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Amino acid sources in the adult diet do not affect life span and fecundity in the fruit-feeding butterfly *Bicyclus anynana*

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Abstract

1. In tropical forests, the adults of many butterfly species feed on fruits rather than nectar from flowers and have long life spans. Rotting fruit and nectar differ from each other in many respects, including sources of amino acids and microbial life. If amino acids in the adult diet can be used for reproduction, this may have facilitated the evolution of extended life spans in this guild.
2. This issue was addressed by investigating effects of banana, yeast, and amino acids in the adult diet of the fruit-feeding butterfly *Bicyclus anynana* (Lepidoptera) on longevity and female reproductive output in two experiments.
3. Results showed that in the fruit-feeding butterfly *B. anynana*: (i) banana juice, but not sliced banana or added amino acids extend life span compared with a sugar solution of similar composition; (ii) compared with this sugar solution, other cohorts (banana juice-amino acid enriched) did not have significantly higher reproductive outputs; (iii) yeast does not represent a valuable source of nutrients; (iv) caloric restriction may cause decreased life span and rate of reproduction; and (v) increased rates of reproduction have a life span cost.

Keywords

Adult diet; caloric restriction; cost of reproduction; fruit feeding; longevity; longitudinal data; protein; reproduction; starvation; tropical butterfly; yeast

Introduction

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 life spans, very little is known about the life history consequences of frugivory. By studying the effect of banana, yeast, and amino acids in the adult diet of the fruit-feeding Satyrine butterfly, *Bicyclus anynana* (Butler), we hoped to uncover some of these consequences. Yeast was included because it is commonly found on rotten fruit, is rich in protein, and may play a role as an attractant to fruit sources (Molleman *et al.*, 2005; Bauerfeind *et al.*, 2007).

In contrast to most butterfly species in temperate regions, many adult butterflies in tropical forests are specialised on feeding on fallen fruits and other alternative substrates, and do not normally visit flowers. 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. Nectar and rotting fruit differ from each other in protein content, sugars, minerals, secondary plant components, and in microbial life (Molleman *et al.*, 2005). Even although nectar can contain significant amounts of amino acids, fruits can be more nitrogen rich (Molleman *et al.*, 2005), although this may not always be the case (Fischer *et al.*, 2004).

There appears to be a wide range in the effects of adult diet on survival and reproduction in female butterflies (Boggs, 1986, 1987; Mevi-Schutz & Erhardt, 2005) which may vary within species 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, 1997a; Fischer & Fiedler, 2001). However, amino acids derived from the adult diet may also play an important role, because in some nectivorous species, amino acids from nectar can be incorporated into eggs (Boggs, 1997a, b; Mevi-Schutz & Erhardt, 2005), but stored protein from the larval stage is viewed as the primary nitrogen source in nectivorous butterflies (Hill, 1989; Hill & Pierce, 1989). 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.

A more balanced adult diet can allow for a smaller proportion of larval resources used for egg laying and thus a higher fecundity and evolution of longer life spans. In some of the long-lived *Heliconius* butterflies that feed on pollen, egg production is sustained by amino acids from the pollen, resulting in a direct relationship between pollen ingestion and egg production (Gilbert, 1972; Dunlap-Pianka *et al.*, 1977; Boggs *et al.*, 1981; O'Brien *et al.*, 2003; Eberhard *et al.*, 2007). Long life spans (up to 10 months) have also been recorded for fruit-feeding butterflies (Beck & Schulze, 2000; Molleman *et al.*, 2007) and these may be associated with use of adult diet-derived amino acids, as hypothesized by Brakefield and Kesbeke (1995).

Apart from food quality, caloric restriction can also affect life history trajectories. In many cases, caloric restriction can extend life span at the cost of reproduction (Gerhard, 2001; Barja, 2004; Crawford *et al.*, 2007), or life span is conserved at the cost of reproduction (Boggs & Freeman, 2005). The presented experiments can provide some insight into the effects of caloric restriction on *B. anynana*.

In life history theory, the allocation of resources to competing goals plays a key role; however, life span and reproduction are of a different nature, as life days are necessary for reproduction (Jervis *et al.*, 2007). Therefore, a life span cost of reproduction is not a simple trade off. Longitudinal data on many individuals can give more insight into the relationship between reproduction and longevity.

