Evolutionary Ecology Research, 2002, **4**: 1229–1240

Protandry in the butterfly *Bicyclus anynana*

Wilte G. Zijlstra,* Fanja Kesbeke, Bas J. Zwaan and Paul M. Brakefield

Section of Evolutionary Biology, Institute for Evolutionary and Ecological Sciences, University of Leiden, PO Box 9516, 2300 RA Leiden, The Netherlands

ABSTRACT

Protandry is the earlier adult emergence of males than females. This is predicted from sexual selection theory because, under certain circumstances, earlier emergence of males will maximize their mating opportunities. The tropical butterfly, *Bicyclus anynana*, is adapted to highly seasonal environments and selection pressures for protandry may vary across seasons. To examine such effects, we compared protandry across a wide range of rearing temperatures in the laboratory which match those that occur in the different seasons. The absolute amount of protandry (in days) remained constant at intermediate and high temperatures (wet season), but increased with temperature at lower temperatures (dry season). Nevertheless, average male development time as a percentage of female development time remained constant. We also compared lines established by artificial selection on development time and pupal weight across temperatures to assess the impact of different selection pressures on protandry. Data for the pupal weight selected lines were inconclusive. The male to female development time ratio did not change across temperatures for the development time selected lines. However, females were relatively slower than males in the slow selected lines (lower male : female development time ratio) than in either the fast lines or the stock. This indicates that sex-specific components to development time are present in *B. anynana* and that there is scope for a response of protandry to selection.

Keywords: *Bicyclus anynana*, life history, protandry, seasonality, selection, sexual differences.

INTRODUCTION

Darwin (1871) observed that 'throughout the great class of insects the males almost always emerge from the pupal state before the other sex' (p. 260). Interest in this phenomenon, called protandry, was rekindled by Wiklund and Fagerström (1977). Two hypotheses to account for protandry have been postulated: (1) sexual selection acts on protandry itself (called the 'adaptive' explanation by Wiklund and Solbreck, 1982) and (2) protandry is a side-effect of natural selection working differently on the sexes (the 'incidental' explanation).

On the sexual selection hypothesis, males that emerge before females are favoured, because this maximizes the number of encounters with females willing to mate and thus their reproductive success. Especially in (near) monandrous species, it is important to be

* Author to whom all correspondence should be addressed. e-mail: zijlstra@rulsfb.leidenuniv.nl

the first to mate, and the amount of protandry is expected to decline with the degree of polyandry (e.g. Zonneveld, 1996). A prerequisite is some discreteness of generations, because were receptive females to be continuously available, the earlier eclosion of males would not be favoured (Singer, 1982). Protandry can also be considered a female reproductive strategy because it minimizes the period females are unmated, thus decreasing the risk of death before reproduction (Fagerström and Wiklund, 1982). An extra factor shaping the degree of protandry is temporal variation in female quality. A lower fecundity for slower developing females would increase selection in favour of an earlier emergence of males (Kleckner *et al.*, 1995; Carvalho *et al.*, 1998). Thus, on this 'adaptive' explanation (Wiklund and Solbreck, 1982), the optimal difference between male and female emergence only depends on conditions in the adult environment, such as adult mortality and mating structure. If the adult life history does not change, the optimal protandry does not change, and different larval conditions should still lead to the same amount of protandry.

An alternative, 'incidental' (Wiklund and Solbreck, 1982) explanation of protandry is based on natural selection. If females could increase their reproductive output by increasing their size, but males could not, and a larger size meant a longer period of development, then males would be expected to emerge before females. In this case, it is not primarily that selection favours males to emerge earlier, but rather that females emerge later to maximize their size and subsequent fecundity (Thornhill and Alcock, 1983). Moreover, when protandry is selected for *per se*, we expect males to be smaller than females, because, given equal growth rates, body size at maturity trades off with development time (Singer, 1982).

Most of the evidence to date points to sexual selection being the driving force in shaping protandry (e.g. Nylin *et al.*, 1993). Using a comparative approach, Nylin and co-workers found a largely constant amount of protandry in Swedish and English populations of the butterfly *Pararge aegeria* reared at different temperatures, in line with the predictions of sexual selection theory. Protandry was absent in Madeiran populations, where seasonality is absent and generations are not discrete (Nylin *et al.*, 1993).

