuals and this regime was sustained from August 2001 to August 2002. In half of the cages, butterflies were presented a 10% (wt/vol) sugar solution made from locally obtained refined cane-sugar and rainwater, in a vial. The other treatments were consecutively a slice of banana (starting between August 2001 and January 2002), a moistened slice of banana (starting between February 2002 and April 2002), and the sugar solution with added amino acids (starting between May 2002 and August 2002). Individuals were kept on one diet only. Bananas are known to contain about 1 g of protein and 14 g of sugar per 100 g (NutritionData, 2008), and the sugar composition is very variable with about 50% sucrose, 25% glucose, and 25% fructose (Molleman et al., 2008). For the amino acid treatment, amino acids were added to the sugar solution using the proportions in *Lantana camara* nectar (Erhardt, 1991). This solution contained per 100 ml: 0.128 g alanine, 0.065 g arginine, 0.112 g asparagine, 0.096 g glutamic acid, 0.272 g glutamine, 0.356 g glycine, 0.512 g proline, 0.288 g serine, 0.161 g threonine, 0.081 g tyrosine, and 0.037 g valine. An *Allophylus dummeri* Baker (Sapindaceae) cutting with one mature leaf was present continuously as oviposition substrate. Three times per week (Monday, Wednesday, and Friday), eggs were collected and counted and food and oviposition substrate were replaced. During the period January–July 2002, eggs were kept in plastic containers and the number of hatchlings was counted 7 days after collection. Rainfall and temperature data were collected by Colin Chapman and colleagues at the field station (Chapman et al., 2005).

Data analysis

Although the sugar diet was randomly assigned to collected butterflies during the entire experiment, the other diets were not randomized in time. The significant difference in the egg reproduction before and after the first of February 2002 within the sugar diet suggests that

seasonal effects may be confounded with diet. To correct for possible seasonal effects, weather information, such as daily temperature and amount of rainfall, was included as covariates in analyses of lifespan and reproduction of butterflies.

We fitted lifespan in captivity by the proportional hazards model (Cox, 1972, 1975) to compare the diet effects on longevity of butterflies, after confirming that the proportionality assumption was satisfied in these data. Wing-wear as well as daily temperature and rainfall were included in the proportional hazards model. Interaction between diet and age of butterflies (wing-wear) was not significant in the initial model and was therefore excluded. After that, fertility was included as a factor to capture the different survival patterns of infertile and fertile butterflies. Butterflies that laid no eggs during the experiment were defined as infertile (63 out of 206 females).

Daily reproduction was calculated assuming a uniform distribution of egg-laying over the 2–3 day collection periods. This (presumably smoothed) daily reproduction during the reproduction period was erratic and had many spikes. All attempts to fit polynomial and linear mixed effect models as well as non-parametric models to these egg-laying patterns failed, and oviposition during the reproduction period had on average a plateau shape. Therefore, for 143 fertile butterflies, we divided the reproduction process into three periods: prior reproduction (capture to first egg-laying), reproduction (first to last egg-laying), and post-reproduction (last egg-laying to death). The rate of reproduction in captivity was calculated for all fertile individuals as the total number of eggs divided by the length of the reproduction period. These reproduction parameters were compared among diets and wing-wear categories in analysis of variance (ANOVA) and regression models.

Models were selected based on P-values of covariates using stepwise selection. Initially, all weather covariates were included in the model. In the first step, the covariate with biggest P-value was excluded, if it is bigger than 0.05. Then we refitted the model with the remaining covariates. In the second step, the first step is repeated to exclude one more covariate. In the third step, the first excluded covariate was added back to the model to see whether it was significant after excluding the second covariate. If so the first excluded covariates were retained in the model. Otherwise, two covariates are both excluded. We repeated steps 2 and 3 until all covariates left in the model were significant. All statistical analyses and figures (except Figure 4) were produced using R (version 2.4.1; R Development Core Team <http://www.r-project.org>) and SAS (version 9.1; SAS Instruments, Cary, NC, USA).

Results

Lifespan in captivity

The longest lifespan recorded in this experiment was 200 days, which therefore compares favourably with the longest observed lifespan in the field (230 days, see above). We report the estimated hazard ratio with 95% confidence interval in Table 1 and survival functions of all diet groups in Figure 1. Considering all 206 butterflies, sugar- and amino acid-fed groups lived significantly longer than banana- and moistened banana-fed groups (proportional hazards model: $P = 0.0041$); while there is no significant difference between the sugar- and amino-acid fed groups, nor between banana- and moistened banana-fed groups ($P = 0.48$). There was no significant difference between wing-wear = 0 and wing-wear = 1 groups ($P = 0.60$; these categories were therefore merged into one ‘young’ age class), but the young butterflies lived significantly longer in captivity than old ones as we expected ($P < 0.0001$). Less rainfall and higher temperatures were associated with higher mortality of butterflies. Furthermore, the interaction between diet and wing-wear is insignificant, which implies that the hazard ratios of old to young were not significantly different among diets. As a result, the observed median

survival time in captivity between young and old butterflies varies with diet as seen in Figure 2, with the sugar cohort having the longest median lifespan among young butterflies.