In this study, we investigated the effect of banana, yeast, and amino acids in the adult diet on life span and on female egg laying in the fruit-feeding Satyrine butterfly, *B. anynana*, from tropical Africa *Bicyclus anynana* is a long-lived butterfly for which adult feeding is important, and is also readily reared in the laboratory.

The results of two experiments were used to address the following questions:

1. How do food sources affect reproductive output and life span?

2. How does the addition of yeast affect reproductive output and life span?
3. How does adult food limitation affect longevity and reproductive output?
4. Is there a phenotypic trade off between reproduction and life span?

Material and methods

Bicyclus anynana

Bicyclus anynana shows seasonal polyphenism in wing pattern and life history parameters (Brakefield & Reitsma, 1991; Brakefield *et al.*, 1998; Pijpe *et al.*, 2007). Individuals bred under wet-season conditions (27 °C) and can live up to about 100 days with an oviposition period of about 30 days at 27 °C in the laboratory when fed *ad libitum* on moist banana (Brakefield & Kesbeke, 1995). The dry season phenotype has a longer life span, and in the field the reproductive period can be postponed for many months until the start of the wet season (Brakefield & Reitsma, 1991; Windig *et al.*, 1994). The caterpillars feed on grasses. The lab population that was used has been bred on *Zea Mays*, while adults were kept on banana diets.

Two separate experiments were performed measuring egg production and longevity in cohorts of adult *B. anynana* feeding on different substrates. Experiment I included six adult diets: sugar solution, banana juice, a slice of banana, banana juice with yeast, yeast solution, and yeast paste. The butterflies were kept in cages in groups of up to 10 individuals of one sex. To corroborate certain findings from this experiment, a second experiment was performed (Experiment II), in which butterflies were kept singly and only two cohorts were used, one fed on a sugar solution and the other on sugar solution with added amino acids.

For both experiments, eggs were collected from a laboratory stock population established in 1988 using over 80 gravid females collected in Malawi. Caterpillars were raised at 27 °C (wet season conditions) using maize (*Z. mays*) in large rearing cages (45 × 45 × 45 cm). The pupae were subsequently sexed and females weighed. Pupae were then kept in small plastic pots (diameter 4 cm), and the emerging butterflies were marked individually on the forewing with a felt-tip pen. Butterflies were kept in cages for up to 3 days before mating. The cages contained vials filled with sugar solution and the butterflies were provided with water using wet cotton wool. All females were mated once by adding males to the cage when the females were 2–3 days old and taking the mating couples out. After copulation, females were randomly allocated to one of the treatments. Both experiments were carried out in a climate room with a constant temperature of 27 °C at 80% humidity and a photoperiod of LD 12:12 h (wet season conditions).

Experiment I—The mated butterflies were put into square cages (12 × 21 × 29 cm) in groups of up to 10 individuals of one sex, four replicates per diet. Food was provided in three small vials placed on the bottom of the cage, or in the case of banana, on a petri-dish. The sugar solution was a mimic of banana juice, containing 150 g sucrose, 50 g glucose and 50 g fructose per litre of demineralised water. The banana juice was prepared by mashing and then centrifuging not-too-ripe bananas. The resulting syrupy supernatant was stored in plastic tubes at –25 °C. Yeast solution and yeast paste were made from baker's yeast (as used for *Drosophila* medium) and demineralised water.

Most of the dead butterflies of each food treatment and sex were used for analyses of fat content. All appendages were removed and the bodies dried for 2 days at 50 °C, weighed, subsequently submerged in ethyl-acetate for 3 days, dried at 50 °C, and weighed again (Zwaan *et al.*, 2001). The difference between initial dry weight and weight after fat extraction was taken as the fat content.

Cuttings of a natural food plant, the African grass species *Oplismenus compositus* (L.), in water were used for egg laying. The butterflies had wet cotton wool in a small petri-dish inside the cage to provide permanent access to water. Butterflies were fed daily when eggs were removed and counted, and deaths recorded. The males were kept under similar conditions, but without an *Oplismenus* cutting.

