Braby, Michael (1994) ***Life history strategies of tropical satyrine butterflies in north-eastern Australia.*** **PhD thesis, James Cook University.**

CHAPTER 5: SEASONAL POLYPHENISM

5.1 Introduction

In tropical environments with contrasting wet and dry seasons, many insects move into and aggregate in moist refugia during the long and unfavourable dry season (Janzen 1973, Monteith 1982, Denlinger 1986, Jones 1987, Scheermeyer 1987, 1993). In these refuges, activity levels drop and adults may enter reproductive diapause. This dry season behaviour might be expected to increase predation risks, and we might expect that species which exhibit it would develop mechanisms to avoid predation. Indeed, many tropical danaine butterflies may be distasteful, sequestering toxic chemicals from their larval food plants (Monteith 1982, Scheermeyer 1987).

An alternative strategy may occur in some butterflies which become more cryptic during the dry season (Owen 1971, Shapiro 1976, Brakefield and Larsen 1984, Brakefield 1987, Brakefield and Reitsma 1991, Windig 1992, Jones 1987, 1992). For example, several tropical satyrine butterflies, such as in the genera *Mycalesis* and *Bicyclus*, show striking seasonal polyphenism in the underside wing pattern: the wet-season forms are characterised by conspicuous submarginal ocelli (eyespot) patterns on the wing underside, whereas the dry-season forms are rather drab, being paler with greatly reduced ocelli (Corbet and Pendlebury 1978, Kirk 1982, Brakefield and Reitsma 1991). In a few of these species it has been established that this type of phenotypic plasticity is associated with marked changes in behaviour and breeding status and associated changes in habitat favourability. The dry-season forms are usually reproductively dormant, less active, aggregate in moist refugia and tend to rest amongst dead brown leaves (Brakefield 1987, Brakefield and Reitsma 1991, Chapter 4); their wing patterns are thus well camouflaged against the resting background during the dormancy period. By contrast, wet-season forms tend to reproduce more directly, are more active, generally do not aestivate in refugia (Brakefield and

Larsen 1984, Chapter 4) and the prominent eyespots possibly function as anti-predator devices, either to startle or to deflect attacks of vertebrate predators from the vulnerable body (Young 1979, 1980a, Brakefield and Larsen 1984, Brakefield 1984 and references therein).

In the Australian tropics the incidence and significance of seasonal polyphenism in butterflies has received little attention, although it has been well documented in several pierids (Rienks 1985, Jones *et al.* 1985, Jones 1992) and has been reported in three common northern satyrines, *Melanitis leda* (Fabricius), *Mycalesis perseus* (Fabricius), *M. terminus* (Fabricius) (Waterhouse and Lyell 1914, Common and Waterhouse 1981). This study examines the precise changes in spot size of the wing phenotype in three satyrine butterflies of the genus *Mycalesis*, the Bush Browns, over a two-year sampling period.

Waterhouse and Lyell (1914) and Common and Waterhouse (1981) described the wet- and dry-season forms of *Mycalesis perseus* and *M. terminus* in northern Australia. The wet-season form *M. perseus* (sometimes referred to as form *infuscata*) has large prominent ocelli and a more distinct white transverse line across the wings, whereas the dry season form has reduced ocelli and tends to be larger in size. In *M. terminus* the dry season form (sometimes referred to as form *tira*) also has reduced ocelli and appears to be less abundant than the wet-season form. In both *M. perseus* and *M. terminus* the seasonal forms are similar between sexes. Seasonal variation has not been described in the third Australian member of *Mycalesis, M. sirius* (Fabricius), though Common and Waterhouse (1981) note that there is some variation in ocelli size and number.

Only fragmentary information is available concerning the temporal incidence of the seasonal forms of *Mycalesis*. Waterhouse and Lyell (1914) commented that there was a gradual transition between the two forms of *M. perseus*, and that the wet- and dry-season forms of *M. terminus* were not strictly confined to their respective seasons. However, since the descriptions were based on specimens collected (i.e. pooled) over locations and years, these data do not exclude the possibility that transitions are in fact rapid, and may vary in timing between locations and years. More recently, Moore (1985) established at Townsville that populations of *M. terminus* (both sexes) gradually shifted from individuals with small ocelli in November to individuals with larger ocelli in March, whereas in *M. perseus* he found that the phenotypic change was similar but far more rapid and dramatic, shifting from a distinct dry-season form in November to a wet-season form in January.

The main aims of this study are therefore to: (1) quantify in more detail the precise seasonal change in hindwing phenotype (spot size) of all three *Mycalesis* spp., (2) compare and contrast the extent of seasonal variation between the species, (3) relate the incidence of seasonal polyphenism of each species to their behaviour and reproductive biology in the field, and to suggest possible factor(s) inducing the phenotypic change.

5.2 Materials and methods

The butterflies analysed for phenotypic variation were based on collections undertaken at Cardwell (18°16'S), north-eastern Australia, during 1989-90 as part of a two-year sampling programme designed mainly to assess the reproductive biology of the three *Mycalesis* species. The climate and sampling procedure are accordingly described in detail elsewhere (Chapter 4). Female butterflies were collected about every 2-3 weeks during the first year (starting in April) and thereafter every 4-6 weeks. The total number of specimens collected and examined for each species was 340 (*M. perseus*), 294 (*M. terminus*) and 341 (*M. sirius*). For *M. perseus* sample sizes for each collecting occasion ranged from 5 to 16 with a mean of 12.2, for *M. terminus* from 3 to 16 with a mean of 10.6, and for *M. sirius* from 6 to 18 with a mean of 13.0. All specimens are lodged in the Australian National Insect Collection, CSIRO, Canberra.

The primary character chosen for quantitative analysis in this study concerned the eyespot diameter, and the basic underside wing pattern of the Australian *Mycalesis* is illustrated in Figure 5.1. There are 13 eyespots, six on the forewing and seven on the hindwing, but there is some variation in spot frequency within and between species (Kirk 1982). In *M. perseus* there are usually only four ocelli on the forewing, spot 1 is generally absent while spot 6 is always absent, but all seven eyespots are present on the hindwing. In *M. terminus* there are five ocelli on the forewing, spot 6 is absent, and six on the hindwing, spot 13 being absent. The number of ocelli in *M. sirius* is variable, the forewing has four (with spots 1 and 6 missing), five (with spots 1 or 6 missing) or all six, while the hindwing has six (with spot 13 missing) or all seven. Measurements were made on four of these characters: ocellus 2 between veins M_1 and M_2 on the forewing, ocellus 5 between veins CuA_1 and CuA_2 on the forewing, ocellus 7 between veins R_5 and M_1 on the hindwing, and ocellus 11 between veins CuA_1 and CuA_2 on the hindwing. The eyespots are not perfectly circular but somewhat ellipsoid so the diameter of each was measured parallel to the veins of the wing. All measurements were made on the left fore- and hindwings, unless either wing was damaged in which case the right wings were used, with a dissecting microscope fitted with a calibrated eyepiece micrometer (0.1 mm accuracy).

Wing length, from the thorax centre to apex of the right forewing, was also measured for each specimen using vernier callipers (0.1 mm accuracy). Spot size did not vary with body size (wing length) within seasons so the data are presented in absolute terms.