Splitting butterflies into infertile and fertile groups, we refitted the survival model for the two groups separately. Infertile butterflies, which laid no eggs in captivity, also differed from others in survival. Even after adjusting diet, wing-wear effects, and weather effects, infertile butterflies lived significantly shorter than fertile butterflies (39 days difference in median given other covariates are at their average values; Figure 3). Furthermore, for those infertile butterflies diet had no significant effect on life time in captivity ($P = 0.46$), while the ‘young’ group had a higher survival rate than the ‘old’ group ($P = 0.040$). For 143 fertile butterflies, the rate of reproduction was initially included as a covariate besides diet and wing-wear for estimating the remaining life time, but this factor had no significant effect and was therefore excluded.

Reproduction

The egg-laying of *C. fulvescens* was erratic with individuals laying no eggs during many periods (74.1% of 2–3-day periods), while on a few days many eggs were counted. The size of the average egg batch was 14.6 and the maximum number of eggs laid over a 2-day period was 80. The largest reproductive output was 356 eggs for a young butterfly of wing-wear 0 fed moistened banana and who lived for 63 days. We found no significant difference in the probability of being infertile among four diet groups, although old butterflies were twice as likely to be infertile than young individuals (Table 2). The longest reproduction period, from the first to the last day of egg-laying, was 103 days, which was from a young butterfly of wing-wear 0 fed moistened banana who lived 140 days and died 5 days after it stopped reproducing. Overall reproduction trajectories were visualized in averaged daily parity plots (Figure 4). These show that: (1) on most days butterflies did not lay eggs; (2) there appears to be a decline in reproduction with age in the sugar cohort that is an artefact of long post-reproduction periods in this cohort; and (3) there appears to be some increase in egg production when sources of amino acids are offered, particularly in the moistened banana treatment, which, however, did not bare out fully statistically (see below).

The analysis of reproduction is confounded by possible weather or seasonal effects that were not countered by a balanced design and therefore the following results should be treated with some caution. Some weather effects were adjusted for by including average temperature and rainfall as covariates in regression models. Model predictions with 95% confidence interval are presented in Table 3 and statistical results of regression analyses on transformed data in Table 4. Butterflies fed with moistened banana started reproducing significantly earlier. The sugar cohort had a significantly longer post-reproduction period than all other diet treatments, indicating that their longer lifespans compared to other diet treatments (Figure 1) can be attributed to longer non-reproductive periods. The reproduction period in captivity of butterflies fed with moistened banana is slightly shorter than those fed with sugar and amino acid (which could be related to the shorter lifespans in the banana cohorts) but not significantly, and this treatment also produced the longest individual reproduction period, thus showing a potential for sustained reproduction on a moistened banana diet. The reproduction rate of butterflies fed with moistened banana is higher (albeit not significantly) than those fed with sugar. Combining rate of reproduction and length of the reproduction period, the total reproduction in captivity was not significantly different from those fed with sugar. Butterflies fed with amino acid reproduced longer (even though they lived slightly shorter; Figure 1) but at a similar rate compared to the sugar cohort, and this resulted in an insignificant difference in total reproduction between the amino acid and sugar cohorts.

Hatchability of the eggs

Some butterflies only laid eggs that never hatched and appeared empty and shrivelled, while others started out laying eggs that hatched and later in life produced such infertile eggs. Starting from 30 November 2001, the number of caterpillars hatched from the eggs of 88 butterflies was counted and used to calculate egg hatchability. After adjusting the average temperature and rainfall, we found that butterflies in the amino acid and moistened banana cohorts had significantly higher egg hatchability than in the sugar and banana cohorts [ANOVA on $\log(x + 1)$ -transformed hatchability: $P = 0.003$; Figure 5]. Wing-wear did not significantly affect hatchability in this analysis.

Discussion

By using individuals directly from the field, there is more variation in our study population than would exist in laboratory populations. By capturing them over an extended period and keeping them near the wild environment, seasonal effects of both individual condition (they had a pre-capture larval and adult diet, lifespan, copulation(s), and reproduction), and weather during the experiment, will add additional variance, which was not countered with a balanced design in this experiment. Despite this variation, analysis of the remaining lifespan and reproduction showed significant effects of adult diet on female lifespan and reproduction parameters.

While butterflies fed with banana (banana and moistened banana) lived shorter than those fed with sugar-based diets (sugar and amino acid; Figure 1), within these groups, there were parallel effects on reproduction: within the banana diets, the moistened banana cohort reproduced earlier, while in the sugar group, females that had added amino acids in their diet reproduced earlier (Table 3). Hatchability of the eggs was also elevated in both moistened banana and amino acid treatment. That the moistened banana diet positively affected reproduction in a similar fashion to the amino acid-enriched diet (shorter pre-reproduction period and higher hatchability of eggs) suggests that *C. fulvescens* can derive amino acids from fruits or benefit from other nutrients available in banana. However, this response to amino acid-enriched adult diet is not comparable to that of some of the long-lived *Heliconius* butterflies that feed on pollen. In these species, egg production is sustained using amino acids from the pollen and nuptial gifts from males (Gilbert, 1972; Dunlap-Pianka et al., 1977; O'Brien et al., 2003; Eberhard et al., 2007), while we found no significant effects of amino acids in the adult diet on the length or intensity of the reproduction period of the fruit-feeding *C. fulvescens*. The lack of such an effect may be due to a too low concentration of amino acids, the composition being inappropriate (Orentreich et al., 1993; O'Brien et al., 2005), or fore-mentioned compromises in the experimental design, but overall our results indicate small effects of amino acids on egg production. The most significant benefits of amino acids in the adult diet may be in increased hatchability, which was only measured for a subset of individuals in an unbalanced design. The observed longitudinal pattern of egg-laying in *C. fulvescens*, however, is akin to the constant reproduction hypothesized for butterflies that use adult diet for egg-production (Boggs, 1987), or the plateau identified in *Drosophila* and Medfly egg laying (Novoseltsev et al., 2004, 2005), although no decline phase was observed (any decline seen in Figure 4A can be attributed to long post-reproductive lifespans in the sugar cohort). Post-reproductive periods were observed and these were significantly longer in the sugar cohort than in other cohorts. Because we did not perform dissections, we do not know whether butterflies had no more eggs to lay (egg-limited), or died before laying all their eggs (time-limited). The high frequency of days without oviposition interspersed with days on which many eggs are laid, contrasts with the more regular oviposition patterns reported for *Heliconius* butterflies (Dunlap-Pianka, 1979). Some of this irregularity was probably caused by weather influences and possibly by varying oviposition substrate quality, and thus more insight into egg-laying patterns may be