Experiment II—Mated females, obtained as in experiment I, were kept singly in plastic pots (diameter 14 cm) with gauze covers on which a cotton wool pad moistened with demineralised water was placed. Food was provided in a small vial. For the amino acid treatment, amino acids were added to the sugar solution (as in Experiment I) in a concentration within the range known from the literature on fruits, and using the amino acid proportions in *Lantana camara* nectar (Erhardt, 1991). This solution contained per 200 ml: 0.1280 g alanine, 0.0645 g arginine, 0.1119 g asparagine, 0.0959 g glutamic acid, 0.2723 g glutamine, 0.3562 g glycine, 0.5121 g proline, 0.2880 g serine, 0.1609 g threonine, 0.0810 g tyrosine, and 0.0369 g valine. Seventy six out of 151 butterflies were fed amino acid. *Oplismenus* cuttings were again used for egg laying.

Eggs and caterpillars were removed from the cuttings at 4 day intervals and stored in vials at -25°C for later counting (some vials were unfortunately lost, hence statistical procedures had to account for missing data), while the food was replenished and dead butterflies collected every other day. The proportion of larvae of the total reproductive output was used to calculate an index of hatchability. This is not hatchability in the strict sense, as it also depends on the temporal distribution of the eggs laid (later eggs in each period were frozen before they could hatch). Random samples of the eggs from two dates (2 and 12 days after the onset of oviposition) were used for measurement of protein content. About 30 dried eggs were suspended in 1 ml of demineralised water, and this solution analysed with the BCA Protein Assay Reagent Kit (Cote-Parmer, Vernon Hills, IL) using the Unicam UV1 spectroscope (Unicam Instruments Cambridge, U.K.).

Data analyses—In experiment I, females were held with up to ten individuals per cage, allowing the measurement of individual survival for both males and females, while egg production could only be counted per cage. Therefore, the average number of eggs per female per day was calculated by dividing the total number of eggs on each day by the number of females present in the cage on that day. Standard errors of egg laying were calculated over the four replicate cages for each treatment. This experimental set-up prevented direct calculation of the correlation between egg production and life span or fat content of individual butterflies. In experiment II, all females were held separately and could be treated individually in all analyses.

The egg laying, adult fat- and egg protein-content data were tested for normality and then analysed with GLM and ANOVA. For analyses of longevity, survival analysis was applied using JMP 5.01 (SAS Instruments, Cary, NC), SAS 9.2 and R 2.4 (R Development Core Team, <http://www.r-project.org>). When overall significant differences were detected, tests were performed for selected contrasts.

For experiment II the intensity of egg laying for each butterfly was defined as the total egg counts divided by the total number of days that generated these egg counts. The intensity of egg laying per 4-day period was calculated as the total number of eggs counted during the 4-day period, divided by the number of females for which egg counts were available. This yields a rough summary measure for average daily egg counts. As fecundity of a butterfly and its relation to longevity are of interest, a second analysis of fecundity was performed. Because some egg counts were missing, such a measure was not observed for all butterflies and it was recovered through an innovative statistical method as described in Yao *et al.* (2005). The approach is based on functional principal component analysis to reconstruct the unobserved

egg-laying trajectories based on the observed incomplete data. This unobserved egg-laying trajectory is assumed to be a smooth curve. This may depict the underlying biological process, while on the other hand, the daily egg production, which is unobserved because of aggregation of the eggs in the 4-day observation periods and the occasional missing periods, may be subject to fluctuations. The actual unobserved fecundity was replaced by the total egg production under this smooth egg-laying trajectory and was referred to as *lifetime reproduction* hereafter. The imputation was performed on $\log(x + 1)$ transformation on egg counts before modelling and then transformed back to egg counts for 148 fertile butterflies (two individuals from sugar group and one from amino acid group were infertile and excluded). After computing reproductive output from these curves, we calculated the recovered reproduction intensity as the ratio of recovered lifetime reproduction over life span and compared this with the observed aforementioned reproductive intensity. The mean difference between the two intensities was 0.93 eggs per day with a SD of 1.79. Given that the mean reproductive intensity is nine eggs per day for the 148 butterflies in experiment II, this small discrepancy suggests that our reproduction recovery procedure is quite reliable. After imputing the lifetime reproduction, diet effects were non-parametrically tested for. To quantify the cost of reproduction, life span was transformed to its logarithm and fitted to a censored regression model with imputed reproduction intensity using the Buckley–James method (1979; Stare *et al.*, 2001).

