EFFECT OF LARVAL FOOD ON DEVELOPMENT TIME AND FAT CONTENT IN THE TROPICAL BUTTERFLY *BICYCLUS ANYNANA* (LEPIDOPTERA, SATYRINAE)

Rinny E. Kooi, Erica van Binnendijk and Paul M. Brakefield

Evolutionary Biology, Institute of Evolutionary and Ecological Sciences, University of Leiden, P.O. Box 9516, 2300 RA Leiden, The Netherlands

Keywords: food quality, larval development time, pupal development time, fat content

Summary

Both food plant quality and temperature influence the size of the polyphenic butterfly *Bicyclus anynana*. Therefore, larvae of this species are used to study the effect of the food plant species (= quality) at a low and high temperature on the larval and pupal development times, and the fat content of the adults. The results obtained at a low temperature are not similar to those at a high one. A food plant which is unsuitable at a low temperature and which leads to insects with an increased development time and a small adult size with a low fat content can be a rather suitable food plant at a high temperature. This may be of importance for the wing pattern induction of *B. anynana*.

INTRODUCTION

Insects adapt to changing environments with predictable environmental fluctuations in a number of highly characteristic ways. Many biochemical and physiological changes have been observed to be associated with arrested development both in summer and winter. Some of these simply reflect broad seasonal changes in metabolism, such as reduction of water content in dormant forms, which may indicate an increase in fat content. These processes are often related to elaborate time measuring mechanisms and associated neuroendocrine control of growth, development, reproduction and behaviour (Tauber *et al.*, 1986, Danks, 1987,). There are indications that the butterfly *Bicyclus anynana* (Butler) also has seasonal adaptations with respect to the fat development. This tropical butterfly from Africa (Malawi) lives in an environment with a warm wet, and a cool dry season. This insect probably sequesters lipid and other reserves in a fat body at the end of the wet season for the survival of the dry period when food is in a more limited amount available (Brakefield & Reitsma, 1991). The environmental temperature during the larval development time seems to be very important for the accumulation of fat. When the larvae were reared at a low temperature the fat content of the butterflies was much higher than when they were reared at a high one (Kooi *et al.*, 1997).

B. anynana shows phenotypic plasticity in the form of seasonal polyphenism. In the wet season, when larval food plants are available, the butterflies are active and conspicuous; in the dry season they are inactive and cryptic. The environmental temperature, the larval development time and genetical factors are important influences on the wing pattern induction. The environmental temperature on the first day after pupation contributes also to this process (see Brakefield *et al.*, 1996, Kooi & Brakefield, 1999a).

B. anynana larvae are oligophagous feeding on a wide range of grass species. The phenotype of *B. anynana* is (indirectly) determined by food quality as the food supply of this insect influences its larval development time. Larvae reared on less suitable food plant species need a relatively long period for their development and give rise to butterflies with a more dry-season wing pattern (Kooi *et al.*, 1996, 1998, Kooi & Brakefield 1999b, Kooi, in press).

Table 1. Effect of food plant species on larval (L.) and pupal (P.) development time (days (S.D.)) of butterflies grown at two different temperatures.

Food plant	Females N	L. devel.	P. devel.	Males N	L. devel.	P. devel.
16°C						
Zea mays	62	65.5(9.3)	19.4(1.3)	51	57.1(4.6)	20.2(1.6)
Oplismenus compositus	45	94.4(5.6)	24.0(2.4)	58	83.0(6.2)	20.3(2.3)
Setaria palmifolia	50	65.2(7.5)	18.8(1.8)	50	61.8(6.0)	18.8(1.5)
27°C						
Zea mays	50	21.7(1.8)	5.5(0.6)	49	21.0(2.4)	6.0(0.5)
Oplismenus compositus	50	26.4(2.4)	5.8(0.4)	50	24.6(2.2)	6.0(0.2)
Setaria palmifolia	49	25.3(1.8)	6.0(0.3)	50	23.1(1.6)	6.0(0.4)

The present study focuses on the effect of food plant on the pupal development time and the fat accumulation. When larvae are fed with a less suitable food plant their developmental period increases and also their pupae and adults become smaller. Therefore, food quality may also affect the pupal developmental period and the fat content of the adults. A longer pupal stage could also effect the fat content perhaps via the metabolism of the pupa.

MATERIAL AND METHODS

A stock of *Bicyclus anynana* was established from about 80 gravid females collected from Nkhata Bay, in Malawi (Africa). This stock has been maintained on *Zea mays* L. (young plants, four to five week old) for more than ten years with an adult population in each generation of several hundreds.