The tropical butterfly, *Bicyclus anynana*, occurs in environments with two pronounced seasons and exhibits seasonal polyphenism. During the warm (>23°C), wet season, ample food is available for caterpillars. Those nearing pupation at these temperatures will develop conspicuous wing patterns that function to deflect attacks of predators away from the vulnerable body (Brakefield and Larsen, 1984). In the dry season, temperatures drop (<20°C) and food-plants are not available. Butterflies in this season have no conspicuous wing patterns and rely on camouflage for survival. They must survive many months before egg laying can begin in the the early wet season. Some mating may occur at the beginning of the dry season, but most occurs shortly before the rains. This seasonal polyphenism also affects other (life-history) traits, including fat reserves and oviposition behaviour (Brakefield and Reitsma, 1991). *Bicyclus anynana* can have two to three generations in the wet season, but only one in the dry season (Windig *et al.*, 1994). In the wet season, there may be some overlap in generations, but this will then be synchronized by the harsh conditions in the dry season. The different life histories during the different seasons may also have repercussions for protandry. Selection pressures regarding protandry will vary across the two seasons. In the wet, warm season, there is strong (sexual) selection for protandry, and the emphasis in this season is on fast reproduction to exploit the available resources for caterpillars and to perhaps establish an extra generation before the dry season arrives. Earlier emergence is predicted to ensure a higher probability of mating for males. At lower

temperatures, in the dry season, survival is more important and early emergence is unlikely to confer any fitness advantages. In contrast, it could decrease chances of survival because of lower body weight and/or fat reserves.

In this study, we investigated the effects on protandry of divergent selection in the field by measuring protandry at different rearing temperatures in the laboratory corresponding to the different seasons. Does the amount of protandry fluctuate within and across environments and does the development time of males relative to females remain constant? To investigate which selection pressures are important in the different seasons, we compared protandry in lines artificially selected for fast or slow development time, and also in lines selected for high or low pupal weight. These lines were highly divergent for these selected traits, which are essential in shaping patterns of protandry. Our main aim was to quantify and compare the amount of protandry at different temperatures (corresponding to the different seasons) and in genetically different lines of *B. anynana* to obtain more information about the causes and consequences of the different selection pressures that occur across sexes and environments.

MATERIALS AND METHODS

Butterflies

The base stock of *Bicyclus anynana* was established in 1988 from approximately 80 gravid females caught at a single locality in Malawi. This stock has been kept in climate-controlled chambers with high humidity at the laboratory in Leiden with population census sizes of >500 individuals. It has retained substantial genetic variation (Saccheri and Bruford, 1993). Caterpillars feed on young maize plants, adults on moist banana.

Stock butterflies at different temperatures

From three different generations (February and April 1998, March 2000) of the base stock population, we collected eggs over a 12 h period. Two of these egg collections (February and April 1998) were reared at an intermediate temperature of 22°C in 15 and 7 replicate population cages, respectively ($22°C_A$ and $22°C_B$). The third collection (March 2000) was reared at 25°C (high, wet seasonal temperature) in 6 replicate cages. Each population cage contained approximately 500 eggs. In addition, for protandry comparisons of the stock across a wider range of temperatures, we used sleeve cages (containing ~100 eggs each). Eggs from a large group of stock females (March 1999) were randomly allocated to be reared at 18°C (low), 22°C (intermediate) and 27°C (high temperature) in 3–4 four sleeve cages at each temperature.

Selection lines for development time and pupal weight

The development time selection lines have been selected for at least 30 generations and have diverged markedly from the stock (Brakefield and Kesbeke, 1997). Some of these lines were selected at 20°C and some at 27°C. Because the selection lines from different temperatures behaved similarly, we reclassified them as ‘fast’ (decreased development time) and ‘slow’ (increased development time). Twenty-one sleeve cages per selection regime and seven sleeve cages with stock animals were reared at both 20°C and 27°C. The selection lines for pupal

weight have been selected at 27°C for 14 generations for either increased pupal weight ('large') or decreased pupal weight ('small'); they also showed a large response to selection (B.J. Zwaan, unpublished). We reared these lines in 10 sleeve cages each, at both 20°C and 27°C, together with 10 sleeves with unselected controls that had been propagated and kept under similar conditions throughout the pupal size selection experiment. The two sets of selection lines were reared in different periods.