gained from experiments under standardized conditions, but the erratic pattern may also be a characteristic trait that plays a role in the ecology of the species. A possible principle difference between the nutritional ecology of pollen feeding and fruit-feeding butterflies may be that in pollen-feeding butterflies intake of sugars (flower nectar) and amino acids (flower pollen) is not necessarily coupled, while fruit-feeding butterflies would extract both from fruit juice. Therefore, given the possible dietary restriction experienced in the field (see below), fruit-feeding butterflies may have little scope to optimize amino acid intake in the field (Simpson et al., 2004).

Our results provide little evidence for potential of the use of amino acids from adult food of fruit-feeding butterflies to sustain egg production over longer periods of time, but there was a significant positive effect on the onset of reproduction after capture and on egg hatchability. Therefore, nutritional benefits of the fruit-feeding habit may in part be responsible for the evolution of long lifespans in this guild of tropical forest butterflies, as extra days of life could be used for extra production of hatching eggs based on amino acids or other nutrients derived from the adult diet. We hypothesize that in the wild, butterflies will use this potential by feeding preferentially on substrates that contain sources of amino acids. However, female charaxine butterflies are rarely encountered on protein-rich substrates such as carrion and preference tests with the same amino acid solution used in this experiment revealed no preferences in *C. fulvescens* or any other fruit-feeding butterfly species (Molleman et al., 2005a), but they may prefer protein as shown by Boggs & Dau (2004) for nectar feeding butterflies. In addition, the diet of fruit-feeding butterflies and in particular Charaxinae in the field is not well known as few *Charaxes* were observed feeding on fruits compared to their relative abundance in traps (Molleman et al., 2005b). Nuptial feeding (males donate large spermatophores) may also contribute to amino acid intake of females of this species and affect egg production as it does in nectar and pollen-feeding species (Boggs & Gilbert, 1979; Boggs, 1990; Mevi-Schutz & Erhardt, 2004).

Some butterflies laid eggs that did not hatch. This could be a result of a lack of certain nutrients not provided in the diets, or a lack of sperm (Ehrlich & Ehrlich, 1978; Wedell et al., 2002). Because butterflies on amino acid and moistened banana diets did have a higher proportion of their eggs hatching, amino acids and possibly other nutrients are probably important for the production of fertile eggs. In addition, the possibility of sperm depletion in long-lived fruit-feeding butterflies needs further exploration, as in the wild, females of most butterfly species mate several times (Molleman et al., 2005a).

Phenotypic effects of diet on reproduction can only be understood in relation to its effects on survival (Piper, 2005). In contrast to several other studies (Müller et al., 2001), including the fruit-feeding butterfly, *B. anynana* (Molleman et al., 2008), we found no lifespan cost of reproduction. Although reproduction rate did not predict survival in fertile butterflies, it is possible to argue that there is an indication for costs of reproduction: both reproduction and lifespan responded to diet (e.g., sugar-fed butterflies living longer and laying fewer eggs than other butterflies), while among infertile butterflies we detected no diet effect on survival. Therefore, the diet effect on survival of fertile butterflies could be via reproduction. However, infertile butterflies lived much shorter than those that laid eggs (which may simply be because a short lifespan offers little opportunity for oviposition), and such short lifespans provided a short time window for experimental diet to affect survival.

It is unclear how adult diet caused significant differences in longevity among the diets. Amino acids decreased survival slightly in female *B. anynana* without affecting reproduction (Molleman, 2004), while it increased survival in males of four out of 13 Bornean rainforest butterflies that had no opportunity to mate (Beck, 2007), and female *Heliconius* butterflies deprived of pollen live shorter (Dunlap-Pianka et al., 1977). Much work has been done on

lifespan extension by dietary restriction (e.g., Phelan & Austad, 1989; Carey et al., 2002, 2005; Mair et al., 2003, 2005; Mair, 2004; Houthoofd et al., 2005; Partridge, 2005; Piper, 2005; Min & Tatar, 2006; Crawford et al., 2007; Weithoff, 2007). However, important model organisms such as rats and *Drosophila melanogaster* Meigen have an evolutionary history on more or less complete diets, while an adult diet based on nectar sugars dominates butterfly evolutionary history (Barth, 1991). Therefore, these studies may not be directly translatable to fruit-feeding butterflies.