Results

Experiment I: longevity

Analyses of the life spans of butterflies revealed differences among diets and heterogeneous diet effects between female and male butterflies (Fig. 1). Both Log-Rank and Wilcoxon's tests identify significant diet effects for male and female butterflies. After graphic model checking, we found that data follow a Weibull distribution. Hence, a more powerful parametric regression model was fitted: the accelerated failure-time (AFT) Weibull regression, with diet, sex, and their interaction as covariates. Because about 10 individual butterflies were nested in each cage, the nested cage effect was included in the model. All diet, sex, interaction, and cage effects were significant (Table 1.) On average, male butterflies lived (marginally) significantly longer than females. Diet effects are detailed in Table 2. Although the last surviving individual belonged to the sugar cohort, the survival rate of the banana juice cohort was overall higher than of the sugar cohort. Both yeast diets resulted in a shorter life span. The combination of banana juice and yeast resulted in fermentation, which caused the vial to overflow, and many butterflies in this cohort were found dead with wings stuck to their food. Therefore, the life span data of this treatment are not relevant to our question of how diet affects life span physiologically. The interaction between diet and sex was caused by males living longer in the sugar and banana cohorts, and shorter in the yeast cohorts.

Although the variation among cages is significant in the AFT Weibull regression, this can be attributed to one cage of males fed with sugar that had longer life spans than those in the other cage of the same treatment. Life span of butterflies in this cage averaged 62.6 days (SE 7.3), but the others averaged 19.5 days (SE 6.0). Refitting the model excluding all 10 butterflies from the cage that yielded the longer life spans, the cage effect was no longer significant in the model and neither was the sex effect (Table 1). However, the diet effect remained similar as before and, therefore, further details of this analysis are not reported here.

Experiment I: egg production

The pattern of egg production in *B. anynana* resembles that of a typical nectar-feeding butterfly with no eggs mature at emergence (Boggs, 1997a). Thus, oviposition does not begin directly after emergence, but after a period of egg maturation. There is then an early peak in egg laying before it gradually declines. In *B. anynana*, the peak in egg production was in week two for all

diet treatments, and most eggs were laid in the first 3 weeks (Fig. 2). The number of individual females included in the measurements decreased gradually and at different rates among the treatments as a result of mortality (Fig. 1a).

The six diet treatments differed in the total number of eggs laid per female (ANOVA: number of cages = 24, $F_{5,18} = 9.08$, $P = 0.0002$; Fig. 2). The yeast-only treatments had the lowest fecundity (ANOVA: $F_{1,22} = 36.3$, $P < 0.0001$), and the addition of yeast to banana juice had no effect on fecundity (ANOVA: $F_{1,6} = 0.03$, $P = 0.86$). There was also no significant difference between the sugar-only-treatment and banana juice (ANOVA: $F_{1,6} = 2.72$, $P = 0.15$).

Experiment I: fat content

The fat content at death differed among diets (ANOVA: $F_{5,18} = 2.92$, $P = 0.014$). The highest fat content was recorded in the sugar treatment, while the lowest average fat content was found in the yeast cohorts. In a GLM analysis with diet and sex as factors, the percentage of fat did not differ significantly between the sexes (Fig. 3; $F = 0.06$, $P = 0.8$), and no interaction between these factors was detected ($F = 0.79$, $P = 0.56$).

Experiment II: longevity

Longevity, measured by the survival function, was not significantly different between the two diets (Fig. 4; $n = 151$; Log-Rank: $P = 0.79$, Wilcoxon: $P = 0.38$). Survival rate in this experiment was lower than in experiment I.

Experiment II: egg production

Figure 5 shows how reproduction intensity changes with butterfly age in both diet treatments. Subsequent recovery of reproduction of individual butterflies showed that female intensity of reproduction and life time reproductive output of the cohort fed on sugar solution supplemented with amino acids was similar to that of the cohort fed sugar solution (Fig. 6a, two-sample Wilcoxon's rank sum test: $P = 0.58$, and Fig. 6b, $P = 0.85$ respectively), with similar mean fecundities of 123.8 (SD = 61.3) and 130.1 (SD = 72.4) eggs, respectively ($n = 75$ and $n = 73$). Fecundity was lower in experiment II than in experiment I. The hatching index of the eggs (number of caterpillars/total fecundity) was similar for the sugar cohort and the amino acid cohort with means of 0.25 (SE = 0.018) and 0.27 (SE = 0.021), respectively, and was not significantly different in ANOVA with P -value 0.39. The hatching index of an individual did not correlate with its life span ($r^2 = 0.04\%$, $F = 0.07$, $P = 0.79$).