In March 1989 and May 1990 several wet-season form *M. perseus* females were held in captivity in an outdoor flight cage at Townsville to obtain eggs for rearing experiments. The eggs were collected 1-2 days after being laid and were transferred to an environmental cabinet at constant temperature of 25°C and light regime of 12L:12D. The larvae were reared to adults on Guinea grass *Panicum maximum* according to the procedure outlined in Chapter 6 and

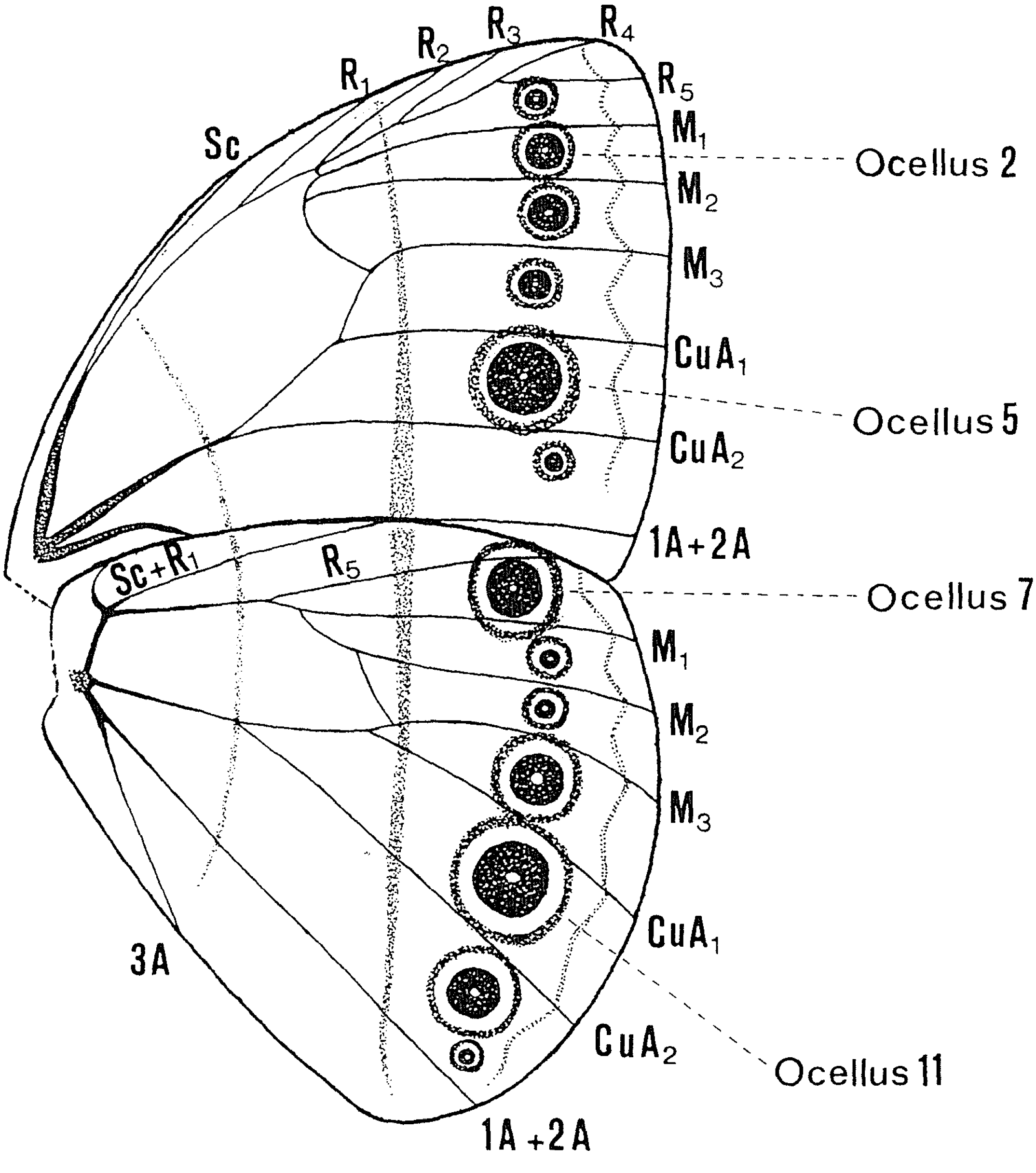

Fig. 5.1 Underside pattern of the left wings of *Mycalesis* (wet-season form) showing the four eyespots characters measured.

the phenotype of each specimen was noted.

5.3 Results

5.3.1 Preliminary observations

Preliminary examination of the wings of all specimens revealed that most of the morphological variation concerned the relative size of the ocelli on the wing underside, with specimens from the dry season generally having substantially reduced eyespots compared with adults taken during the wet season (Plate 9). The seasonal change appeared more dramatic in *M. perseus* and least pronounced in *M. sirius*. In no species was there any obvious seasonal change in wing shape, though in *M. perseus* the termen of both wings was a little more rounded in wet-season forms and the tornus in dry-season forms tended to be slightly more produced. The ground colour of the wing underside was slightly variable, particularly in *M. perseus* dry-season forms (which varied from purplish-brown, dark brown to light brown), but otherwise this character showed little consistent seasonal change. In all three satyrines the terminal and subterminal lines (i.e. between the wing termen and ocelli) on the hindwing underside were generally less distinct in dry-season forms, particularly in *M. perseus* which lost the lines completely. The white postmedian band (stretching between the costa and tornus) was also less prominent in *M. perseus* dry-season morphs. *M. terminus* and *M. sirius* showed a slight reduction in the size of the ocelli on the wings above during the dry season, but in general there was no pronounced morphological change in colour or marking pattern on the wing upperside in all species.

5.3.2 Spot size

Numerical changes in spot size for the four ocelli wing underside characters for the three species are presented in Figures 5.2-5.4. Within species all four characters fluctuated seasonally and simultaneously, and the pattern of seasonal

Plate 9 The three species of *Mycalesis* in Australia: *perseus* (top row), *terminus* (middle) and *sirius* (bottom row). Shown are representative examples of the wet-season form (left column), intermediate forms (middle) and the dry-season form (right column). All specimens are females. Scale = 20 mm.