To measure the effect of food plant quality, the larvae were fed with three different plant species as previous experiments indicated that food quality was correlated with plant species (Kooi *et al.*, 1996). Food plants of uniform age and leaf quality were all cultivated under greenhouse conditions prior to the experiments. The plants used in the experiments were also regularly replaced with fresh plants. Mass rearing was performed on *Zea mays* (=Zm), *Oplismenus compositus* (L.) Beauv. var. *rariflorus* (Presl.) U (=Oc) and *Setaria palmifolia* (J.G. Koenig) Stapf (=Sp). A large number of eggs were collected form the stock and placed on the three plant species in gauze cages (50x50x50cm) in climate controlled rooms at 16°C or 27°C (L12:D12). Per temperature and per food plant about fifty females and fifty males were collected on the first day of emergence and stored at -20°C. Prior to the extraction their wings, legs, antennae and galeae were removed, the bodies were weighed and placed for 48 hours at 45-50°C to desiccate before reweighing. For the extraction the bodies were placed in a glass tube with a solution (2.25 ml) of dichloromethane: methanol = 2:1. The tubes were sealed and incubated at room temperature in a shaker. After 48 and 96 hours the solution was renewed. After the incubation the butterflies were dried and weighed again. The difference between the dry and extracted weight was used as a measure of the fat content of the individual.

Table 2. Effect of food plant species on fresh and dry weight (mg(S.D.)) of butterflies grown at two different temperatures.

Food plant	Females N	fresh	dry	Males N	fresh	dry
16°C						
Food plant						
Zea mays	62	64.5(7.8)	23.0(2.6)	51	45.9(5.5)	13.2(1.4)
Oplismenus compositus	45	52.7(8.3)	17.6(2.6)	58	40.7(7.9)	11.0(1.3)
Setaria palmifolia	50	64.2(8.4)	23.5(2.8)	50	43.9(6.2)	13.2(1.6)
27°C						
Zea mays	50	56.3(10.4)	20.5(3.8)	49	35.2(6.9)	10.3(2.2)
Oplismenus compositus	50	54.5(7.1)	19.5(2.9)	50	35.3(4.8)	10.1(1.7)
Setaria palmifolia	49	62.4(10.0)	23.0(4.2)	50	37.4(5.4)	11.0(2.1)

The Minitab (1993) package was used for the statistical analysis of the data (ANOVA, Pearson correlation coefficient or Regression; significance for $P<0.05$).

RESULTS

Development time

Food plant quality influences the larval and pupal development times. At 16°C for females both the larval and pupal development time on Oc were significantly longer than on the other two food plants species (All results with respect to the larval and pupal developmental period are presented in table 1). The results differ between the sexes. Although the larval development time for males on Oc was significantly shorter than on Zm and Sp, this was not so for the pupal period. Male pupae had a significantly shorter developmental period on Sp than when raised on the other two food plant species.

At 27°C, a significantly shorter larval developmental period was found for both sexes on Zm than on Oc and Sp. At this temperature there is no significant effect of the food plant on the duration of the pupal stage.

Body weight of butterflies

Food plant quality influences the fresh and dry body weights of the butterflies at both temperatures. At 16°C the specimens obtained from Oc had the lowest weight (All data of the weights are presented in table 2). In both sexes the increase of the larval developmental period on Oc resulted in butterflies with lower fresh and dry body weights (in most cases $P<0.05$) (Pearson corr. females: $r = -0.202$ (not significant), dry weight $r = -0.352$; males: fresh weight $r = -0.241$, dry weight $r = -0.292$). At 16°C the results for the butterflies from Zm and Sp are more or less similar.

At 27°C the fresh and dry weights of female and male butterflies reared on Sp were significantly higher than those from Zm and Oc while those from Oc did not differ from Zm.

Table 3. Effect of food plant species on fat content (S.D.) of butterflies grown at two different temperatures.