Statistical analysis

Statistical analyses were performed using JMP 4.0.2 and MINITAB 13. In analyses of covariance (ANCOVA), cage was always a random factor and temperature was treated as a discrete factor. Groups were *post-hoc* compared using Tukey comparisons or contrasts. The absolute amount of protandry was calculated by subtracting the mean development time of males from the mean development time of females. Using bootstrapping (i.e. repeatedly taking the difference between a randomly drawn male and female) yielded similar values. A relative measure of protandry (independent of development time) was derived by taking values for male development time as a percentage of female development time. This relative measure was normally distributed and always had equal variances (Levene's test, $P > 0.05$). Therefore, we used analyses of variance (ANOVA) to analyse these data. The variable overall development time was calculated by averaging the mean values of male and female development time of a cage, to capture development time of a cage in one value. Furthermore, we also used orthogonal regressions to determine if the relationships between male and female development time remained constant over a range of environments and subsequent development times (in which case the slope is unity). We used orthogonal regression to adjust for variability in both traits, and assumed equal variances.

RESULTS

Stock butterflies at intermediate/high temperature

In all 28 population cages, males developed faster than females (ANOVA: $F_{1,10602} = 1641$, $P < 0.0001$; Fig. 1). Egg batches ($F_{2,10602} = 53.92$, $P < 0.0001$) and cages, which were nested within batch ($F_{25,10602} = 79.16$, $P < 0.0001$), differed from each other. Protandry (female – male development time) was 1.73 ± 0.08 days for $22°C_A$, 1.63 ± 0.16 days for $22°C_B$ and 1.75 ± 0.15 days at 25°C (mean ± standard error). The two collections at 22°C also differed from each other in development time (Tukey, $P < 0.05$). Presumably, $22°C_A$ was raised at a slightly lower average temperature, leading to generally slower development. Furthermore, in an ANCOVA on protandry, there was no interaction between collection and the covariate of overall development time ($F_{2,22} = 2.02$, $P = 0.16$), and overall development time (mean of male and female development time) did not explain variance in protandry ($F_{1,24} = 1.40$, $P = 0.25$). To examine further whether protandry changed with development time, we performed an orthogonal regression on male and female development time. The slope of the regression was 1.02, with the lower 95% confidence limit at 0.95 (correlation = 0.985; Fig. 1). Because the slope is not significantly different from unity, there is no change in the relationship at these intermediate to high temperatures between male development time and female development time with increasing overall development time. Although development times

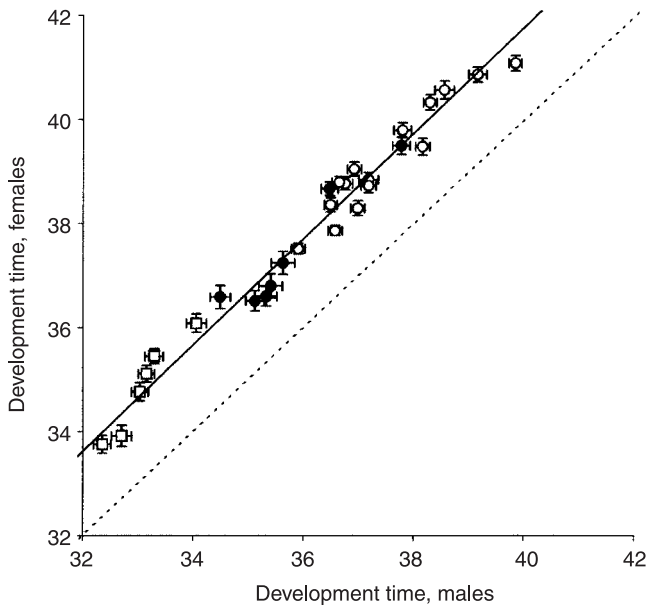

Fig. 1. Development time for population cages (mean ± standard error). Three different egg collections were used: 22°C$_A$ (○), 22°C$_B$ (●) and 25°C (□). Dashed line = development times of males and females are equal; solid line = fitted orthogonal regression line (female development time = 0.96 + 1.02 × male development time).

varied considerably between the different cages and egg batches (Fig. 1), the amount of protandry remained constant at these intermediate to high temperatures (wet seasonal conditions).