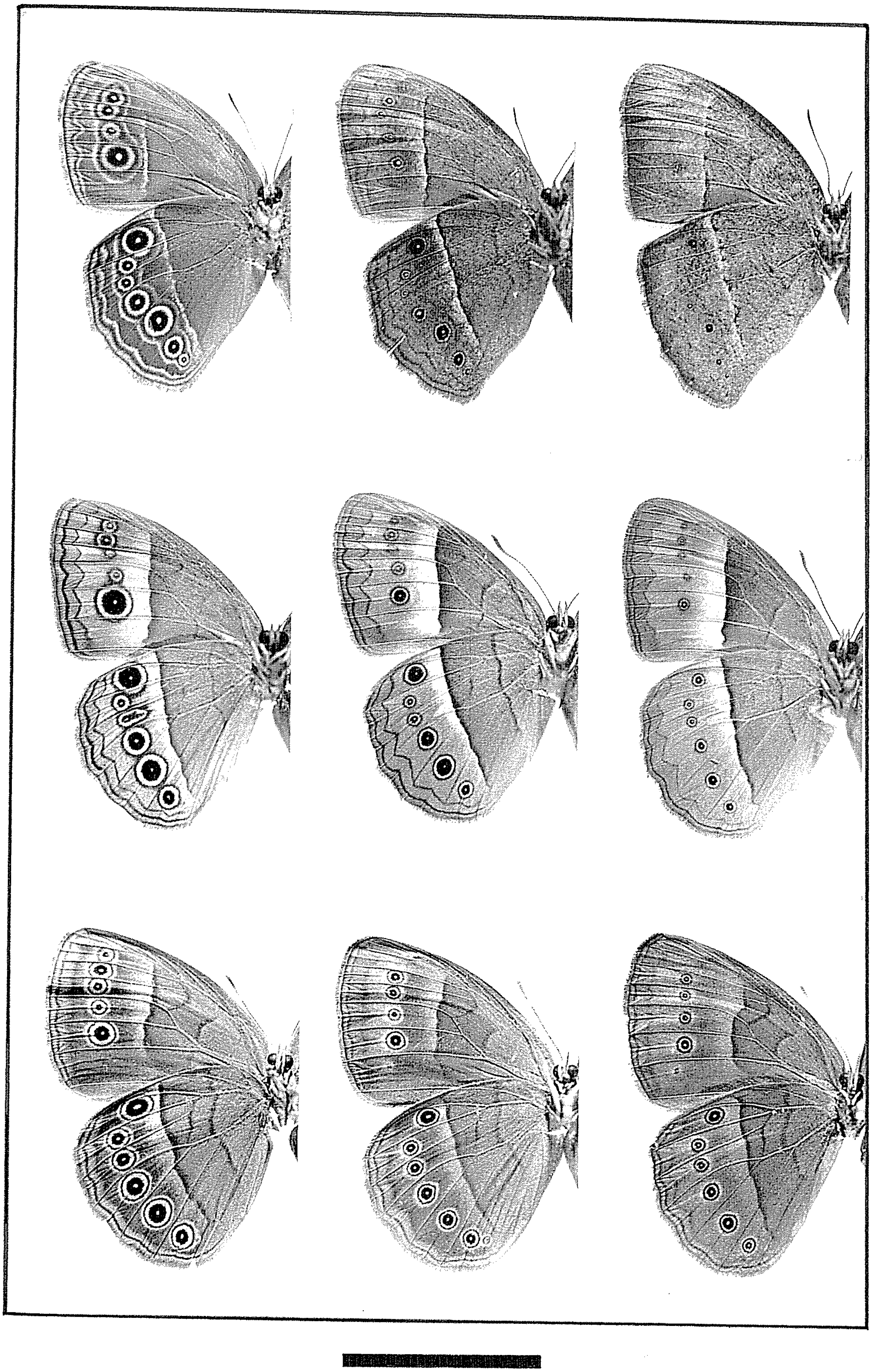

Plate 9

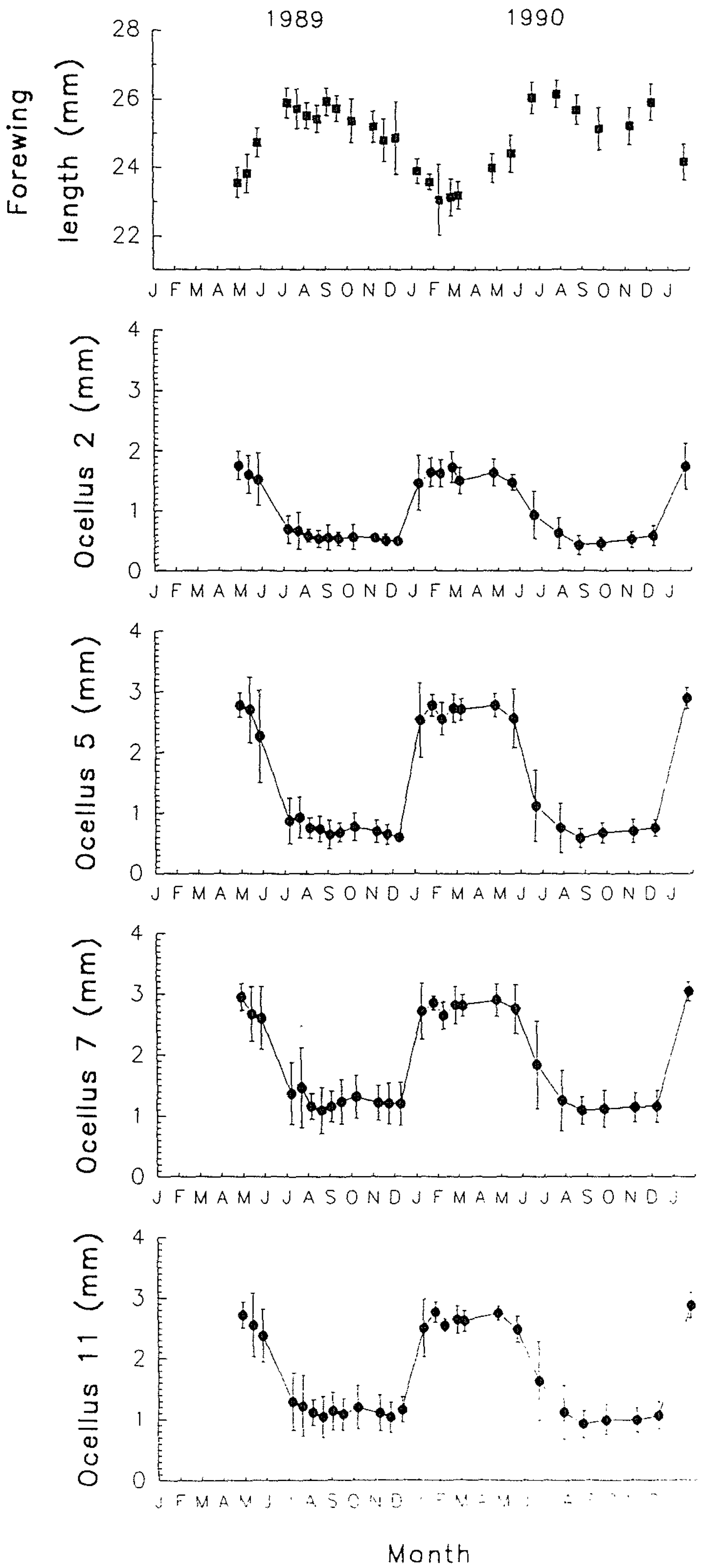

Fig. 5.2 Seasonal variation in forewing length (mean ± 2 S.E.) and eyespot diameter for ocelli 2, 5, 7, 11 (mean ± s.d.) in adult female *Mycalesis perseus* collected over a two-year period at Cardwell.

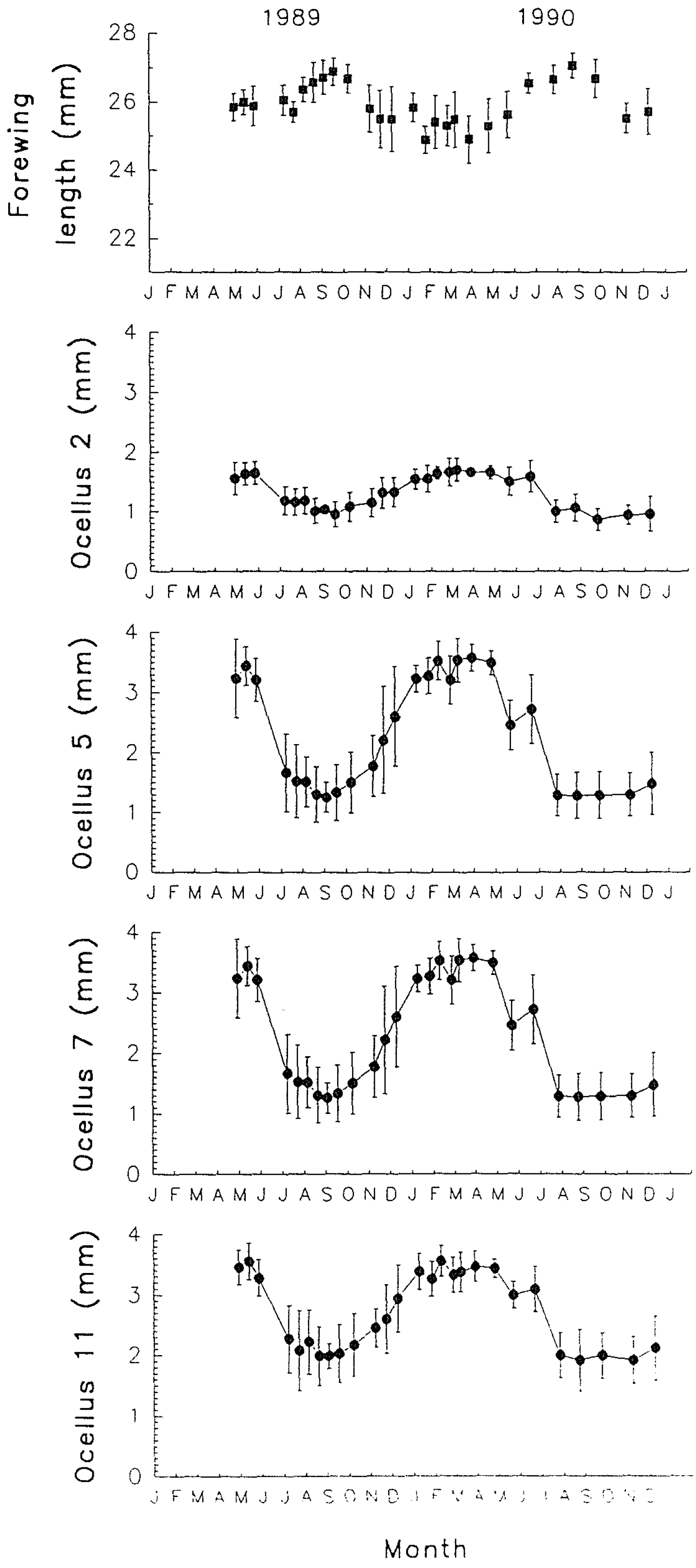

Fig. 5.3 Seasonal variation in forewing length (mean ± 2 S.E.) and eyespot diameter for ocelli 2, 5, 7, 11 (mean ± s.d.) in adult female *Mycalesis terminus* collected over a two-year period at Cardwell.