Experiment II: protein content of eggs

There was no significant difference in protein content of eggs between the two diets (ANOVA: $F_{1,98} = 2.38$, $P = 0.13$). In a GLM analysis, the timing of egg sampling (in week 1 or week 3) had no significant effect on the protein content ($r^2 = 5\%$, $F = 0.52$, $P = 0.47$), and there was no interaction between diet and timing of sampling ($F = 0.42$, $P = 0.52$). Protein content averaged 13.65 μg ($n = 54$, SE = 0.49) and 12.61 μg ($n = 46$, SE = 0.45) per egg in the amino acid and the sugar cohorts, respectively.

Experiment II: egg production-life span nexus

The life span of individuals with a lower intensity of egg laying was longer ($P < 0.0001$, Fig. 7). Therefore, the cost of reproduction was significant, while adult diet is not a significant factor in this analyses ($P = 0.9$). The linearity between log of survival time and reproduction intensity is implied by Fig. 7 and has been tested statistically after taking censoring of observation into account. The Buckley–James estimation for reproduction intensity is -0.054 (SD = 0.010). Thus, if a butterfly was to lay one more egg per day, its life span would be expected to decrease with a factor 0.95 with a 95% CI of 0.93–0.97. However, a positive correlation between life

time reproduction and longevity was detected as was expected, because the longer a butterfly lives, the longer the available period to lay eggs.

Discussion

Life span of *B. anynana* was longer on a banana juice diet than on a slice of banana or a sugar solution of similar composition. However, we found no differences between the life span of the sugar and sugar with added amino acid cohorts, and therefore any extended life span can not be attributed to amino acids in banana. Males lived longer than females on diets that did not contain yeast. In both yeast-only treatments, butterflies were practically starved (as indicated by low fat content at death) and that males had shorter life spans on these diets than females suggest lower starvation resistance in males (Pijpe *et al.*, 2007). The addition of yeast to banana juice produced a sticky substance which was a hazard for the butterflies that appeared to affect males and females similarly, thus masking any physiological effect of added yeast on life span.

Sugar in the adult diet seems to be the most important component influencing egg production in these experiments. Amino acids and other potential nutrients of banana did not affect the total number, hatching success or nitrogen content of the eggs. Other studies also failed to identify components that would enhance egg production in this species: Bauerfeind *et al.* (2007) used sucrose (not a combination of sugars mimicking banana) to contrast with banana and artificial diets. Our results corroborate results from several nectar-feeding butterflies (Hill, 1989; Hill & Pierce, 1989). Thus, Hill (1989) comments that the lack of a positive amino acid effect is surprising in *Euploea core* because this species is extremely long lived. Our data suggest that *B. anynana* mainly relies on stored protein for egg production despite the significant amount of protein found in the adult diet. This corroborates with isotopic evidence from the species under study: that adult diet only contributes carbohydrates to egg production (Fischer *et al.*, 2004). These results suggest that a change in food quality that potentially accompanies a shift from nectar to fruit feeding has had limited life history consequences, at least in *B. anynana*. Feeding on fruit may thus be primarily an adaptation to flower and fruit availability in the understory of tropical forests rather than representing any active switching to a more protein-rich diet.

It remains intriguing why the extra protein in fruit is apparently not utilized by adults of *B. anynana*. Because the lab population has always been fed banana, it could be expected to retain any adaptation to use nutrients in banana for survival and reproduction. However, this selection pressure may have been weak as most egg collections for next generations were done early during the peak of egg production, when resources carried over from the larval food would be expected to be the most important for egg production, and little mortality had taken place. It is also possible that a positive effect of amino acids in the diet will only be detected under conditions of larval food limitation as was found in the nectar-feeding butterfly *Araschnia levana* (Mevi-Schutz & Erhardt, 2005). Lastly, the amino acid concentration or composition of the experimental diets may not have been appropriate for detecting any effects in this species.

In this lineage of fruit-feeding butterflies there may have been little selection for the utilization of amino acids derived from the adult diet, or alternatively such utilization is constrained in some way by physiology. In contrast, conservation of egg weight with age was associated with fruit feeding in the Australian satyrine *Mycalesis terminus* (Braby & Jones, 1995) and unpublished data on the fruit-feeding butterfly *Charaxes fulvescens* indicate mildly positive effects on fecundity when amino acids are added to a sugar diet (F. Molleman, J. Ding, J.-L. Wang, B.J. Zwaan, J.R. Carey, & P.M. Brakefield, unpublished data). Utilization of amino acids from fruits thus probably varies among butterfly species, possibly depending on host plant characteristics, larval ecology, and adult life span.