Stock butterflies over the whole range of temperatures

At all three temperatures spanning the relevant range, the egg-to-adult development time of males was shorter than that of females (ANOVA: 18°C, $F_{1,113} = 50.01$; 22°C, $F_{1,356} = 60.31$; 27°C, $F_{1,218} = 16.79$; all $P < 0.0001$), but females had a significantly shorter pupal phase than males (18°C, $F_{1,113} = 15.54$; 22°C, $F_{1,356} = 58.69$; 27°C, $F_{1,218} = 16.61$; all $P < 0.0001$). The results for differences in length of (components of) development time for the two sexes are shown in Table 1. Protandry increased significantly with decreasing temperature ($F_{2,28} = 21.54$, $P < 0.0001$). Differences in larval time between the sexes were significant between temperatures ($F_{2,28} = 29.29$, $P < 0.0001$), but differences in pupal time were not ($F_{2,28} = 3.26$, $P = 0.054$). Tukey comparisons showed that differences between the sexes were always larger at 18°C than at either 22°C or 27°C (Table 1). Addition of the covariate overall development time did not increase the explanatory power of the model, and at no temperature was there a significant relationship between protandry and development time (regressions, $P > 0.30$). Although the difference in development time increased with decreasing temperature, male development time as a percentage of female development time showed no change across temperatures ($F_{2,28} = 0.79$, $P = 0.47$). This was true for each component of development time (larval time, $F_{2,28} = 0.61$; pupal time, $F_{2,28} = 0.53$; both $P > 0.50$, Table 1). Thus,

Table 1. Differences between stock females and males in components of development time (days; mean ± standard error)

Temperature	*n*	Development time	Larval time	Pupal time
18°C	8	6.37 ± 0.99^a	6.85 ± 0.74^a	-0.92 ± 0.23^a
		93.4 ± 0.9%	90.2 ± 1.2%	104 ± 1.0%
22°C	12	2.20 ± 0.26^b	2.50 ± 0.14^b	-0.59 ± 0.10^b
		94.9 ± 0.9%	91.8 ± 0.6%	107 ± 1.1%
27°C	11	1.79 ± 0.20^b	1.84 ± 0.30^b	-0.36 ± 0.13^b
		94.4 ± 0.9%	91.5 ± 1.2%	106 ± 2.1%

Note: *n* = the number of cages used. Percentages refer to the male component of development time as a percentage of female development time. Identical letters indicate no significant differences in a Tukey comparison ($P > 0.05$). Larval and pupal time do not sum to development time because of pupal mortality.

the absolute amount of protandry (in days) was significantly greater at 18°C, a temperature representative of the dry season, than at intermediate or high temperatures, while the relative difference between male and female development time did not change across temperatures.

Selection lines

The protandry results for the different selection lines at 20°C and 27°C are shown in Table 2. The control groups for the two different selection regimes, development time and pupal weight, differed significantly from each other in protandry ($F_{1,30} = 4.72$, $P = 0.038$). Therefore, we examine development time and pupal weight selection lines separately.

Protandry in selection lines for development time differed between temperatures and between selection lines (temperature: $F_{1,92} = 6.58$, $P = 0.012$; line: $F_{2,92} = 12.82$, $P < 0.0001$; Table 2). The interaction between temperature and selection line was also significant ($F_{2,92} = 4.78$, $P = 0.011$). Protandry for the 'slow' line increased much more from 27°C to 20°C than for the 'fast' line and the stock (contrast, $t = 2.81$, $P = 0.006$; Table 2).

Alternatively, we can analyse protandry using overall development time. Overall development time ($F_{1,94} = 21.75$, $P < 0.0001$) and temperature ($F_{1,94} = 4.71$, $P = 0.033$) were significant, as well as their interaction ($F_{1,94} = 7.57$, $P = 0.007$); protandry increased more with development time at 20°C than at 27°C (Fig. 2). None of the slopes of orthogonal regressions on male and female development time were significantly different from unity at 27°C (slope of all lines combined = 0.92; upper 95% confidence limit = 1.03). At 20°C, however, the slopes for both the 'fast' and 'slow' lines were significantly lower than 1, being 0.77 (upper 95% confidence limit = 0.99) and 0.43 (upper 95% confidence limit = 0.93), respectively. The slope for all lines combined was 0.73, with an upper 95% confidence limit of 0.82 (see Fig. 2). This implies that, at 20°C, females became relatively slower than males with increasing development times of both sexes.