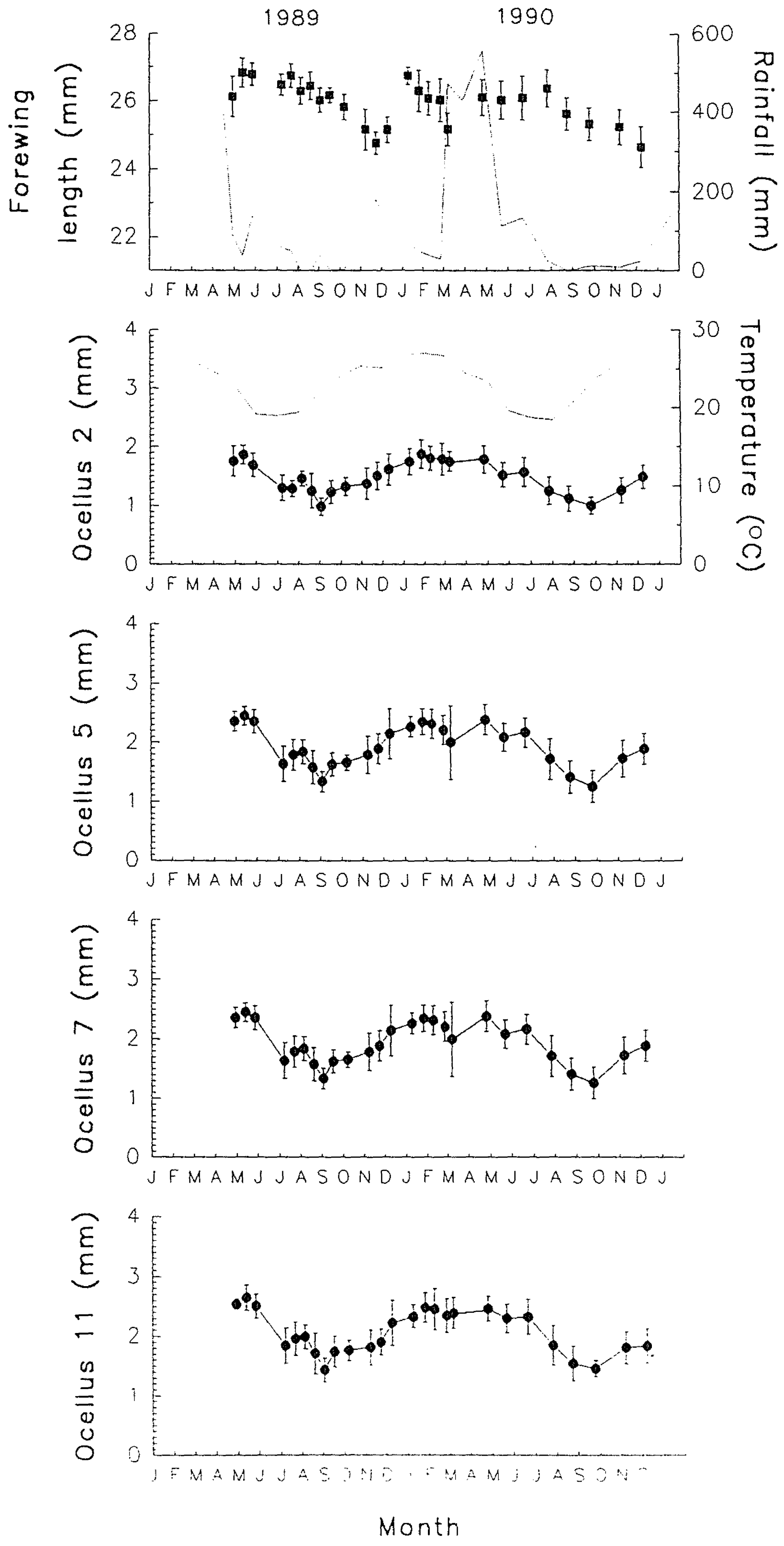

Fig. 5.4 Seasonal variation in forewing length (mean ± 2 S.E.) and eyespot diameter for ocelli 2, 5, 7, 11 (mean ± s.d.) in adult female *Mycalesis sirius* collected over a two-year period at Cardwell. Rainfall and mean monthly temperature values are also shown.

variation was remarkably similar between the three *Mycalesis* spp. Eyespots were largest from January to April during the hot wet season, and smallest in August-September during the cooler dry season. However, there were some differences between species. The amplitude of size variation was far greater in *M. perseus* and *M. terminus* than in *M. sirius*, which showed only slight seasonal variation in spot size (the mean difference between the largest and smallest was of the order of about 1 mm for each ocellus).

The species also differed in the rate of change between seasons, although the transition period from large eyespots to small eyespots was the same for each. In general, seasonal variation in spot size in *M. terminus* (Fig. 5.3) and *M. sirius* (Fig. 5.4) was, with one small exception, gradual and consistent between years. The exception occurred in *M. terminus* during the latter part of 1990 when spot size showed negligible increase during the late dry season compared with the previous year. By contrast, in *M. perseus* the smaller spots were prevalent for a longer period during the dry season (July-December), and the transition from small to large was far more rapid (Fig. 5.2). The increase in spot size occurred in December-January in both years and corresponded with an overall change in phenotype from the dry-season morph to the wet-season morph. The decrease in spot size in *M. perseus*, however, occurred at the same time as in *M. terminus* and *M. sirius* (i.e. May-June), and involved presence of 'intermediate' morphs (Plate 9). These intermediate phenotypes occurred in very low frequency; only 15 (4.4%) individuals were sampled during the May-June transition period in the two years of the study, and they were not detected when spot size increased again later in the season.

The seasonal and consistent change in spot size in *M. terminus* and *M. sirius* suggest these characters are under strong environmental control (e.g. temperature and/or photoperiod). Indeed, correlation analyses between these climatic variables and mean spot size (ocellus 7) 2-3 weeks before collection of specimens revealed that both mean temperature and photoperiod were significantly correlated with mean spot size (Table 5.1). Limited laboratory data

Table 5.1 Correlations between mean spot size (ocellus 7) and ambient temperature and photoperiod 2-3 weeks before capture for female *Mycalesis* at Cardwell. For *M. perseus*, samples collected during the turnover period from wet-season to dry-season morph (April to July) are analysed only.

Species		Temperature	Photoperiod
M. perseus	*r*	0.917	0.973
	d.f.	10	10
	P	<0.001	<0.001
M. terminus	*r*	0.845	0.717
	d.f.	25	25
	P	<0.001	<0.001
M. sirius	*r*	0.778	0.665
	d.f.	24	24
	P	<0.001	<0.001

on *M. terminus* also indicate that spot increases with higher rearing temperature in males but less so for females (Table 5.2).

Similar correlation analyses were performed for *M. perseus* but only at the transition period from wet-season form to dry-season form (Table 5.1). The reduction in spot size from April to July correlated with both temperature and photoperiod. Of 68 adults reared in captivity from eggs laid in May, 13 (19.1%) were of the dry-season form, whereas all adults reared from eggs laid earlier in the season in March were of the wet-season form (Table 5.3). The incidence of dry-season morphs in this late autumn sample suggest that climatic conditions experienced during the embryonic stage may play a role in determining adult morphology.

Table 5.2 Effect of developmental temperature on eyespot size in *M. terminus* (modified from Moore 1985). Individuals were reared from egg to adult on *Panicum* at 12 hr photoperiod. Percentages given in parentheses.

Temp. (°C)	Eyespot size small	 intermediate	 large	N
Males				
21	1 (20.0)	4 (80.0)	0	5
25	0	1 (12.5)	7 (87.5)	8
28	0	0	37 (100)	37
Females				
21	0	2 (66.7)	1 (33.3)	3
25	0	1 (10.0)	9 (90.0)	10
28	0	0	28 (100)	28

Table 5.3 Proportion of wet- and dry-season *M. perseus* adults reared from eggs laid under field conditions at Townsville during March 1989 and May 1990. Larvae were reared on *Panicum maximum* at 25°C and 12L:12D.