Before we can fully understand the evolutionary implications of effects of dietary restriction on lifespan, two important caveats have to be considered: (1) amino acids in the adult diet could change the lifespan of butterflies in ways that are not relevant in the captive environment, for example, by maintaining predator avoidance capacity (e.g., flight muscles), and (2) a possibly underestimated phenomenon in lifespan studies is the influence of behaviour on lifespan, because active flight in cages can cause impairments and may shorten lifespan (Carey et al., 2007), and behaviour may be affected by diet (Piper, 2007). For example, amino acid-deprived individuals may search for amino acids, and thus incur damage, or alternatively become weak flyers and spend less time beating their wings (Karlsson, 1994). Therefore, future lifespan studies would be more informative if behaviour was included (Papadopoulos et al., 2002; Piper, 2007).

Mark recapture studies have shown an increase in wing-wear with age in the wild (e.g., Benson, 1972). Our results showed a decrease in survival with increasing wing-wear at capture, and thus either an impairment effect on survival in these cages, or aging in the wild. The latter may be more likely as butterflies had little use for flight in these cages. Because wing-wear did roughly correlate with remaining lifespan in captivity, it may be used as a surrogate for age at the cohort level (Müller et al., 2004). However, this remains problematic for two reasons: (1) butterflies can suffer injuries at any age and can also be handled by birds without showing beak marks (Wourms & Wasserman, 1985), thus generating extensive variation in wing-wear within age classes, and (2) the average actual age difference between wing-wear categories is difficult to estimate using remaining lifespan in captivity, as this estimate will depend on diet and possibly other experimental conditions.

Although diet did affect reproduction parameters, wing-wear (and thus age) only slightly influenced length of reproduction period and therefore also total reproduction (Table 3). This is remarkable because field captured butterflies can be expected to already have laid eggs in the wild, and this apparently did not affect length of prior reproduction period and reproduction intensity. This indicates that in the wild these butterflies commonly experience a restricted diet and egg laying rates in the wild may be low, as no eggs appear to be ready for oviposition upon capture. Alternatively, individuals need time to adjust to the captive environment, but then we would not expect diet to affect the length of the pre-reproductive period as it did in our study, as this time difference indicates a diet effect on egg maturation rate. That longevity in the banana cohort was very similar to that in the moistened banana cohort, although reproduction was affected by the wetting, indicates that this species is able to conserve lifespan at the cost of reproduction under dietary restriction, similar to *Speyeria mormonia* (Boggs & Ross, 1993). In general, information on lifespan and reproduction in captivity of wild-caught insects of different ages could provide insights into life-history trajectories in the wild.

Conclusions

Lifespan is not significantly affected by the addition of amino acids, but is longer on sugar water- than on banana-based diets. Long-lived *C. fulvescens* butterflies may use amino acids in the adult diet to produce fertile eggs, as it shortens their prior oviposition period and increases hatchability of the eggs, while egg numbers were non-significantly higher. Because extra days

of life can then be used for the production of hatching eggs, the use of amino acids in the adult diet for female reproduction may be partly responsible for the evolution of extended lifespan in fruit-feeding butterflies. More wing-wear is related to lower survival in captivity in this species. Our results indicated that fruit-feeding butterflies are dietary restricted in the field. No lifespan cost of reproduction was detected in these data.

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References

- Barth, FG. Insects and Flowers: The Biology of a Partnership. Princeton University Press; Princeton, NJ, USA: 1991.
- Bauerfeind SS, Fischer K. Effects of adult-derived carbohydrates, amino acids and micronutrients on female reproduction in a fruit-feeding butterfly. *Journal of Insect Physiology* 2005;51:545–554. [PubMed: 15894001]
- Bauerfeind SS, Fischer K, Hartstein S, Janowitz S, Martin-Creuzburg D. Effects of adult nutrition on female reproduction in a fruit-feeding butterfly: the role of fruit decay and dietary lipids. *Journal of Insect Physiology* 2007;53:964–973. [PubMed: 17493631]
- Beck J. The importance of amino acids in the adult diet of male tropical rainforest butterflies. *Oecologia* 2007;151:741–747. [PubMed: 17318609]
- Beck J, Schulze CH. Diversity of fruit-feeding butterflies (Nymphalidae) along a gradient of tropical rainforest succession in Borneo with some remarks on the problem of ‘pseudo-replicates’. *Transactions of the Lepidopterological Society of Japan* 2000;51:89–98.
- Benson WW. Natural selection for Müllerian mimicry in *Heliconius erato* in Costa Rica. *Science* 1972;176:936–939. [PubMed: 17829303]
- Boggs CL. Reproductive strategies of female butterflies: variation in and constraints on fecundity. *Ecological Entomology* 1986;11:7–15.
- Boggs, CL. Ecology of nectar and pollen feeding in Lepidoptera. In: Slansky, FJR.; Rodriguez, JG., editors. *Nutritional Ecology of Insects, Mites, Spiders and Related Invertebrates* (ed. John Wiley and Sons; New York, NY, USA: 1987. p. 369-391.
- Boggs CL. A general model of the role of male-donated nutrients in female insects reproduction. *American Naturalist* 1990;136:598–617.
- Boggs CL. Dynamics of reproductive allocation from juvenile and adult feeding: radiotracer studies. *Ecology* 1997a;78:192–202.
- Boggs CL. Reproductive allocation from reserves and income in butterfly species with differing adult diets. *Ecology* 1997b;11:181–191.
- Boggs CL, Dau B. Resource specialization in puddling Lepidoptera. *Environmental Entomology* 2004;33:1020–1024.
- Boggs CL, Gilbert LE. Male contribution to egg production in butterflies: evidence for transfer of nutrients at mating. *Science* 1979;206:83–84. [PubMed: 17812454]
- Boggs CL, Ross CL. The effect of adult food limitation on life-history traits in *Speyeria mormonia* (Lepidoptera, Nymphalidae). *Ecology* 1993;9:433–441.
- Braby MF, Jones RE. Reproductive patterns and resource allocation in tropical butterflies: influence of adult diet and seasonal phenotype on fecundity, longevity and egg size. *Oikos* 1995;72:189–204.