Male development time as a percentage of female development time did not differ across temperatures, but was significantly different between selection lines (ANOVA: $F_{2,95} = 4.64$, $P = 0.012$). This was confirmed by a Kruskall-Wallis test ($H = 13.84$, d.f. = 2, $P = 0.001$). Tukey comparisons with temperatures pooled ($P < 0.05$) showed that male development time as a percentage of female development time was significantly larger for the 'fast' than

Table 2. Protandry (mean female development time – mean male development time) for different selection lines at 20°C and 27°C (days; mean ± standard error)

		20°C		27°C	
	n	Protandry	%	Protandry	%
Development time selection					
'Fast'	21	1.85 ± 0.20^{a}	95.8 ± 0.4	1.58 ± 0.23^{a}	94.1 ± 0.9
'Slow'	21	5.86 ± 0.86^{b}	90.4 ± 1.4	2.59 ± 0.49^{a}	93.0 ± 1.4
Stock	7	2.56 ± 0.86^{a}	94.8 ± 1.8	1.99 ± 0.48^{a}	93.6 ± 1.5
Pupal weight selection					
'Large'	10	1.29 ± 0.67^{ef}	97.8 ± 1.1	0.82 ± 0.36^{ef}	97.6 ± 1.0
'Small'	10	2.18 ± 0.71^{f}	96.2 ± 1.2	-0.06 ± 0.34^{e}†	100.1 ± 1.0 †
Control	10	1.73 ± 0.62^{ef}	97.0 ± 1.1	0.28 ± 0.34^{ef} †	99.2 ± 1.0 †

Note: *n* = the number of cages used. Percentages refer to the mean male development time as a percentage of mean female development time. Identical letters denote no differences after ANOVA on either development time or pupal weight selected lines at both temperatures (Tukey, $P > 0.05$). † Does not differ significantly from zero, or 100%.

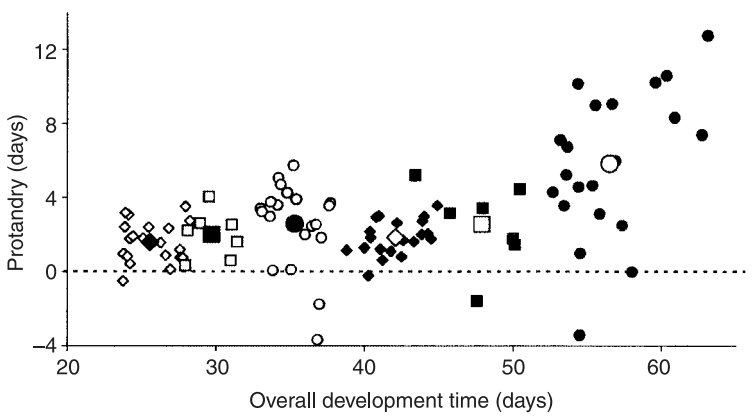

Fig. 2. Protandry (female development time – male development time) versus overall development time (average of male and female development time) at 20°C (closed small symbols) and 27°C (open small symbols) for the development time selection lines 'fast' (◇), 'slow' (○) and for stock (□). Large symbols represent the means per selection line and temperature (closed symbols for 27°C, open symbols for 20°C).

for the 'slow' lines. That is, the difference between the sexes was less for the 'fast' lines (closer to equal development times for males and females). Stock was intermediate and did not differ from either selection line (Table 2; Tukey results not indicated).

The pupal weight selected lines only differed in the amount of protandry between temperatures. At 20°C, the difference in development time between males and females was greater than at 27°C (ANOVA: $F_{1,56} = 10.01$, $P = 0.003$; Table 2). Neither selection line nor the line × temperature interaction were significant ($F_{2,56} = 0.01$, $P = 0.99$ and $F_{2,54} = 1.39$,

$P = 0.26$, respectively). Furthermore, the control and the 'small' populations did not show significant protandry at 27°C (Table 2). When overall development time was used, protandry of the pupal weight selected lines differed between temperatures ($F_{1,57} = 6.07$, $P = 0.017$), and protandry decreased with increasing development time ($F_{1,57} = 4.44$, $P = 0.040$). The interaction was not significant ($F_{1,55} = 0.42$, $P = 0.52$). The decrease in protandry with increasing development time was not observed in the orthogonal regression analyses. At both temperatures, the orthogonal regression slope did not differ significantly from unity (20°C: slope = 2.03, lower 95% confidence limit = 0.85; 27°C: slope = 1.30, lower 95% confidence limit = 0.69).