Month eggs laid	Sex	Wet-season form	Dry-season form	N
March	Male	27	0	27
	Female	25	0	25
May	Male	32 (91.4%)	3 (8.6%)	35
	Female	23 (69.7%)	10 (30.3%)	33

5.3.3 Body size

All three satyrines exhibited seasonal changes in adult size, measured in terms of forewing length, and this was most pronounced in *M. perseus* (Figs. 5.2-5.4). In *M. terminus* and *M. perseus* the changes were inversely related to spot size, that is, wet-season forms were generally smaller. Adult size in *M. terminus* peaked in August-September, whereas in *M. perseus* adult size peaked over a longer period, from about June to December, corresponding with the longer incidence of dry-season morphs. In contrast, size variation in *M. sirius* was less precisely seasonal and the timing did not correspond to phenotypic shifts (Fig. 5.4). Females tended to be smaller in November-December during the late dry season and somewhat larger in the early dry period, around May-July, but they were also very large in January-February. Adult size in *M. sirius* may be more closely tied to rainfall than temperature. The peaks in adult size recorded during May-July 1989, January-February 1990 and June-July 1990 follow high rainfall periods, while the declines observed in late 1989, March 1990 and late 1990 follow periods of low rainfall. Forewing lengths ranged from 20.3 mm to 27.7 mm (*M. perseus*), 22.9-28.7 mm (*M. terminus*), and 23.3-28.9 mm (*M. sirius*).

5.4 Discussion

5.4.1 Seasonal polyphenism

Adult females of all three Australian *Mycalesis* species show phenotypic variation in the wing underside eyespot elements. The variation is strongly seasonal, smaller ocelli (dry-season forms) occur during the dry cooler winter period and larger ocelli (wet-season forms) occur during the wet hotter summer-autumn. The extent of change is most pronounced in *M. perseus* and *M. terminus*, and considerably less dramatic in *M. sirius*. The seasonal forms in *M. perseus* and *M. terminus* had been noted by earlier workers (Waterhouse and Lyell 1914, Moore 1985) but the extent and timing of seasonal change had not previously been established for sympatric populations over an entire season.

Moreover, seasonal variation in *M. terminus* and *M. sirius* is more gradual so that the wet- and dry-season forms essentially represent extremes of a continuum of variation, whereas in *M. perseus* the forms are more distinct and the seasonal change is in general more abrupt.

Similar patterns of phenotypic change to *Mycalesis* have been reported for several tropical Satyrinae elsewhere (Brakefield 1987, Brakefield and Reitsma 1991), and although the incidence of seasonal forms is well known for many taxa (see Shapiro 1976, Brakefield and Larsen 1984 for examples), few studies have documented the extent and precise nature of the change over several seasons at a single locality. Brakefield (1987) examined 155 specimens of *Melanitis leda* collected by N. Manders on Mauritius from January to December 1905, and Moore (1985) studied phenotypic variation in *Mycalesis perseus* and *M. terminus* at Townsville, north-eastern Australia, over a five month period from the late dry season (November 1984) to the end of the wet season (March 1985). Although Moore did not precisely quantify changes in spot size his results are broadly similar to those obtained at Cardwell; individuals of both species sampled in the late dry season were characterised by small ocelli while those taken during the wet season possessed larger ocelli, and the change over was more rapid in *M. perseus* than in *M. terminus*. More recently Brakefield and Reitsma (1991) studied seasonal polyphenism in *Bicyclus safitza* (Hewitson) and *B. anynana* (Butler) in Malawi, east Africa, over a three month period at the transition between the wet and dry seasons. The seasonal change in phenotype in these two species is very similar to that of Australian *Mycalesis*, particularly *M. perseus*, and the turnover from wet-season to dry-season forms coincided with declining temperatures and a general increase in drying of the habitat, including the host plants (grasses) on which larvae feed.

The factors inducing phenotypic change in tropical butterflies have been partly established for only a few species (McLeod 1968, 1984, Jones *et al.* 1985, Rienks 1985, Brakefield and Reitsma 1991, Windig 1992, Jones 1992). Although phenotypic variation in *Mycalesis* correlated well with temperature, it must be emphasised that under field conditions temperature in most tropical regions is also correlated with many other potential cues, including photoperiod (the annual variation in daylength at Cardwell is 2 hr 8 mins.), humidity, and host plant and microhabitat conditions. McLeod (1968, 1984) concluded that temperature was the only factor involved in the control of adult phenotypic variation in the nymphalid *Precis octavia* in east Africa; low temperatures produced dry-season forms. Brakefield (1987) studied seasonal polyphenism in *Melanitis leda* and made a detailed analysis of 16 climatic variables for all field collected samples; he found that mean daily temperature at 0900 hrs 2 weeks before capture accounted for most (40.7%) of the phenotypic variance. Brakefield and Reitsma (1991) and Windig (1992) showed that different temperatures (or varying developmental time) in final instar larvae of *Bicyclus safitza* induced different seasonal forms. Jones (1992) also discovered that in four *Eurema* spp. (Pieridae) temperature strongly influenced polyphenism in three species, although temperature alone could not account for all the variation observed. Rienks (1985) and Jones *et al.* (1985) have also demonstrated that small changes in photoperiod in conjunction with temperature can affect wing phenotype in two tropical pierids, even for populations at low latitudes (<20°). However, in *Eurema herla* (W.S. Macleay) neither temperature, photoperiod, or humidity affects seasonal polyphenism (including both the darker markings and pink ground colour of the wing underside - characters typical of the dry-season form) (Jones 1992).

Owen (1971) and Jones (1987, 1992) suggested that dry-season forms in several nymphalid and pierid butterflies may be a mechanism to enhance survival through crypsis as the savanna environments they occupy dry off and change colour. Brakefield and co-workers (Brakefield and Larsen 1984, Brakefield 1987, Brakefield and Reitsma 1991) have developed this hypothesis further for the tropical Satyrinae and Nymphalinae, and proposed that the alternative phenotypes (i.e. wet- and dry-season forms) represent responses to seasonal differences in environmental conditions and selective pressures such as the nature of predation. The wet-season form is thought to rely on anti-predator devices (prominent eyespot patterns) which are displayed at rest and function principally in the deflection of attacks from vertebrate predators such as lizards. In contrast, dry-season forms have reduced or no eyespots and probably rely wholly on crypsis for survival.

However, very little information on the reproductive phenology and seasonal behaviour of the forms of these species is available. In two satyrines, *Melanitis leda* and *Bicyclus safitza*, the dry-season forms seek moist refugia, remain relatively inactive for long periods, and females may stop breeding during the unfavourable dry period when host plants (grasses) deteriorate, whereas wet-season forms are probably more mobile and reproductively active when breeding conditions are generally more favourable (Brakefield 1987, Brakefield and Reitsma 1991). In two pierid butterflies (*Eurema herla*, *E. laeta*), Jones (1987, 1992) has also observed that seasonal polyphenism is linked with seasonal changes in breeding status and aggregation behaviour, which in turn are associated with changes in habitat favourability.

The same pattern is also found in the three *Mycalesis* species for which the breeding phenology and reproductive seasonality are well understood (Chapter 4). Both *M. terminus* and *M. sirius* breed for most of the year, egg production declines as the dry season progresses and only late in the season, around September-November, do females (all dry-season form) enter reproductive dormancy. The extent of reproductive arrest appears to be slightly more pronounced in *M. terminus* than *M. sirius*, which also shows greater tendency to aggregate in moist refugia (*M. terminus* females typically rest amongst dry leaf litter in creek beds supporting dense gallery rainforest). By contrast, breeding in *M. perseus* is confined largely to the short wet season, and during the long dry season females (dry-season form) retreat to moist refugia and remain in reproductive diapause, from about July to December at Cardwell depending on the season. During the dry period *M. perseus* adults often form relatively large aggregations and rest amongst dead leaves on the ground in dry creek beds and gullies in the savanna.

Hence, the incidence of dry-season forms and the timing of phenotypic change in *Mycalesis* spp. are well synchronised with changes in female reproductive behaviour. Moreover, differences in the degree of phenotypic variation within *Mycalesis* correspond with differences in the extent of aggregation behaviour, implying there is greater selection pressure on *M. perseus* and weaker selection on *M. sirius* to produce a dry-season morph. Such phenotypic plasticity (i.e. the ability to produce different phenotypes in response to variable environmental conditions) in these butterflies must have a substantial influence on fitness (Bradshaw 1965, Stearns 1989). If Brakefield and Larsen's (1984) adaptive hypothesis is correct, then seasonal polyphenism in *Mycalesis* may be a dry season strategy to enhance survival (i.e. minimise predation through crypsis) during the unfavourable period when adults aggregate in refugia and remain reproductively dormant: it should not be viewed as an alternative strategy for escape in time or space.