- Brakefield PM, Kesbeke F. Raised adult lifespan and female fecundity in tropical fruit-feeding *Bicyclus* butterflies. *Proceedings of the Section Experimental and Applied Entomology of the Netherlands Entomological Society (NEV)* 1995;6:93–98.
- Carey JR. Insect biodemography. *Annual Review of Entomology* 2001;46:79–110.
- Carey JR, Liedo P, Harshman L, Müller H-G, Partridge L, et al. Life history response of Mediterranean fruit flies to dietary restriction. *Aging Cell* 2002;1:140–148. [PubMed: 12882344]
- Carey JR, Liedo P, Müller H-G, Wang J-L, Zhang Y, Harshman L. Stochastic dietary restriction using a Markov-chain feeding protocol elicits complex, life history response in medflies. *Aging Cell* 2005;4:31–39. [PubMed: 15659211]
- Carey JR, Pinter-Wollman N, Wyman M, Müller HG, Molleman F, Zhang N. A search for principles of disability using experimental impairment of *Drosophila melanogaster*. *Experimental Gerontology* 2007;42:166–172. [PubMed: 17118600]
- Carey JR, Papadopoulos NT, Müller HG, Katsoyannos BI, Kouloussis NA, et al. Age structure changes and extraordinary lifespan in wild medfly populations. *Aging Cell* 2008;7:426–437. [PubMed: 18363903]
- Chapman CA, Chapman LJ, Struhsaker TT, Zanne AE, Clark CJ, Poulsen JR. A long-term evaluation of fruiting phenology: importance of climate change. *Journal of Tropical Ecology* 2005;21:31–45.
- Cox DR. Regression models and life-tables. *Journal of Royal Statistical Society, Series B* 1972;34:187–220.
- Cox DR. Partial likelihood. *Biometrika* 1975;62:264–276.
- Crawford D, Libina N, Kenyon C. *Caenorhabditis elegans* integrates food and reproductive signals in lifespan determination. *Aging Cell* 2007;6:715–721. [PubMed: 17711560]
- Crespon L, Harris MP, Lebreton JD, Wanless S. Increased adult mortality and reduced breeding success with age in a population of common guillemot *Uria aalge* using marked birds of unknown age. *Journal of Avian Biology* 2006;37:273–282.
- Descamps S, Boutin S, Berteaux D, Gaillard JM. Best squirrels trade a long life for an early reproduction. *Proceedings of the Royal Society B – Biological Sciences* 2006;273:2369–2374.
- Dunlap-Pianka HL. Ovarian dynamics in *Heliconius* butterflies: correlations among daily oviposition rates, egg weights, and quantitative aspects of oogenesis. *Journal of Insect Physiology* 1979;25:741–749.
- Dunlap-Pianka HL, Boggs CL, Gilbert LE. Ovarian dynamics in Heliconiine butterflies: programmed senescence versus eternal youth. *Science* 1977;197:487–490. [PubMed: 17783249]
- Eberhard SH, Hrassnigg N, Crailsheim K, Krenn HW. Evidence of protease in the saliva of the butterfly *Heliconius melpomene* (L.) (Nymphalidae, Lepidoptera). *Journal of Insect Physiology* 2007;53:126–131. [PubMed: 17210163]
- Ehrlich AH, Ehrlich PA. Reproductive strategies in the butterflies. I. Mating frequency, plugging, and egg number. *Journal of the Kansas Entomological Society* 1978;51:666–697.
- Erhardt A. Nectar sugar and amino-acid preferences of *Battus philenor* (Lepidoptera, Papilionidae). *Ecological Entomology* 1991;16:425–434.
- Fischer K, Fiedler K. Effects of adult feeding and temperature regime on fecundity and longevity in the butterfly *Lycaena hippothoe* (Lycaenidae). *Journal of the Lepidopterists' Society* 2001;54:91–95.
- Fischer K, O'Brien DM, Boggs CL. Allocation of larval and adult resources to reproduction in a fruit-feeding butterfly. *Functional Ecology* 2004;18:656–663.
- Gilbert LE. Pollen feeding and reproductive biology of *Heliconius* butterflies. *Proceedings of the National Academy of Sciences of the USA* 1972;69:1403–1407. [PubMed: 16591992]
- Hill CJ. The effect of adult diet on the biology of butterflies. 2. The common crow butterfly, *Euploea core corinna*. *Oecologia* 1989;81:258–266.
- Hill CJ, Pierce NE. The effect of adult diet on the biology of butterflies. 1. The common imperial blue, *Jalmenus evagoras*. *Oecologia* 1989;81:249–257.
- Houthoofd K, Johnson TE, Vanfleteren JR. Dietary restriction in the nematode *Caenorhabditis elegans*. *Journals of Gerontology Series A – Biological Sciences and Medical Sciences* 2005;60:1125–1131.