The pupal weight selection lines did not differ in male development time as a percentage of female development time, but this ratio was significantly lower at 20°C than at 27°C ($F_{1,58} = 5.07$, $P = 0.028$). The interaction was not significant ($F_{2,54} = 1.86$, $P = 0.17$). A non-parametric Mann-Whitney test confirmed the differences in ratio between temperatures ($W = 1064$, $P = 0.028$). The ratio between male and female development time was similar for the stock used together with the development time selection lines (mean = 94.2%; Table 2) and the stock butterflies measured across temperatures (mean = 94.7%; cf. Table 1). However, male development time as a percentage of female development time was significantly higher for the controls in the pupal weight selected lines (mean = 98.1%; Table 2) than for either stock population.

The main findings were that development time of stock males relative to females remained constant over temperatures, but that this ratio was significantly lower for 'slow' selected lines. Pupal weight selected lines differed across temperatures in this relative measure (ratio between male and female development time).

DISCUSSION

Protandry at intermediate/high temperatures

Protandry (in absolute terms, i.e. days) in stock *Bicyclus anynana* did not differ across intermediate and high temperatures (22°C and 27°C). Even though mean development times varied from 30 to 41 days over this temperature range (Fig. 1), the difference in development time between females and males was fairly constant at about 2 days. This is consistent with sexual selection being the primary force shaping the degree of protandry in these (wet season) conditions, because this selective factor is expected to be largely independent of environment. The difference in development time between the sexes is probably the outcome of balancing two selective factors in the adult stage: males are selected to increase the difference between the sexes because it increases the number of mating opportunities, but this is opposed by the increase in pre-reproductive mortality (assuming adults have higher mortality) (Wiklund and Fagerström, 1977). It is beneficial to be the first male to mate with a female, since in the field about one-third of *B. anynana* females remate (Brakefield and Reitsma, 1991) and in the laboratory about one-quarter of females remate, without strict last male sperm precedence (Brakefield *et al.*, 2001). The 2 day difference between males and females in these circumstances presumably represents the optimal balance between these two selective pressures. Furthermore, there may be a premium on rapid reproduction rather than long-term survival in the wet season, because an extra generation confers large fitness benefits in an environment rich in larval food plants (grasses).

Protandry from low to high temperatures

In the experiment in which we examined a wider range of temperatures (from 18°C to 27°C), we did find changes in protandry. At 18°C (dry season conditions), females emerged much later (approximately 6 days) than males than at temperatures >22°C (protandry is around 2 days; Table 1). However, because of longer development times, the ratio between male development time and female development time remained constant across temperatures (~95%; Table 1). Thus we have two measures of the relationship between male and female development time – a relative one (ratio between males and females) and an absolute measure (difference between males and females) – that appear at odds with one another; one is variable and the other is constant. The adaptive explanation of protandry (based on sexual selection) predicts a fixed absolute difference and a varying relative measure under different larval conditions (assuming adult conditions remain constant). The incidental explanation predicts variation in the absolute amount of protandry and a fixed ratio between male and female development time across temperatures. Here, we found evidence for both explanations; absolute protandry remains constant at intermediate and high temperatures, but the relative measure also remains constant across a wider range of temperatures. Further research is needed to determine which of these two findings should be given the most weight and under what (natural) conditions.

To retain (aspects of) the sexual selection hypothesis, we need to explain why the absolute amount of protandry is so much higher at 18°C than at intermediate to high temperatures (22–27°C). Four possible reasons, which are not mutually exclusive, are:

1. Natural selection (the 'incidental' explanation) is much more important at 18°C than the sexual selection hypothesis (the 'adaptive' explanation).
2. Adult circumstances are different at 18°C; therefore, the optimal difference between male and female development time is different (this is in accordance with the 'adaptive' explanation).
3. In both (seasonal) environmental circumstances (low and high temperature), there is selection for protandry of ~2 days. However, the physiological mechanism is constrained such that the same difference cannot be produced at different temperatures.
4. There is no selection in favour of protandry at 18°C and the observed difference at this low temperature is a by-product of the fine-tuned physiological mechanism to produce an approximately 2 day difference at high temperatures.

