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An Artificial Diet for Butterflies, Including *Bicyclus* Species, and its Effect on Development Period, Weight and Wing Pattern

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Abstract. A semi-artificial diet based on bean flour suitable for the laboratory rearing of butterflies is described. The effect of the diet on development period, pupal weight and wing colour pattern was examined. Butterflies of the species *Bicyclus anynana* reared on living maize plants developed faster and were heavier, but there was no significant effect of diet on colour pattern.

Introduction

Bergomaz and Boppré (1986) described a simple semi-artificial diet that could be used to rear moths in the laboratory or, in a pre-prepared form, in the field. Although some species refused the diet, 38 species were reared successfully on the diet from egg to adult with no apparent effects on size, colour or other characteristics. Hence the diet provided them with a cost effective means of rearing moths in the field. A further advantage to using a pre-prepared diet is that rearing conditions can be standardised. These circumstances are attractive where a balanced data set is preferred in order to make statistical tests more robust. Also, the use of diet may reduce environmental variation which could interfere with certain types of study, such as in quantitative genetics.

Butterflies are frequently used as experimental material, usually with the use of living plants for larval food. This is expensive both in terms of space and time. We experimented with the use of the diet described by Bergomaz and Boppré (1986) using particularly the tropical African, brown butterfly, *Bicyclus anynana* (Butler), but also *B. safitza* and *B. ena*. A number of pilot experiments showed that the larvae could develop on the diet with over 90% pupating, but the majority of the emerging butterflies were crippled, i.e. the wings failed to expand properly, if at all.

The purpose of the present study was to modify the diet to make it more suitable for use with *B. anynana* by reducing the number of crippled butterflies developing. *B. anynana* has a striking plastic response to certain environmental cues which results in the production of dry season or wet season butterflies (Brakefield & Reitsma, 1991). These seasonal forms differ in their appearance, development time, physiology and behaviour; the most conspicuous difference is the much larger, submarginal eyespots present in wet season butterflies. Therefore, an examination of the diet reared adults was performed to assess the influence of the artificial conditions on size and wing pattern.

Materials and Methods

The recipe of the diet used in the current study is shown in Table 1. The diet differed from that devised by Bergomaz and Boppré (1986) in that p-formaldehyde and formaldehyde were omitted and linseed oil was added. Linseed oil provided extra essential fatty acids, the lack of which in the original diet presumably caused the observed crippling. The agar was dissolved in hot water. The other ingredients (thoroughly mixed together) were then added to the agar and stirred well to disperse the material evenly throughout. The gelling mixture was then poured into a suitable tray to a depth of about 1.5cm and allowed to set at room temperature for about one hour. The diet was then covered and transferred to the fridge for storage.

Two alternative quantities of sorbic acid, methyl-p-hydroxybenzoate and streptomycin were used (Table 1). Two types of diet were prepared: one containing the higher amounts of the preservatives and antibiotics (normal diet) and a second containing the lower quantities (low diet). This was done since Bergomaz and Boppré (1986) suggested that these materials may have an adverse effect on the development rate.

The experiment was carried out at 28°C and 80% relative humidity (12 hours D:L) which normally produces only extreme wet season form butterflies (Brakefield & Reitsma, 1991). For each type of diet, 100 newly hatched, unfed larvae from eggs of a large mixed population stock laid on maize plants were placed individually onto cubes of diet (approximately 1.5cm wide and 1.0cm high) in 9.5cm diameter petri dishes. 50 of the larvae were presented with fresh diet two times per week and the other 50 only once a week. The old diet was always removed from the petri dishes and discarded. Clean petri dishes were only provided if fungus developed on the diet or, more often, on the frass. In addition, to examine density effects, for each diet type 48 larvae (two per petri dish) and 120 larvae (five per petri dish) were also set up. Cannibalism is not a problem in *Bicyclus* species. As before, half of the dishes had the diet renewed two times a week and the other half only once a week.

Larvae pupated on the underside of the lids of the petri dishes. The resulting pupae could easily be removed after a day or two by scraping the silken holdfast from the petri dish lid. The day of pupation and the weight (to the nearest 0.1mg) of the pupae two days after pupation were recorded. Pupae were transferred after weighing to small cardboard containers (height 4cm, diameter 5cm), covered with a piece of gauze and returned to the 28°C cabinet. Butterflies were able to develop and emerge normally from pupae lying down rather than hanging as is invariably the case. Emergent butterflies were sexed and the widths of the 2nd forewing eyespot and the 5th hindwing eyespot were measured under a Wild microscope at 25x magnification using a graduated eyepiece. The length of the forewing was also measured using the eyepiece at 6x magnification. Butterflies successfully developing on the diet were placed together in a large breeding cage at 28°C and 90% relative humidity to assess whether they were capable of laying fertile eggs.