Food plant	Females N weight (mg)	% dry weight	Males N weight (mg)	% dry weight
16°C Food plant				
Zea mays	62 3.7(0.8)	15.7(2.3)	51 3.1(0.5)	23.3(2.8)
Oplismenus compositus	45 2.4(0.5)	13.4(1.6)	58 1.9(0.4)	17.6(1.8)
Setaria palmifolia	50 3.8(0.7)	16.0(1.2)	50 3.1(0.7)	23.5(3.0)
27°C				
Zea mays	50 3.0(0.9)	14.3(2.9)	49 2.2(0.7)	21.2(3.6)
Oplismenus compositus	50 3.5(1.2)	17.5(4.5)	50 2.2(0.8)	21.1(4.7)
Setaria palmifolia	49 3.1(1.0)	13.4(2.4)	50 2.3(0.8)	20.3(4.0)

In general, the butterflies reared at 16°C are significantly heavier than those from 27°C. This contrasts to females from Oc: their weights are significantly lower at 16°C than at 27°C.

Fat content

Food plant quality appears to have an effect on the fat synthesis. Larvae fed at 16°C with Oc yielded butterflies in each sex with a significantly lower absolute and relative fat content than those fed with Zm or Sp (All data of the fat contents are presented in table 3). Remarkably, the females of 27°C had a significantly higher fat content when reared on Oc, than on Zm or Sp.

On Zm and Sp the absolute and relative fat content at 16°C was significantly higher than at 27°C. This contrasts to the specimens fed with Oc. When reared at 16°C their fat content was significantly lower.

The increase in larval development time on Oc resulted in a significant decrease in the absolute and relative fat content. This was observed for both sexes at both temperatures (Regression, $P < 0.05$). No comparable change in fat content was observed for the material from the other two food plant species at both temperatures. Remarkably, the females from Oc had the highest fat content at 27°C.

DISCUSSION

In *B. anynana* there are complex interactions between wing pattern induction and the larval food, development time, growth rate and pupa or adult weight (Kooi & Brakefield, 1999b). The present results indicate that the influence of the environmental temperature on the food plant species is also of importance as the food plant quality is temperature dependent. The results indicate that at 16°C (but not at 27°C!) Oc is a less suitable food plant than the other two species. At this temperature the larvae raised on Oc had the longest development time, and produced the smallest butterflies with the lowest fat content. This strong increase in the larval developmental period may be of importance for the wing pattern induction. Future work will pay attention to this aspect.

In previous experiments the food plant quality of Oc was more or less comparable with that of Zm and Sp at 27°C and 20°C.

Our laboratory stock of *B. anynana* has been bred for many generations on Zm. There are indications of a shift in oviposition preference of *B. anynana*. Initially, the butterflies showed a preference for Oc over Zm. After rearing the larvae on Zm for seven years that strong preference was no longer observed (Kooi, 1996). Rearing the stock on Zm for more than ten years may have changed the larval performance on Oc.

The three plant species used in the experiment occur in Malawi. However, Zm is not a natural host for *B. anynana*. This species is imported and used for agricultural purposes. Although sometimes butterflies from *B. anynana* lay eggs on this species no severe infestations are known and *B. anynana* is not recorded as a pest on Zm.

In the field Oc and Sp are used as natural hosts. In contrast to Oc, Sp is more adapted to the dry season. Even at the end of the dry period green Sp still can be found (N. Reitsma, R. de Groot pers. comm.). Although this plant species is still present in that time of the year, females appear to lay no eggs on it (Brakefield & Reitsma, 1991).

As the pupal development time of females grown at 16°C was influenced by the food plant this can result in two effects: Firstly, the longer pupal development of females from Oc can influence the fat content of their butterflies as the pupae may use some of their fat for their metabolism. Secondly, the first day after pupation the pupae are still sensitive for the environmental temperature (Kooi & Brakefield, 1999b). An increase of this sensitive period caused by a less suitable food plant may effect the wing pattern induction.

Danks (1987) presents fat contents for diapausing and non-diapausing butterfly species. In an earlier experiment when only Zm was used as a food plant (Kooi *et al.*, 1997) the data of Danks were compared with the fat content of *B. anynana*. In that experiment the fat contents (percentage of dry weight) for *B. anynana* were: 17°C=12.1%:19.5%, 27°C=6.2%:13.8% (females:males). At that time it was concluded that the values for *B. anynana* were rather low in comparison with those given by Danks. The values of the present experiment (table 3) are higher. In our former experiment a different solvent was used, namely chloroform. This compound is rather toxic and a carcinogen. For this reason it was decided to replace chloroform by dichloromethane. This compound may enhance the solvability of the fat in the butterflies thus raising the percentages of fat. This issue will be investigated further.

Both, the data of the former experiment and the present one indicate that the fat content of females is lower than that of males. Also in both experiments it was found that at a low temperature more fat is synthesised than at a high one. However, this process is food plant dependent as it did not occur on Oc.