No matings occur in the middle period of the dry season, and males have to wait until just before the onset of the wet season to become reproductively active (Brakefield and Reitsma, 1991; N. Reitsma, personal communication). Hence, protandry may not present any fitness benefits in the dry season, and this season can effectively be regarded as an 'adult diapause'. However, there could be some selection on protandry in the early stages of the dry season by males mating with dry season females followed by sperm storage before aestivation. During the dry season, the emphasis is more on survival than on reproduction, since dry season females have larger and fatter bodies, fewer mature eggs in storage, and have longer delays before oviposition than wet season forms (Brakefield and Reitsma, 1991). Butterflies of the same family in Australia exhibit various degrees of adult reproductive dormancy during the dry season, ranging from complete reproductive diapause throughout the season to a decline in reproductive activity as the dry season progresses (Jones, 1987; Braby, 1995).

Protandry in selection lines

The development time selection lines of *B. anynana* showed that both the absolute and relative difference between male and female development time had at least changed in the 'slow' selected lines. This agrees with work on the pitcher-plant mosquito, in which protandry also increased for lines selected for slow development (Bradshaw *et al.*, 1997). The differences in protandry were especially clear at 20°C; the difference between 'slow' males and females was about 4 days more than for the 'fast' lines or the stock (Table 2, Fig. 2). Furthermore, the development time of 'slow' males only constituted ~92% of female development time, compared to >94% for the other lines. Although this is not a huge difference, it does imply that protandry for 'slow' lines is 1.33 times that of the other lines. The response to selection for a decreased development time seems to have been greater in females, presumably because of a contribution of sex-specific components of development time. Such components must have been present at some stage for protandry to arise during evolution. This also suggests that development time can be manipulated independently within a single sex and that selection on the trait protandry itself is possible. We intend to test specifically for this in selection experiments on protandry. The converse did not occur; the relation between male and female development time was similar for 'fast' lines and stock, although 'fast' lines tended to have a slightly higher ratio. For all development time selection lines, male development time as a percentage of female development time was constant over temperatures, analogous to the stock butterflies (cf. Tables 1 and 2). This consistent relationship across temperatures ('reaction norm') has been observed previously for reaction norms in this species. For example, Wijngaarden and Brakefield succeeded in changing the elevation of reaction norms for wing pattern via artificial selection, but did not change the shape of the reaction norm (Wijngaarden and Brakefield, 2001; Wijngaarden *et al.*, 2002).

At 20°C, we observed orthogonal regressions of male and female development time that did not equal unity, but these may be a by-product of the fact that the range of developmental times is wider at 20°C (Fig. 2), in combination with a constant relationship between male and female development time. The main conclusion from the development time selection lines is that the relationship between male and female development time can be changed (compare 'slow' with 'fast' and unselected), but that this relationship remains consistent across temperatures.

We cannot explain why the pupal weight selection lines should be sensitive to temperature in their relationship between male and female development. The change in male development time as a percentage of female development time for unselected controls (from 94–95% to 98%; Tables 1, 2) perhaps indicates some inadvertent selection during the selection experiment, but it is unlikely that it caused such a major shift. Differential environmental circumstances are also an improbable explanation, because the male : female development time relation is conserved and environment-independent (see above and Fig. 1). Pupal weight selection did not affect the sexes differently; the relation between male and female development time remained unaltered between the selection lines. There was a trend for the 'small' selection line to be more temperature-sensitive than the control, and for the 'large' line to be less sensitive (Table 2). This might be due to physiological processes being more or less buffered from temperature in large or small pupae, respectively.

In conclusion, the absolute amount of protandry is constant over a range of intermediate and high temperatures (Fig. 1), which correspond to the characteristics of the wet season

and supports the sexual selection hypothesis for protandry. More research is needed to determine how this can be reconciled with the finding that the relative relationship between male and female development time is conserved across all temperatures. Only artificial selection for slow development time has changed the value of this ratio, so that females develop even more slowly than males. This underlines the fact that development time of a sex can be changed independently of the other sex in *B. anynana*, and suggests that there is a potential for a response to selection on protandry.

ACKNOWLEDGEMENTS

We thank Els Schlatmann, Bert de Winter and Wil van der Hoeven for maize cultivation. This research was supported by the Netherlands Organization for Scientific Research (NWO), grant 805-36-033 to W.G.Z. and grant PULS-805-48-002 to B.J.Z.