5.4.2 Body size

There are also pronounced changes in body size (wing length), which in *M. perseus* and *M. terminus*, are seasonal and associated with other phenotypic shifts. In these two species winter-caught females (dry-season forms) were much larger than summer-caught females (wet-season forms), particularly in the former species. Similar findings have been reported for the satyrines *Melanitis leda* (Brakefield 1987) and two *Bicyclus* spp. (Brakefield and Reitsma 1991) and in four of the five *Eurema* spp. (Jones 1992) in which adults of the dry-season form tend to be larger than those of the wet-season form. In most of the pierids, the seasonal switch in body size among field populations is consistent with laboratory studies: larger adults arise when larvae are reared at lower temperatures, and in at least two species (*Eurema laeta, E. herla*) shorter photoperiods also produce larger size (Jones 1992). In most (70%) of the above species the larger sized dry-season forms diapause during the dry-season (often for several months or more). There may therefore be a number of advantages associated with this increase in size of diapausing individuals: longevity may be greater so that life expectancy is increased during the unfavourable period, or the capacity for accumulation and storage of fat body is enhanced.

By contrast size variation in *M. sirius* was not related to seasonal changes in adult phenotype, but appeared to be more influenced by variation in the rainfall pattern, with progressively smaller sizes being recorded during the long dry spells. The most likely explanation for this is that *M. sirius* larvae suffer from severe food limitation or poor host quality so that large adult size cannot be attained. Jones (1992) has suggested a similar scenario for *Eurema hecabe* in which body size declines as the dry season progresses; larval overcrowding and resource depletion at high densities possibly prevent the potential size increase during the cooler dry season.

CHAPTER 6: DEVELOPMENTAL BIOLOGY

6.1 Introduction

In this chapter I examine the survival rates, developmental times and sizes of the three *Mycalesis* spp. under laboratory conditions. Developmental time and adult size at maturity are two crucial components of a species' life history strategy. Covariation of these two traits may occur: for example, selection favouring an increase in developmental rate may be accompanied by a corresponding decrease in adult size because not all traits can be maximised simultaneously (Roff 1981, Sibly *et al.* 1985, Stearns and Koella 1986, Smith *et al.* 1987, Forrest 1987).

The geographic distributions and habitat requirements of each species were described and compared in Chapters 2 and 3. Although the three taxa overlap in some areas of north-eastern Queensland, the habitat preferences and distributions of each are quite distinct. *M. sirius* favours the swampy paperbark woodlands and reaches its southern distributional limit near Rollingstone (approx. 65 km NW of Townsville), although a small disjunct population appears to have formerly occurred further south at Mackay, N. Qld. (Chapter 2). *M. perseus* and *M. terminus* occur primarily in open forest/woodland and rainforest edge respectively. They extend considerably further in range and have similar southern limits, the Kingaroy-Imbil district and the Bundaberg-Childers district respectively, in south-eastern Qld. (Common and Waterhouse 1981, cf. Dunn and Dunn 1991, P. Fox, pers. comm. 1991, Chapter 2). Table 6.1 gives the mean monthly temperature profiles at the localities close to the southern limits of each.

Laboratory reared females of *M. perseus* and *M. terminus* are larger than males (Moore 1985), and field caught females of all species are somewhat the larger sex though there is considerable size variation, some of which is seasonal (Chapter 5). In common with many other Australian Satyrinae the life cycles

Table 6.1 Mean monthly temperatures at selected sites close to the edge of the geographic range of the three satyrine species studied (source, Bureau of Meterology 1988a).

Site	Latitude		J	F	M	A	M	J	J	A	S	O	N	D	Year
Townsville	19°15'	mean	27.6	27.3	26.3	24.7	22.3	20.1	19.0	20.5	22.4	24.9	26.7	27.4	24.1
(*M. sirius*)		max.	31.3	30.8	30.1	29.4	27.3	25.5	24.9	26.0	27.5	29.3	30.7	31.2	
		min.	23.8	23.7	22.4	20.0	17.3	14.7	13.1	15.0	17.2	20.5	22.7	23.6	
Childers	25°14'	mean	25.5	25.0	24.2	22.2	19.2	16.9	16.0	17.7	19.5	22.1	24.0	24.7	21.4
(*M. terminus*)		max.	30.5	29.6	29.2	27.4	24.3	22.1	21.6	23.5	25.4	27.8	29.7	30.0	
		min.	20.4	20.4	19.2	16.9	14.0	11.7	10.3	11.8	13.5	16.3	18.2	19.4	
Imbil	26°28'	mean	24.9	24.5	23.5	21.0	17.8	15.2	14.1	15.8	17.8	20.8	23.3	23.9	20.3
(*M. perseus*)		max.	30.5	29.6	29.3	27.5	24.3	21.9	21.3	23.3	25.4	27.7	30.2	30.0	
		min.	19.3	19.3	17.7	14.5	11.3	8.5	6.9	8.3	10.2	13.9	16.3	17.8	

and general biology of the three *Mycalesis* spp. are very poorly documented, and there is little reliable information concerning larval host plant preferences (see Chapter 2). Manski (1960) and Common and Waterhouse (1981) stated that larvae of all three species feed on *Imperata* and other coarse grasses. Females oviposit on a range of grasses (Poaceae), including native Kangaroo grass, *Themeda triandra* Forrskal, and introduced Guinea grass, *Panicum maximum* Jacq. (Moore 1986, Valentine 1988, Chapter 2). These two hostplants are widespread and occur abundantly where the butterflies frequent. *T. triandra* typically occurs in the drier open woodland/forest areas, while *P. maximum* forms larger tussocks that have invaded many of the moister gullies and creeks. Waterhouse (1932) gave the pupal duration for *M. terminus* at Cairns, N. Qld, as lasting 12 days in April-May. Unlike many of their univoltine temperate counterparts (Edwards 1973, Fisher 1978, Pearse 1978, Braby and New 1988a, 1994) populations of *Mycalesis* are presumed to be multivoltine with shorter generations of the order of several weeks, as are several other Australian tropical satyrines (De Baar 1981, Wood 1984, 1988).

I asked three questions with respect to the ecology of these three tropical species. (1) Do they respond similarly to temperature variation? To investigate this the survival and developmental rates of eggs at different temperatures were examined. (2) Does the species of grass on which they are reared affect their performance in terms of three fitness components: survival, rate of larval development and pupal weight attained? (3) Is the developmental biology of these species related to their distribution and preferred habitats, and how does it compare with temperate satyrines from the more southern regions?

6.2 Materials and methods

The three butterfly species were collected as adult wet-season form females from several coastal populations in north-eastern Queensland during 1989-1992. *M. sirius* was obtained from Cardwell (18°16'S, 146°02'E) and Rollingstone (19°03'S, 146°23'E), while both *M. perseus* and *M. terminus* were collected from Rollingstone and Townsville (19°15'S, 146°45'E). The females were transported to the university campus on the day of capture and held in captivity to obtain eggs for the two developmental experiments described below. For *M. perseus* the females were transferred to 0.7 X 0.7 X 0.7 m flight cages which were kept in an outdoor shade house. The flight cages were supplied with potted tussocks of *P. maximum* on which the butterflies were allowed to lay eggs. Adults were supplied intermittently with rotting fruit as a food source. The cages were watered daily. *M. terminus* and *M. sirius* females were kept individually in small plastic cages (100 mm diam. X 100 mm height) lined with moistened filter paper and fitted with muslin cloth over the roof. Rotting fruit was supplied as an adult food source and freshly cut blades of soft grass were provided as an oviposition substrate. The fruit and grass blades were changed every 1-2 days. The plastic cages were kept at 25°C ± 0.5°C and a 12:12 L:D cycle. All cages containing females were checked twice each day.

6.2.1 Temperature

Egg developmental rates and survival were examined at seven temperatures, *viz.* 17, 20, 23, 26, 30, 32, 35°C ± 0.5°C. The three *Mycalesis* spp. usually oviposit in the late afternoon and at the end of each day (i.e. within 4-5 hours of the first eggs being laid) eggs were carefully removed from the grass blade with a fine pair of tweezers, placed into plastic petri dishes with filter paper and then immediately transferred to one of the seven temperature cabinets (Labec incubator). Humidity was kept high by placing dishes of water on the bottom of each cabinet. The incubators were fitted with light sources which were kept on throughout the experiment. At least 25 eggs of each species were reared at each temperature; normally, 40 eggs were used, but at temperatures where mortality (as indicated by shrivelled chorion and failure of larvae to hatch) was high, more were reared. The eggs were monitored every 12 h until hatching.