50 larvae were raised in the same 28°C cabinet on maize to enable comparisons to be made with butterflies reared on a more natural diet. The larvae were reared in groups of 10. The growing plants were coiled up into 12.5cm diameter plastic cages. Availability of space limited us to rearing larvae in groups, which is also the technique used generally in our experimental work. The same morphometric measurements were made on the maize-reared butterflies.

Table 1. Diet recipe (modified from Bergomaz and Boppré, 1986)

	Ingredients	Amounts
	White bean flour	75g
	Brewers yeast	17.5g
	Ascorbic acid	3.5g
	Cholesterol	0.5g
	Sorbic acid	0.5g or 0.2g
	Methyl-p-hydroxybenzoate	0.5g or 0.2g
	Streptomycin	0.4g or 0.1g
	10% tocopherol in germ oil	3ml
	linseed oil	4ml
Above added to agar solution:		
	High strength agar	15g
	Deionised water	400ml

Results

The larvae on the low diet performed very poorly with no butterflies emerging from diet changed once per week. This was probably due to the development of mould on the diet, which may not have been toxic to the larvae *per se* but rather formed a barrier between the larva and the food. From the diet that was changed two times per week about 50% developed from the petri dishes containing single and two larvae. Survival dropped to 15% in petri dishes containing 5 larvae. The purpose of the study was to develop an acceptable diet and to determine a suitable way of presenting the diet to the larvae, therefore only data from the normal diet will be considered from here on.

Eighty percent of the solitary larvae on the normal diet changed two times per week developed successfully and 74% of the larvae on normal diet changed once per week. The percentage of larvae developing normally (i.e. into adults with fully expanded wings) also remained high with two larvae per dish when the diet was changed two times per week (79%), but dropped to 58% when the diet was changed only once per week. The percentage of larvae successfully developing from five larvae per dish was 55% when the diet was changed two times per week, but only 42% when the diet was changed once per week. Therefore, overall survival was slightly improved by keeping the diet fresh (i.e. by renewing the diet two times per week) and, under these conditions, survival was not adversely affected by increasing the density from one to two larvae per petri dish. In total, 77 males and 91 females developed, which is a sex ratio not significantly different from unity ($\chi^2 = 1.17$).

Table 2 shows the means for the development period (egg hatching to pupation) for the various treatments and by sex. The anova in Table 2 shows a significant density effect with individuals held singly in petri dishes developing slightly faster than higher densities. The frequency with which the diet was changed did not have a significant effect on

Table 2. Means and analysis of variance of development periods (emergence from egg to emergence of adult in days) of butterflies reared from artificial diet in petri dishes at a variety of densities and where the diet was renewed either one or two times per week.

Diet renewal rate per week	Sex	Density 1	2	5
1	♂	28.3	30.6	31.2
	♀	33.0	34.3	34.2
2	♂	28.6	32.3	28.5
	♀	31.3	31.3	34.8

Analysis of variance of development periods

Source	DF	MS	F	p
Density (d)	2	57.90	3.79	0.025
Renewal (r)	1	29.97	1.90	0.170
sex (s)	1	337.78	22.11	<0.001
d * r	2	0.17	0.01	0.989
d * s	2	37.69	2.47	0.088
r * s	1	7.09	0.47	0.497
Error	153	15.28		
Total	162			

development period. Males develop on average faster, are lighter and have a different pattern than females, thus it is not surprising to find significant sex effects throughout.

Table 3 shows the mean fresh pupal weights at two days old. There were significant effects of density and the frequency with which the diet was changed; the heaviest animals coming from the petri dishes containing one larva in which the diet was renewed two times per week.

Tables 4 and 5 compare the wing patterns by considering the ratios of the width of the 2nd forewing spot (2spot) (25x magnification) over the length of the forewing (6x magnification) and the ratio of 2spot over the width of the 5th hindwing spot (5spot), respectively. Absolute spot size was not considered as wing size and spot size are allometrically related, but the pattern may be the same irrespective of the size of the butterfly. There is a clear sex effect for both measures, as expected. Density had no effect on pattern, but there was a marginally statistically significant effect of frequency of diet renewal on the size of the 2spot/5spot ratio. However, with a significant interaction between frequency of diet renewal and sex there was not a single method of diet presentation producing the largest ratio.