REFERENCES

Braby, M.F. 1995. Reproductive seasonality in tropical Satyrine butterflies – strategies for the dry season. *Ecol. Entomol.*, **20**: 5–17.

Bradshaw, W., Holzapfel, C., Kleckner, C. and Hard, J. 1997. Heritability of development time and protandry in the pitcher-plant mosquito, *Wyeomyia smithii. Ecology*, **78**: 969–976.

Brakefield, P.M. and Kesbeke, F. 1997. Genotype–environment interactions for insect growth in constant and fluctuating temperature regimes. *Proc. R. Soc. Lond. B*, **264**: 717–723.

Brakefield, P.M. and Larsen, T.B. 1984. The evolutionary significance of dry and wet season forms in some tropical butterflies. *Biol. J. Linn. Soc.*, **22**: 1–12.

Brakefield, P.M. and Reitsma, N. 1991. Phenotypic plasticity, seasonal climate and the population biology of *Bicyclus* butterflies (Satyridae) in Malawi. *Ecol. Entomol*, **16**: 291–303.

Brakefield, P.M., El Filali, E., Van der Laan, R., Breuker, C.J., Saccheri, I.J. and Zwaan, B.J. 2001. Effective population size, reproductive success and sperm precedence in the butterfly, *Bicyclus anynana*, in captivity. *J. Evol. Biol.*, **14**: 148–156.

Carvalho, M., Queiroz, P. and Ruszczyk, A. 1998. Protandry and female size–fecundity variation in the tropical butterfly *Brassolis sophorae. Oecologia*, **116**: 98–102.

Darwin, C. 1871. *The Descent of Man, and Selection in Relation to Sex*. London: John Murray.

Fagerström, T. and Wiklund, C. 1982. Why do males emerge before females? Protandry as a mating strategy in male and female butterflies. *Oecologia*, **52**: 164–166.

Jones, R.E. 1987. Reproductive strategies for the seasonal tropics. *Insect Sci. Applic.*, **8**: 515–521.

Kleckner, C., Hawley, W., Bradshaw, W., Holzapfel, C. and Fisher, I. 1995. Protandry in *Aedes sierrensis* – the significance of temporal variation in female fecundity. *Ecology*, **76**: 1242–1250.

Nylin, S., Wiklund, C., Wickman, P.-O. and Garcia-Barros, E. 1993. Absence of trade-offs between sexual size dimorphims and early male emergence in a butterfly. *Ecology*, **74**: 1414–1427.

Saccheri, I.J. and Bruford, M.W. 1993. DNA fingerprinting in a butterfly, *Bicyclus anynana* (Satyridae). *J. Hered.*, **84**: 195–200.

Singer, M. 1982. Sexual selection for small size in male butterflies. *Am. Nat.*, **119**: 440–443.

Thornhill, R. and Alcock, J. 1983. *The Evolution of Insect Mating Systems*. Cambridge, MA: Harvard University Press.

Wijngaarden, P.J. and Brakefield, P.M. 2001. Lack of response to artificial selection on the slope of reaction norms for seasonal polyphenism in the butterfly *Bicyclus anynana. Heredity*, **87**: 410–420.

Wijngaarden, P.J., Koch, P.B. and Brakefield, P.M. 2002. Artificial selection on the shape of reaction norms for eyespot size in the butterfly *Bicyclus anynana*: direct and correlated responses. *J. Evol. Biol.*, **15**: 290–300.

Wiklund, C. and Fagerström, T. 1977. Why do males emerge before females? A hypothesis to explain the incidence of protandry in butterflies. *Oecologia*, **31**: 153–158.

Wiklund, C. and Solbreck, C. 1982. Adaptive versus incidental explanations for the occurrence of protandry in a butterfly, *Leptidea sinapsis* L. *Evolution*, **36**: 56–62.

Windig, J.J., Brakefield, P.M., Reitsma, N. and Wilson, J.G.M. 1994. Seasonal polyphenism in the wild: survey of wing patterns in five species of *Bicyclus* butterflies in Malawi. *Ecol. Entomol.*, **19**: 285–298.

Zonneveld, C. 1996. Being big or emerging early? Polyandry and the trade-off between size and emergence in male butterflies. *Am. Nat.*, **147**: 946–965.