Adult *B. anynana* butterflies are fruit-feeders. The energy income through fruit-feeding seems to be sufficient for fat body maintenance, but not for egg production as egg-laying depletes fat reserves (Zijlstra *et al.*, 2000). The higher fat content of males may also contribute to their longer (25%) potential lifespan (Brakefield & Kesbeke, 1995).

In general, our work indicates that the development of *Bicyclus* butterflies is not only temperature or food plant dependent; there can also be an interaction between food plant quality and temperature which influences the outcome of experiments. This may also be important in the wing pattern induction of *B. anynana*. The effect of a food plant species at a high temperature can be markedly different from its effect at low temperature.

ACKNOWLEDGEMENTS

We thank Els Schlatmann, Bert de Winter, Wil van der Hoeven and Marjanda van Splunter for their helpful contributions. Rinny E. Kooi received some support from the Uyttenboogaart-Eliasen foundation.

REFERENCES

BRAKEFIELD P.M., J. GATES, D. KEYS, F. KESBEKE, P.J. WIJNGAARDEN, A. MONTEIRO, V. FRENCH & S.B. CARROLL, 1996. Development, plasticity and evolution of butterfly eyespot patterns. *Nature* **384**:236-242.

BRAKEFIELD, P.M. & F. KESBEKE, 1995. 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 (N.E.V.)* **6**:93-98.

BRAKEFIELD, P.M. & N. REITSMA, Phenotypic plasticity, seasonal climate and the pupation biology of Bicyclus butterflies (Satyridae) in Malawi. *Ecological entomology* **16**:291-303.

DANKS, H.V., 1987. *Insect dormancy: an ecological perspective.* Biological survey of Canada (Terrestrial Arthropods). National Museum of Natural Sciences. Ottawa. 439 pp.

KOOI, R.E., 1996: Factors influencing food plant acceptance by the tropical butterfly Bicyclus anynana (Satyrinae). *Proceedings of the section Experimental and Applied Entomology of the Netherlands Entomological Society (N.E.V.)* **7**:117-122.

KOOI, R.E. Does bottleneck survival on a poor plant species influence the larval performance and wing pattern induction in the polyphenic butterfly Bicyclus anynana? *Netherlands Journal of Zoology* (in press).

KOOI, R.E., C. BERGSHOEFF, W. E.M.-T. ROSSIE & P.M. BRAKEFIELD, 1997. *Bicyclus anynana* (Lepidoptera, Satyrinae): comparison of fat content and egg-laying in relation to dry and wet season temperatures. *Proceedings of the section Experimental and Applied Entomology of the Netherlands Entomological Society (N.E.V.)* **8**:17-22.

KOOI, R.E. & P.M. BRAKEFIELD, 1999a: The critical period for wing pattern induction in the polyphenic butterfly Bicyclus anynana (Satyrinae). *Journal of Insect Physioloy* **45**:201-212.

KOOI, R.E. & P.M. BRAKEFIELD, 1999b: Effect of larval food on the reaction norm for wing pattern polyphenism in the tropical butterfly Bicyclus anynana. *Proceedings of the section Experimental and Applied Entomology of the Netherlands Entomological Society (N.E.V.)* **10**:43-48.

KOOI, R.E., P.M. BRAKEFIELD & W.E.M.-TH.ROSSIE, 1996: Effects of food plant on phenotypic plasticity in the tropical butterfly Bicyclus anynana. *Entomologia Experimentalis et Applicata* **80**:149-151.

KOOI, R.E., M. DE VRIES & P.M. BRAKEFIELD, 1998. The effect of drought stress in food plants on wing pattern induction of the polyphenic tropical butterfly Bicyclus anynana (Lepidoptera, Satyrinae). *Proceedings of the section Experimental and Applied Entomology of the Netherlands Entomological Society (N.E.V.)* **9**:217-222.

MINITAB, 1993. *Minitab for windows*, **release 9**. Sowers Printing Company, Lebanon, PA.

TAUBER, M.J., C.J. TAUBER & S. MASAKI, 1986. *Seasonal adaptations of insects.* Oxford university press, 411 pp.

ZIJLSTRA, W.G., B. J. ZWAAN & P.M. BRAKEFIELD, 2000. Consequences of delayed mating for life-history traits in the tropical butterfly Bicyclus anynana. *Proceedings of the section Experimental and Applied Entomology of the Netherlands Entomological Society (N.E.V.)* **11**(in press).