Overall, neither density nor frequency of diet renewal had a clear effect on wing pattern. However, if the quality of the diet is determined by fast development and large butterflies, from Tables 2 and 3 it appears that

Table 3. Means and analysis of variance of pupal weights (grams) at two days old from larvae reared from artificial diet in petri dishes at a variety of densities and where the diet was renewed either one or two times per week.

Diet renewal rate per week	Sex	Density 1	2	5
1	♂	0.117	0.113	0.109
	♀	0.145	0.118	0.135
2	♂	0.125	0.113	0.119
	♀	0.153	0.150	0.137

Analysis of variance of pupal weights

Source	DF	MS	F	p
Density (d)	2	0.0020	6.84	0.001
Renewal (r)	1	0.0036	12.43	0.001
sex (s)	1	0.0206	71.73	<0.001
d * r	2	0.0004	1.23	0.294
d * s	2	0.0002	0.79	0.458
r * s	1	0.0001	0.47	0.497
Error	153	0.0003		
Total	162			

one larva per petri dish (at least on the size of diet blocks used in the current study) and diet renewed two times per week is best.

The results from the best diet treatment were compared with the performance of a sample of larvae reared on maize at 28°C. 52% of the larvae on maize reached the 4th instar and of the 26 larvae assigned to 28°C, 20 produced butterflies. Thus about 40% emergence from egg to adult survival (however, if sufficient space was available to rear larvae individually on living maize plants, much higher survival could certainly be achieved). For none of the comparisons made between the diet results and the results from maize was there a significant interaction between food (maize or diet) and sex. Therefore, single F values are presented. The mean development periods (emergence from egg to adult) for males (20.4 days) and females (22.2 days) on maize were significantly shorter than on diet ($F=86.4$, $p<0.001$). The mean pupal weight was also higher from maize (males: 0.142g, females: 0.173g) than from diet ($F=8.67$, $p<0.05$), although, curiously, there was not a significant difference between the lengths of the forewings from the two foods ($F=1.81$, p=n.s.). The ratios of 2spot/wing were 0.372 (males) and 0.370 (females) from maize and were not significantly different from the diet butterflies ($F=0.21$, p=n.s.). The 2spot/5spot ratios for maize butterflies were 0.76 (males) and 0.71 (females) and again there was no difference between the two groups of butterflies ($F<0.01$, p=n.s.).

The butterflies developing on diet were placed together to crudely

Table 4. Means and analysis of variance of ratio of the width of the 2nd forewing spot (25x magnification) over length forewing (6x magnification) in butterflies reared from artificial diet in petri dishes at a variety of densities and where the diet was renewed either one or two times per week.

Diet renewal rate per week	Sex	Density 1	Density 2	Density 5
1	?	0.38	0.37	0.38
	/	0.34	0.32	0.33
2	?	0.37	0.39	0.35
	/	0.37	0.35	0.37

Analysis of variance of ratio of 2nd spot over wing length

Source	DF	MS	F	p
Density (d)	2	0.0015	0.55	0.577
Renewal (r)	1	0.0092	3.30	0.071
sex (s)	1	0.0292	10.44	0.002
d * r	2	0.0007	0.24	0.787
d * s	2	0.0031	1.11	0.331
r * s	1	0.0209	7.46	0.007
Error	153	0.0028		
Total	162			

assess the effect of the diet on breeding potential. Although eggs were laid, there were very few considering the number of females which suggested that either only a small number of butterflies laid eggs or that fecundity was generally much reduced. Most of the eggs hatched normally.

Discussion

A large number of artificial diets suitable for the rearing of many species of moths can be found in the literature (e.g. Vanderzant, 1967; 1974; Singh and Moore, 1985; Bergomaz and Boppré, 1986). However, it is generally not as easy to rear butterflies on artificial diets (Wielgus, 1974; Morton, 1979). There could be several reasons for this. For example, many moth species are generalists with respect to host plant whilst butterflies tend to be more specialized. Also, some moths are capable of boring into materials; a behaviour that butterflies, on the whole, do not possess. However, we do know of a few instances where butterflies are being reared on artificial diets. Among these workers it is generally believed that butterflies may be unable to find certain essential fatty acids in diets designed for moths. Indeed, this seems to have been the cause of the extensive crippling of wings noted during all of our earlier trials. The addition of linseed oil to the present diet (Table 1) removed

Table 5. Means and analysis of variance of ratio of the width of the 2nd forewing spot over 5th hindwing spot in butterflies reared from artificial diet in petri dishes at a variety of densities and where the diet was renewed either one or two times per week.