- Karlsson B. Feeding habits and change of body composition with age in three Nymphalid butterfly species. *Oikos* 1994;69:224–230.
- Larsen, TB. The Butterflies of Kenya and Their Natural History. Oxford University Press; Oxford, UK: 1991.
- Mair W. Lifespan extension by dietary restriction in female *Drosophila melanogaster* is not caused by a reduction in vitellogenesis or ovarian activity. *Experimental Gerontology* 2004;39:1011–1019. [PubMed: 15236760]
- Mair W, Goymer P, Pletcher SC, Partridge L. Demography of dietary restriction and death in *Drosophila*. *Science* 2003;301:1731–1733. [PubMed: 14500985]
- Mair W, Piper MDW, Partridge L. Calories do not explain extension of life span by dietary restriction in *Drosophila*. *PLoS Biology* 2005;3:1–7.
- Mevi-Schutz J, Erhardt A. Larval nutrition affects female nectar amino acid preference in the map butterfly (*Araschnia levana*). *Ecology* 2003;84:2788–2794.
- Mevi-Schutz J, Erhardt A. Mating frequency influences nectar amino acid preference of *Pieris napi*. *Proceedings of the Royal Society of London Series B – Biological Sciences* 2004;271:153–158.
- Mevi-Schutz J, Erhardt A. Amino acids in nectar enhance butterfly fecundity: a long-awaited link. *American Naturalist* 2005;165:411–419.
- Min KJ, Tatar M. Restriction of amino acids extends lifespan in *Drosophila melanogaster*. *Mechanisms of Ageing and Development* 2006;127:643–646. [PubMed: 16616772]
- Molleman, F. PhD Thesis. Leiden University; Leiden, The Netherlands: 2004. Patterns of biodiversity and life history in fruit-feeding butterflies.
- Molleman F, Ding J, Wang J-L, Brakefield PM, Carey JR, Zwaan BJ. Dietary protein does not affect life span and egg numbers in the fruit-feeding butterfly, *Bicyclus anynana*. *Ecological Entomology* 2008;33:429–438. [PubMed: 19081752]
- Molleman F, Grunsven RHA, Liefting M, Zwaan BJ, Brakefield PM. Is male puddling behaviour of tropical butterflies targeted at sodium for nuptial gifts or activity? *Biological Journal of the Linnean Society* 2005a;86:345–361.
- Molleman F, van Alphen ME, Brakefield PM, Zwaan BJ. Preferences and food quality of fruit-feeding butterflies in Kibale Forest, Uganda. *Biotropica* 2005b;37:657–663.
- Molleman F, Kop A, Brakefield PM, DeVries PJ, Zwaan BJ. Vertical and temporal patterns of biodiversity of fruit-feeding butterflies in a tropical forest in Uganda. *Biodiversity and Conservation* 2006;15:107–121.
- Molleman F, Zwaan BJ, Brakefield PM, Carey JR. Extraordinary long life spans in fruit-feeding butterflies can provide window on evolution of life span and aging. *Experimental Gerontology* 2007;42:472–482. [PubMed: 17360139]
- Moore RA, Singer MC. Effects of maternal age and adult diet on egg weight in the butterfly *Euphydryas editha*. *Ecological Entomology* 1987;12:401–408.
- Müller HG, Carey JR, Wu D, Liedo P, Vaupel JW. Reproductive potential predicts longevity of female Mediterranean fruit flies. *Proceedings of the Royal Society B – Biological Sciences* 2001;268:445–450.
- Müller HG, Wang JL, Carey JR, Caswell-Chen EP, Chen C, et al. Demographic window to aging in the wild: constructing life tables and estimating survival functions from marked individuals of unknown age. *Aging Cell* 2004;3:125–131. [PubMed: 15153180]
- Müller HG, Wang J-L, Yu W, Delaigle A, Carey JR. Survival in the wild via residual demography. *Theoretical Population Biology* 2007;72:513–522. [PubMed: 17727909]
- Norris MJ. The feeding-habits of the adult Lepidoptera Heteroneura. *Transactions of the Royal Entomological Society of London* 1936;85:61–90.
- Novoseltsev VN, Arking R, Carey JR, Novoseltseva JA, Yashin AI. Individual fecundity and senescence in *Drosophila* and medfly. *Journals of Gerontology Series A – Biological Sciences and Medical Sciences* 2005;60:953–962.
- Novoseltsev VN, Carey RJ, Novoseltseva JA, Papadopoulos NT, Blay S, Yashin AI. Systemic mechanisms of individual reproductive life history in female Medflies. *Mechanisms of Ageing and Development* 2004;125:77–87. [PubMed: 14706240]