6.2.2 Hostplants

The effects of two hostplant species, native Kangaroo grass, *Themeda triandra*, and introduced Guinea grass, *Panicum maximum*, on larval survival, developmental time and pupal weight were studied at 25°C ± 0.5°C and a 12:12 L:D cycle. Females of each butterfly species were kept in outdoor 0.7 X 0.7 X 0.7 flight cages, as described above for *M. perseus*. Eggs were collected each morning, before 900 hrs, weighed and transferred individually to petri dishes, then checked daily until hatching. On eclosion the newly emerged larvae were transferred with a fine brush to freshly cut strips of the hostplant and reared individually in the petri dish with moistened filter paper until pupation. Only soft growth of the two grass species were tested and compared in this study because larval survival was found to be very poor on tougher leaf material, particularly of *P. maximum* (Chapter 8). Larvae were checked every 24 h for condition and for presence of discarded head capsule as an indication of instar stage. The leaf strips and filter paper were changed every 1-3 days. Pupae were weighed 24 hrs after pupation on a Cahn 21 automatic electrobalance (0.1 mg accuracy). On eclosion the newly emerged adults were sexed and the length of the right forewing (from centre of thorax to apex) was measured using callipers (0.1 mm accuracy). Some additional body size measurements were made on *M. perseus* from other rearing trials.

6.2.3 Statistical analyses

To describe and compare the relationship between egg development rate (D) [measured as 1/(developmental time in days)] and temperature (T) for the three satyrines the linear regression model $D=bT+a$ (where b represents the slope and a the D-intercept) was fitted to the data. The 35°C data for *M. perseus*, and the 32°C and 35°C data for *M. terminus* and *M. sirius* were excluded from the regression calculations because these temperatures were outside the linear range. The linear model allows estimation of the threshold temperature or developmental zero T_0 (i.e. $T_0=-a/b$, the temperature below which the regression predicts that no development will occur) and the physiological time or thermal constant K (i.e. $K=1/b$, which predicts the number of degree-days above T_0 required to complete development) (Jones *et al.* 1987). Standard errors of T_0 and K were calculated as described by Campbell *et al.* (1974).

Several non-linear models which describe the relationship between insect development rate and temperature have been developed (see reviews by Wagner *et al.* 1984, Rae and De'ath 1991). Although these give more accurate estimates of developmental times at extreme temperatures, where departures from linearity are often observed, the linear model is preferred in this study because it provides a simple, straight-forward comparison of the development patterns between these closely related species if it is assumed that field temperatures normally lie within the more linear portion of the development rate function (Worner 1992).

The influence of HOSTPLANT species (2 levels), butterfly SPECIES (3 levels) and SEX (2 levels) on larval developmental time, pupal weight and pupal developmental time were analysed with three-way analyses of variance using the generalised linear models procedure (SAS Institute Inc. 1988). Initial inspection of the data suggested that males and females of each species behaved very differently in terms of the parameters measured in this study so SEX was therefore included in the model as an independent factor.

6.3 Results

6.3.1 Egg developmental rate and survival

The effect of temperature on rate of egg development for the three butterfly species is illustrated in Figure 6.1. Table 6.2 summarises the regression statistics, temperature thresholds and physiological times. *M. perseus* eggs developed substantially faster than *M. terminus* or *M. sirius* at the temperatures examined. Development was slowest *erminus* but this was not significantly different from *M. sirius* (The mean developmental times

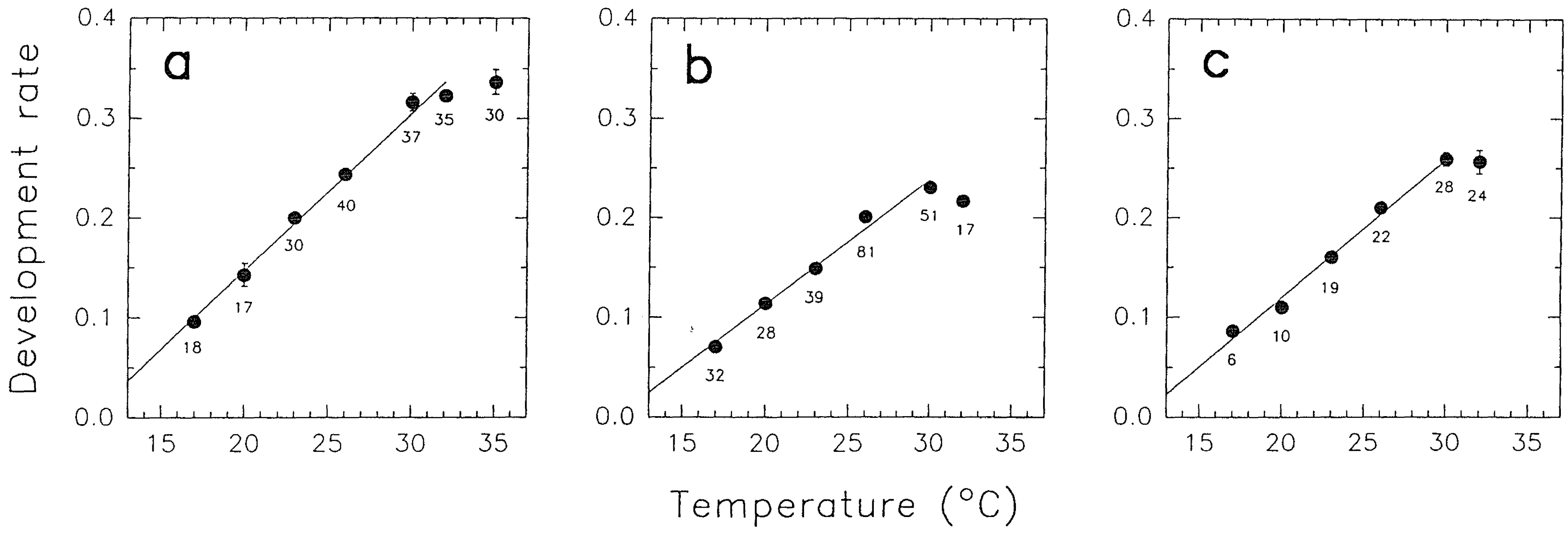

Fig. 6.1 Egg development rates (mean ± S.E.) at a range of temperatures for the three tropical satyrine species: (a) *M. perseus*, (b) *M. terminus*, (c) *M. sirius*. Sample sizes are given under each data point. Regression equations are summarised in Table 1.

Table 6.2 Relationships between egg development rate and temperature for the satyrine species studied, and the temperature thresholds (mean ± S.E.) and physiological times (mean ± S.E.) for egg development. Developmental rates at 35°C for *M. perseus* and at 32°C for *M. sirius* and *M. terminus* were excluded from the linear regression analyses.

Species	N	Slope (*b*)	Intercept (*a*)	r^2	Threshold (°C)	Physiological time (degree-days)
M. perseus	177	0.0158	-0.169	0.991	10.7 ± 0.7	63.3 ± 3.1
M. terminus	231	0.0126	-0.139	0.984	11.0 ± 0.9	79.4 ± 5.8
M. sirius	85	0.0139	-0.158	0.989	11.4 ± 0.8	71.9 ± 4.3