Diet renewal rate per week	Sex	Density 1	Density 2	Density 5
1	M	0.73	0.76	0.76
	F	0.65	0.66	0.65
2	M	0.76	0.80	0.74
	F	0.71	0.72	0.70

Analysis of variance of 2nd spot forewing over 5th spot hindwing

Source	DF	MS	F	p
Density (d)	2	0.0073	0.86	0.426
Renewal (r)	1	0.0406	4.76	0.031
sex (s)	1	0.2086	24.44	<0.001
d * r	2	0.0052	0.61	0.542
d * s	2	0.0018	0.21	0.812
r * s	1	0.0169	1.98	0.161
Error	153	0.0085		
Total	162			

this problem and we successfully reared *B. anynana*, *B. safitza* and *B. ena*. However, it is of note that the earlier trials often resulted in survivals of over 90%, sometimes even over 95%. The reason for this higher survival may have had little to do with the addition of linseed oil, but rather due to the handling and transfer of larvae onto the new blocks of diet. During prior experiments the larvae were left to find the new diet for themselves and the old diet was left in the petri dish. Therefore, it may be better to avoid disturbing the larvae as much as possible, particularly during the first instar when the larvae is most prone to damage.

Another factor shown to be of importance, to *Bicyclus* butterflies at least, was the freshness of the diet. Butterfly larvae usually feed on fresh material with a high water content. It is possible that the diet dried out over a week to the extent that it was detrimental to the insect. Here again is a factor to which many moth species are less susceptible. We successfully reared the warehouse moth, *Ephestia kuhniella*, on the diet (minus linseed oil) without renewing the diet throughout the entire development period. Of course, *E. kuhniella* is a stored product insect and, as such, is extremely resistant to low moisture conditions.

The diet described in Table 1 opens up the possibility of certain types of experiments, particularly involving the study of colour patterns, that would be difficult to perform using living plants. Examples include the manipulation of development time independent of temperature to examine the subsequent effect on wing pattern, and the study of pigments sequestered from the host plant in larvae, pupae and adults. However, the diet does not appear to be suitable for the maintenance of stock animals. Diet reared butterflies had a low fecundity and living plants are also required for the deposition of eggs.

A second important reason for developing artificial diets is to enable amateur entomologists to rear a few mint condition specimens through to adult to be added to collections without the need to use living plants. Morton (1979) experimented with rearing butterflies on artificial diets and presented a recipe with which he successfully reared 50 species. However, Morton's recipe is considerably more complicated than ours (see Table 1) and the need to develop a simple diet without expensive and difficult to obtain chemicals is of paramount importance if it is to be widely used. Furthermore, Morton found that phagostimulants were usually required in the diet in the form of dried natural host plant material. Only *Bicyclus* species are considered in the present study, but no dried maize leaves were needed in the diet to induce feeding. With edge feeding species, Morton shredded his diet through a cheese grater to facilitate feeding. *Bicyclus* species are also edge feeders (grasses), but did not require the presentation of the diet to be modified in any way beforehand.

The simplicity of the diet presented here and the ease with which it can be used warrents further investigation into its applicability with other species of butterflies. The colour pattern of the species used here did not appear to be effected by the diet, but the size of the resulting animal did. This may be an important consideration if collectors' specimens are to be reared, although it may be possible to overcome the problem through slight modifications of the basic diet.

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Literature Cited

BERGOMAZ, R. & M. BOPPRÉ, 1986. A simple instant diet for rearing Arctiidae and other moths. J. Lepidopt. Soc. 40: 131-137.

BRAKEFIELD, P.M. & N. REITSMA, 1991. Phenotypic plasticity, seasonal climate and the population biology of *Bicyclus* butterflies (Satyridae) in Malawi. Ecol. Entomol. (in press).

MORTON, R.S. 1979. Rearing butterflies on artificial diets. J. Res. Lepid. 18: 221-227.

SINGH, P. & R.F MOORE, 1985. Handbook of insect rearing (Vol. II). Elsevier Science Publishers, Amsterdam.

VANDERZANT, E.S. 1967. Wheat germ diets for insects: rearing the boll weevil and the saltmarsh caterpillar. Ann. Entomol. Soc. Am. 60: 1062-1066.

——. 1974. Development., significance, and application of artificial diets for insects. Ann. Rev. Entomol. 19: 139-160.

WIELGUS R.S. 1974. Artificial diet: the key to the mass rearing of *Megathymus* larvae. J. Res. Lepid. 13: 271-277.