- NutritionData. Nutrition facts: know what you eat (USDA's national nutrient database for standard reference). 2008. available at <http://www.nutritiondata.com/facts-C00001-01c20Tm.html>
- O'Brien DM, Boggs CL, Fogel ML. Pollen feeding in the butterfly *Heliconius charitonia*: isotopic evidence for essential amino acid transfer from pollen to eggs. *Proceedings of the Royal Society of London Series B – Biological Sciences* 2003;270:2631–2636.
- O'Brien DM, Boggs CL, Fogel ML. The amino acids used in reproduction by butterflies: a comparative study of dietary sources using compound-specific stable isotope analysis. *Physiological and Biochemical Zoology* 2005;78:819–827. [PubMed: 16096984]
- O'Brien DM, Fogel ML, Boggs CL. Renewable and non-renewable resources: amino acid turnover and allocation to reproduction in Lepidoptera. *Proceedings of the National Academy of Sciences of the USA* 2002;6:4413–4418.
- Orentreich N, Matias JR, DeFelice A, Zimmerman JA. Low methionine ingestion by rats extends life span. *Journal of Nutrition* 1993;123:269–274. [PubMed: 8429371]
- Papadopoulos NT, Carey JR, Katsoyannos BI, Kouloussis NA, Muller HG, Liu XL. Supine behaviour predicts the time to death in male Mediterranean fruitflies (*Ceratitis capitata*). *Proceedings of the Royal Society of London Series B – Biological Sciences* 2002;269:1633–1637.
- Partridge L. Dietary restriction in *Drosophila*. *Mechanisms of Ageing and Development* 2005;126:938–950. [PubMed: 15935441]
- Phelan JP, Austad SN. Natural-selection, dietary restriction, and extended longevity. *Growth Development and Aging* 1989;53:4–5.
- Piper MDW. Counting the calories: The role of specific nutrients in extension of life span by food restriction. *Journals of Gerontology Series A – Biological Sciences and Medical Sciences* 2005;60:549–555.
- Piper M. Comment by Matthew Piper, William Mair, Linda Partridge on Min KJ, Flatt T, Kulaots I, Tatar M (2006) 'Counting calories in *Drosophila* dietary restriction'. *Experimental Gerontology* 2007;42:253–255. [PubMed: 17317064]
- Simpson SJ, Sibly RM, Lee KP, Behmer ST, Raubenheimer D. Optimal foraging when regulating intake of multiple nutrients. *Animal Behaviour* 2004;68:1299–1311.
- Watanabe M. Egg maturation in laboratory-reared females of the swallowtail butterfly, *Papilio xuthus* L. (Lepidoptera: Papilionidae), feeding on different concentration solutions of sugar. *Zoological Science* 1992;9:133–141.
- Wedell N, Gage MJG, Parker GA. Sperm competition, male prudence and sperm-limited females. *Trends in Ecology and Evolution* 2002;17:313–320.
- Weithoff G. Dietary restriction in two rotifer species: the effect of the length of food deprivation on life span and reproduction. *Oecologia* 2007;153:303–308. [PubMed: 17453249]
- Wourms MK, Wasserman FE. Bird predation on Lepidoptera and the reliability of beak-marks in determining predation pressure. *Journal of the Lepidopterists' Society* 1985;39:239–261.

Figure 1. Estimated survival functions of wild-caught *Charaxes fulvescens* females on one of four diets at averaged wing-wear, rainfall, and temperature values.

Figure 2.

Box plot of observed remaining lifetime in captivity for *Charaxes fulvescens* females fed with one of four diets with sample size in brackets. The median is indicated by the solid line in each box, whose lower and upper bounds are the first and third quartiles, respectively. The difference between the first and third quartiles is referred to as interquartile range (IQR). Data points that are at least 1.5 IQR smaller than the first quartile or 1.5 IQR larger than the third quartile are indicated by circles and referred to as outliers. A whisker connects the boundary of the box with the largest value that is not an outlier.

Figure 3. Estimated survival functions for fertile and infertile *Charaxes fulvescens* butterflies, at averaged covariates, with 95% confidence intervals (dotted lines).

Figure 4.

Averaged temporal patterns in reproduction, plotted as daily parity averaged per week for *Charaxes fulvescens* fed on (A) sugar, (B) banana, (C) moistened banana, and (D) sugar with amino acids; white: 0 eggs, light grey: 1–5 eggs, dark grey: 6–10 eggs, and black: 11–40 eggs. (A) uses data on all sugar-fed individuals. Note that eggs were counted every 2–3 days and, to represent daily parity, these eggs were evenly distributed over these periods and then averaged over 7-day periods. On most days, individuals did not lay eggs (y-axis here truncated at 0.5). Only in the sugar-only group do we see some decline in reproductive output with age, which can be explained by the longer post-reproductive periods recorded in this cohort, and the larger sample size and high survival rate gave a higher chance on long-lived individuals. Amino acid-enriched treatments show some elevated reproduction compared to the sugar-only treatment, which may be confounded by seasonal effects.

Figure 5.

Box plot of observed hatchability for *Charaxes fulvescens* eggs laid by females fed with one of four diets with sample size in brackets. The median is indicated by the solid line in each box, whose lower and upper bounds are the first and third quartiles, respectively. The difference between the first and third quartiles is referred as inter quartile range (IQR). Data points that are at least 1.5 IQR smaller than the first quartile or 1.5 IQR larger than the third quartile are indicated by circles and referred to as outliers. A whisker connects the boundary of the box with the largest value that is not an outlier.

Table 1

Estimated hazard ratios with 95% confidence intervals of *Charaxes fulvescens* females, treating young butterflies fed with sugar-only as baseline group. After adjusting for rainfall and average daily temperature, the diet significantly affects the survival rate ($P = 0.0002$).