Bicyclus anynana had reduced longevity on a diet of yeast. The rate of egg laying was also significantly lower than when sugar-containing substances were offered, and butterflies in the yeast cohorts had the lowest mean percentage of fat at death, indicating starvation. When yeast was added to banana juice, survival was significantly lower than without yeast. However, the lower survival of butterflies on the banana juice-with-yeast diet than for the banana juice cohort can be explained by the sticky substance produced by fermentation in which the butterflies frequently became trapped by their wings. This would fit the observation that egg-laying rate was not affected by addition of yeast and is consistent with fruit-feeding butterflies being yeast tolerant. The observation that yeast is not a source of nutrition contrasts with findings for larvae and adults in several species of fruit-flies (Hendrichs *et al.*, 1993; Jacome *et al.*, 1999; Mangan, 2003; Carey *et al.*, 2005). However, in some species of fruit-flies, yeast can have a negative impact on survival and reproduction (McCaffrey *et al.*, 1994). Our results with *B. anynana* corroborate those in the Caribbean fruit-fly, *Anastrepha suspensa* (Loew), in which high rates of male sexual activity were observed in flies provided with either a mixture of hydrolysed torula yeast and cane sugar, or with pure sucrose, but not with yeast alone or when deprived of all food (Landolt & Sivinski, 1992).

Our results allow us to reflect on the effect of food limitation on the life history of *B. anynana*. Because sugar was the only component in our experiment with apparent life history effects we can speak of caloric restriction. First, the yeast diet offered little sugar and the egg laying patterns resemble those found in *Speyeria mormonia* under food limitation (Boggs & Ross, 1993). However, while *S. mormonia* was able to maintain longevity, this was not true for *B. anynana*, possibly because food was more limited as no sugar was offered. Second, food was provisioned daily in experiment I, while in experiment II food was only replenished every other day. Even although there was still food available on the other days, the butterflies may not have found it as acceptable or were not able to access it, thus representing lower food availability. Possibly caloric restriction was the cause of the differences in rate of reproduction and longevity between experiment I and II. However, other differences between the experiments, most notably group cages versus individuals kept singly, prevent a clear conclusion. That reducing the provisioning of new food from daily to once every 2 days could produce a decrease in rate of reproduction indicates that females of this species may need to feed daily in the wild, not unlike Mediterranean fruit-flies (Carey *et al.*, 2005).

There appears to be a quandary in life-history theory on the relationship between life span and reproduction. Longer lives provide an opportunity for increased reproduction, but on the other hand there could be a life span-reproduction trade off. Several studies show that it is not total reproductive output, but age patterns in reproduction that are associated with long life span (Müller *et al.*, 2001, 2002). For *B. anynana* a cost of reproduction was demonstrated by the negative correlation between life span and intensity of reproduction in experiment II. This corroborates results in Mediterranean fruit-flies in which the rate of decrease in egg production with age was found to predict life span (Müller *et al.*, 2001). These results indicate that a trade off between investing in early reproduction and survival could play a role in the evolution of life span and ageing.

The presented results show the utility of more detailed analyses of longitudinal data for gaining insight into the structure of life history (Müller *et al.*, 2001). The results showed that in the fruit-feeding butterfly *B. anynana*: (i) banana juice, but not sliced banana or added amino acids extend life span compared with a sugar solution of similar composition; (ii) compared with this sugar solution, other cohorts (banana juice-amino acid enriched) did not have significantly higher reproductive outputs; (iii) yeast does not represent a valuable source of nutrients; (iv) caloric restriction may cause decreased life span and rate of reproduction; and (v) increased rates of reproduction have a life span cost.

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Fig. 1. Kaplan-Meier survival plots for six *Bicyclus anynana* cohorts kept on different diets in cages with up to 10 individuals; (a) females, and (b) males.

Fig. 2.

Average egg production of *Bicyclus anynana* on six different diets. Egg numbers per cage on each day were divided by the number of females alive in that cage and then averaged per week. Error bars among the four cages are attached when multiple cages of the treatment contain live females.