Variable	Hazard ratio (P)	95% confidence interval
Old vs. young	1.814 (<0.001)	1.347–2.441
Banana vs. sugar	1.948 (<0.001)	1.380–2.749
Moistened banana vs. sugar	2.045 (<0.001)	1.412–2.961
Amino acid vs. sugar	1.425 (0.238)	0.791–2.565
Increase 1 mm rainfall	0.975 (0.012)	0.956–0.994
Increase 1 unit temperature	1.113 (0.089)	0.984–1.260

Table 2

Odds ratio estimation for logistic regression on the probability of being infertile (laying no eggs). In this model, young butterflies fed with sugar were treated as baseline group. An effect is significant when the confidence interval does not contain one

Effect	Odds ratio	95% confidence interval
Banana	1.296	0.641–2.619
Moistened banana	0.704	0.313–1.587
Amino acid	0.431	0.088–2.103
Old	2.038	1.101–3.772

Table 3

95% confidence interval for reproduction of female *Charaxes fulvescens* fed with one of four diets: prior-reproduction, reproduction, and post-reproduction period, and reproduction rate (number of eggs/length of reproduction period). All response variables have been transformed by $\log(x + 1)$ to symmetrize and stabilize variation in the ANOVA model, and estimated values here have been transformed back to its original scale. All other covariates in the model are evaluated at their average values

	Days			Eggs/day
	Prior-reproduction period	Reproduction period	Post-reproduction period	Reproduction rate
Sugar	12.73 (9.31–17.29)	19.87 (14.71–26.74)	10.22 (7.91–13.14)	3.05 (2.50–3.70)
Banana	10.55 (7.04–15.60)	13.92 (9.41–20.38)	4.68 (3.24–6.61)	3.46 (2.70–4.38)
Moistened banana	5.56 (3.55–8.46)	15.06 (10.18–22.08)	4.92 (3.41–6.95)	4.12 (3.24–5.18)
Amino acid	7.76 (3.21–17.25)	31.51 (14.70–66.33)	4.38 (1.97–8.71)	2.95 (1.71–4.77)

Table 4

Results of regression analyses of reproduction by *Charaxes fulvescens* using log transformed data (see text). Treatment and factors are contrasted with data for young butterflies (no to little wing damage, see text) on a sugar diet at zero rainfall and zero temperature. Significant effects are in bold and other results of special interest in italics. Due to unbalanced design, these results can be confounded by seasonal effects that could have affected the results through the quality of individuals captured from the field, and weather influences on daily oviposition. Therefore, these numbers should be interpreted with some caution

	Estimate	SE	t-value	Pr (> t)
Prior reproduction period (log days + 1)				
Intercept	0.9148	3.57252	0.256	0.7983
Wing-wear	-0.0516	0.20647	-0.250	0.8030
Banana	-0.1729	0.23497	-0.736	0.4632
Moistened banana	-0.7384	0.23846	-3.096	0.0024 ^{**}
Amino acids	-0.4497	0.40294	-1.116	0.2664
Rainfall	0.0817	0.05585	1.464	0.1456
Average temperature	0.0676	0.17110	0.395	0.6933
Post-reproduction period (log days + 1)				
Intercept	6.0471	2.8778	2.101	0.0375 [*]
Wing-wear	-0.0827	0.1663	-0.497	0.6197
Banana	-0.6810	0.1893	-3.598	0.0004 ^{***}
Moistened banana	-0.6387	0.1921	-3.325	0.0011 ^{**}
Amino acids	-0.7360	0.3246	-2.268	0.0249 [*]
Rainfall	-0.0075	0.0450	-0.167	0.8675
Average temperature	-0.1790	0.1378	-1.299	0.1962
Reproduction period (log days + 1)				
Intercept	7.4675	3.5417	2.108	0.0368 [*]
Wing-wear	-0.4224	0.2047	-2.064	0.0409 [*]
Banana	-0.3356	0.2329	-1.441	0.1520
Moistened banana	-0.2622	0.2364	-1.109	0.2694
Amino acids	0.4430	0.3995	1.109	0.2694
Rainfall	0.0468	0.0554	0.845	0.3995
Average temperature	-0.2261	0.1696	-1.333	0.1848
Rate of reproduction (eggs/days)				
Intercept	4.6081	1.8414	2.503	0.0135 [*]
Wing-wear	-0.0091	0.1064	-0.086	0.9320
Banana	0.0964	0.1211	0.796	0.4273
Moistened banana	0.2335	0.1229	1.900	0.0596
Amino acids	-0.0252	0.2077	-0.121	0.9036
Rainfall	-0.0337	0.0288	-1.170	0.2442
Average temperature	-0.1531	0.0882	-1.736	0.0848
Total reproduction in captivity (log eggs + 1)				
Intercept	12.8098	3.6415	3.518	0.0006 ^{***}

	Estimate	SE	t-value	Pr (> t)
Wing-wear	-0.4405	0.2105	-2.093	0.0382 *
Banana	-0.2137	0.2395	-0.892	0.3738
Moistened banana	0.0271	0.2431	0.112	0.9113
Amino acids	0.4899	0.4107	1.193	0.2350
Rainfall	0.0080	0.0569	0.141	0.8881
Average temperature	-0.4363	0.1744	-2.502	0.0135 *

* 0.01 < P < 0.05

** 0.001 < P < 0.01

*** P < 0.001.