Fig. 3. Mean fat content of females and males of *Bicyclus anynana* with standard deviations by adult diet.

Fig. 4. Kaplan-Meier survival plots for cohorts of individual adult females kept singly and feeding on sugar solution or sugar solution with amino acids. Dashed lines represent 95% CI.

Fig. 5. Measured reproduction intensity per 4-day period for *Bicyclus anynana* females fed on sugar and sugar with amino acid solutions with error bars attached.

Fig. 6. Histograms of recovered reproduction of female of *Bicyclus anynana* feeding on either sugar solution or sugar solution with amino acids; (a) intensity of reproduction, and (b) imputed life time reproduction.

Fig. 7.
Relationship between intensity of reproduction and life span in the fruit-feeding butterfly *Bicyclus anynana*.

Table 1

Results of AFT Weibull regression on life span of *Bicyclus anynana* butterflies fed six different diets and kept in cages of around 10 individuals, four cages per diet for females and two cages per diet for males ($n = 325$). AFT Weibull model formula is: $\log(T_{ij}) = \beta_0 + \beta_1 Banana + \beta_2 BJuice + \beta_3 BJYeast + \beta_4 Yeast + \beta_5 YPaste + \beta_6 Male + \beta_7 BananaMale + \beta_8 BJiceMale + \beta_9 BJYeastMale + \beta_{10} YeastMale + \beta_{11} YPasteMale + cage_i + \sigma \epsilon_{ij}$, where T_{ij} is the life span of the j th butterfly in the i th cage and ϵ_{ij} is the error term, which has extreme value distribution. The β 's are the coefficients describing the covariate effects on the life span. Female butterflies of the sugar diet group were the baseline group. The σ is the scale parameter and its inverse can be interpreted as the Weibull shape parameter. Note that cage effect is nested within the interaction of diet and sex since butterflies in the same cage were same sex and fed the same diet.

Effect	Including outlier cage			Excluding outlier cage		
	DF	χ^2	Pr > χ^2	DF	χ^2	Pr > χ^2
Diet	5	361.344	<0.0001	5	333.382	<0.0001
Sex	1	3.484	0.0620	1	0.252	0.616
Diet*sex	5	1.072	0.0008	5	25.834	<0.0001
Cage (diet*sex)	24	44.232	0.0072	23	25.991	0.3013

Parameter	Including outlier cage					χ^2	Pr > χ^2
	DF	Estimate	SE	95% CI	Lim		
Intercept	1	3.9309	0.1660	3.6056	4.2563	560.80	<0.0001
Banana	1	-0.7675	0.2523	-1.2620	-0.2731	9.26	0.0023
b. juice	1	-0.3604	0.2217	-0.7948	0.0740	2.64	0.1040
b.j. + yeast	1	-0.6153	0.2216	-1.0497	-0.1809	7.71	0.0055
Yeast	1	-1.3951	0.2421	-1.8695	-0.9206	33.21	<0.0001
Yeast paste	1	-0.8521	0.2335	-1.3098	-0.3945	13.32	0.0003
Male	1	-0.7439	0.2522	-1.2383	-0.2496	8.70	0.0032
Banana*male	1	1.3993	0.3524	0.7086	2.0901	15.76	<0.0001
b. juice*male	1	1.1694	0.3275	0.5276	1.8113	12.75	0.0004
b.j. + y*male	1	1.1505	0.3275	0.5086	1.7923	12.34	0.0004
Yeast*male	1	0.7968	0.3494	0.1121	1.4815	5.20	0.0226
Y paste*male	1	0.1677	0.3394	-0.4975	0.8329	0.24	0.6213
Scale	1	0.4670	0.0205	0.4286	0.5089		
Weib. Shape	1	2.1412	0.0939	1.9648	2.3334		

Table 2

Results of Wald tests on life span of *Bicyclus anynana* cohorts compared with the sugar cohort. *Banana c.* is combination of the cohort on banana and banana juice, *yeast comb.* is combination of the two yeast cohorts.

Diets	Model structural difference	SE	P-value
Banana	0.200	0.111	0.072
b. juice	0.433	0.108	<0.001
b.j. + yeast	0.029	0.108	0.786
Yeast -0.957	-0.957	0.110	<0.001
Yeast paste	-0.825	0.110	<0.001
Bananana	0.221	0.092	0.016
Yeast comb.	-0.891	0.097	<0.001