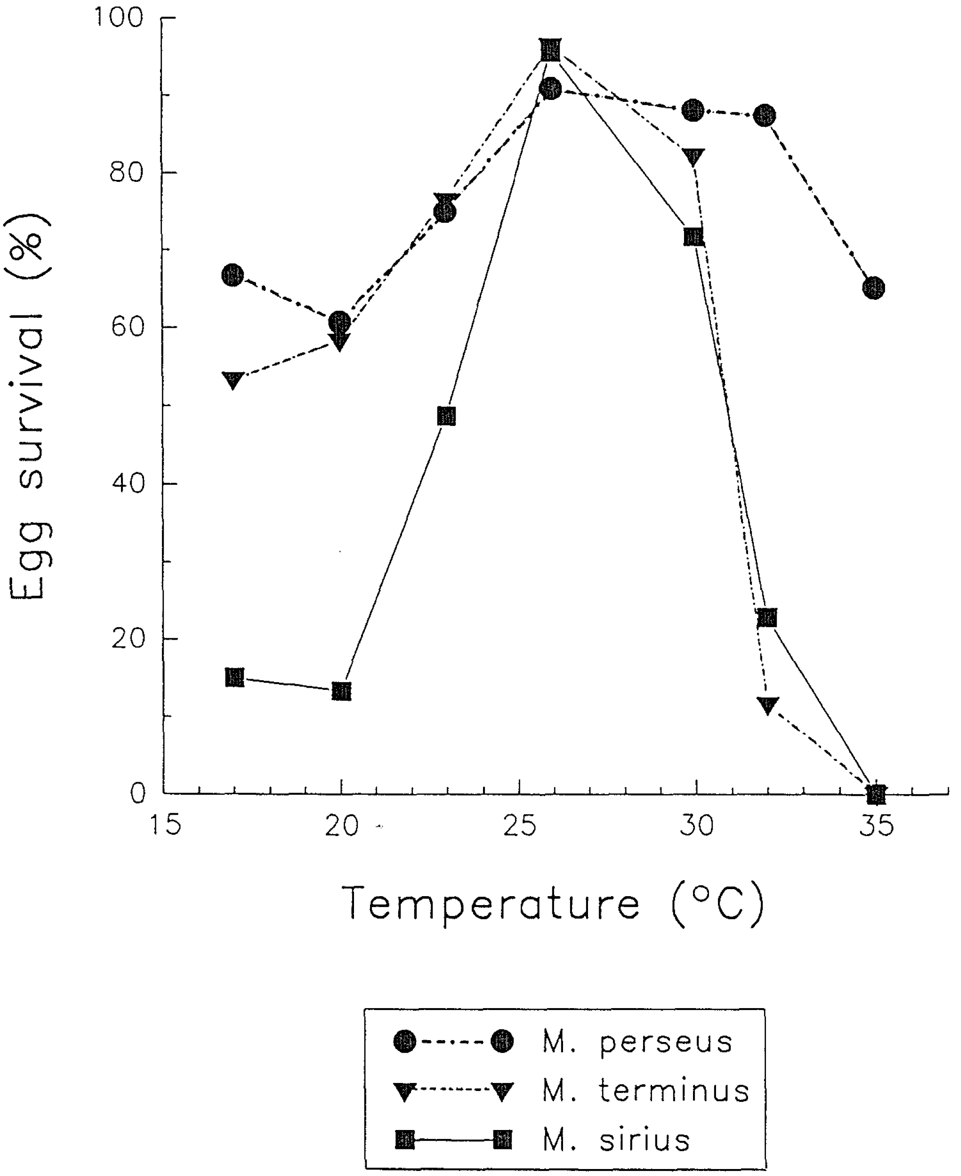

Fig. 6.2 Egg survival at a range of temperatures for the three *Mycalesis* species.

varied from 2.97 ± 0.60 (sd) days at 35°C to 10.39 ± 2.78 days at 17°C for *M. perseus*, and for *M. terminus* from 4.34 ± 0.43 days at 30°C to 14.14 ± 0.65 days at 17°C. In both *M. terminus* and *M. sirius* rate of development was highest at 30°C and at 32°C the rate declined. In contrast, egg development in *M. perseus* reached a maximum at 35°C, although the rate departed from linearity at this temperature (Fig. 6.1a). Estimated temperature thresholds were around 11°C and did not differ significantly between species.

Egg survival varied considerably with temperature, and the effect of temperature extremes varied between species (Fig. 6.2). Survival was highest at 26°C in all three (>90%) and declined at temperatures above and below this value. *M. perseus* showed the broadest tolerance with higher survival rates (>65%) at the extreme temperatures examined, while *M. sirius* exhibited a much narrower tolerance with very few eggs (<25%) surviving at temperatures of 20 and 32°C. In both *M. terminus* and *M. sirius* no eggs survived at 35°C, but at the lower temperatures *M. terminus* survived significantly better, similar to *M. perseus*.

Thus, the three species showed different temperature requirements with respect to optimal egg development and survival. *M. perseus* survived well between 23 and 32°C and developed most rapidly between 30 and 35°C. By comparison, *M. terminus* and *M. sirius* had a much smaller and lower optimal temperature range of around 25-30°C, although *M. terminus* survived well at 23°C.

6.3.2 Larval survial and developmental time

Larvae of all three butterfly species reared in captivity at 25°C survived considerably better to third instar on native *Themeda* than on introduced *Panicum* (*M. perseus* $X^2=27.16$, 1 *d.f.*, $P<0.0001$; *M. terminus* $X^2=20.75$, 1 *d.f.*, $P<0.0001$; *M. sirius* $X^2=19.04$, 1 *d.f.*, $P<0.0001$) (Fig. 6.3). Despite the relatively high survival rates on *Themeda*, larvae on this hostplant developed

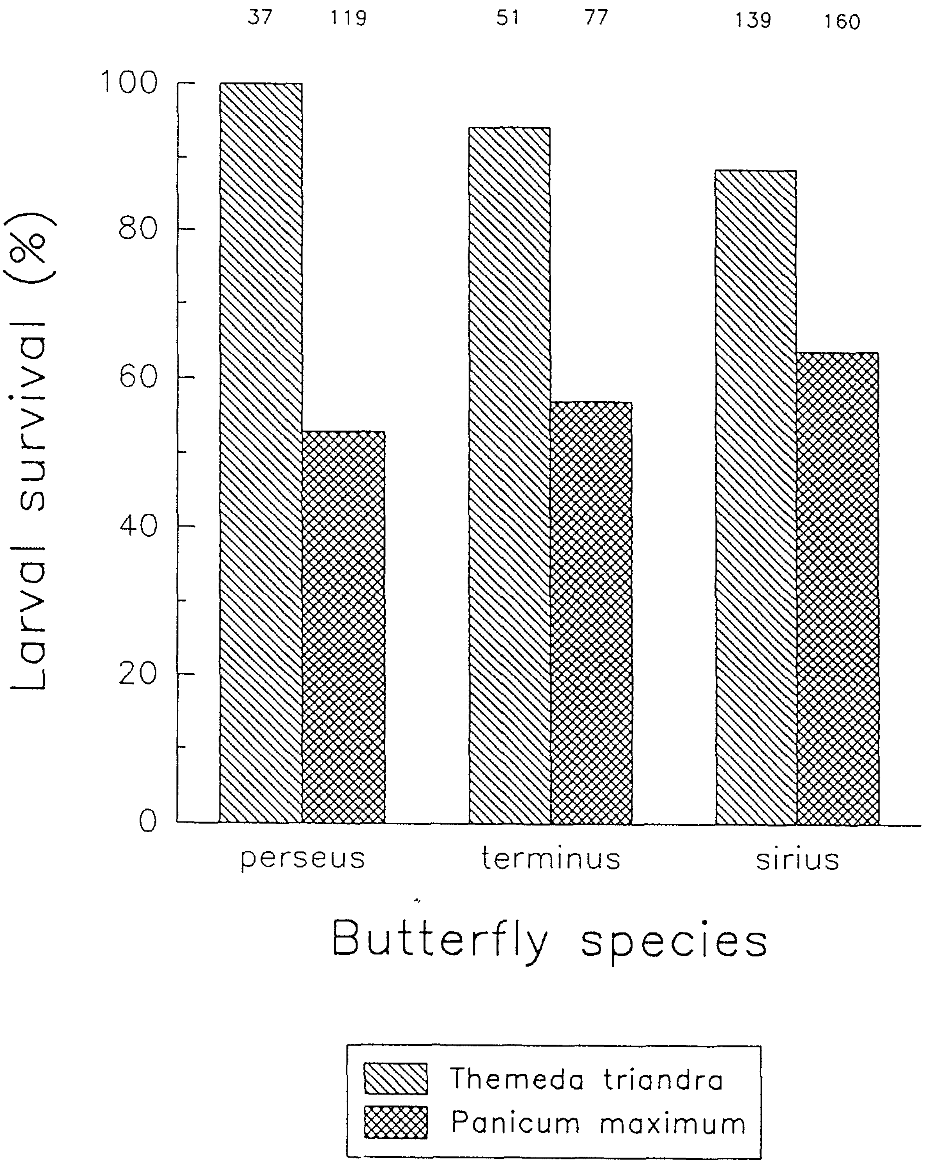

Fig. 6.3 Survival rates of *Mycalesis* larvae reared on young growth of two grass species, introduced *Panicum maximum* and native *Themeda triandra*, at 25°C and 12:12 L:D. Survival was determined by the proportion of larvae which survived to the third instar. Sample sizes given above bars.

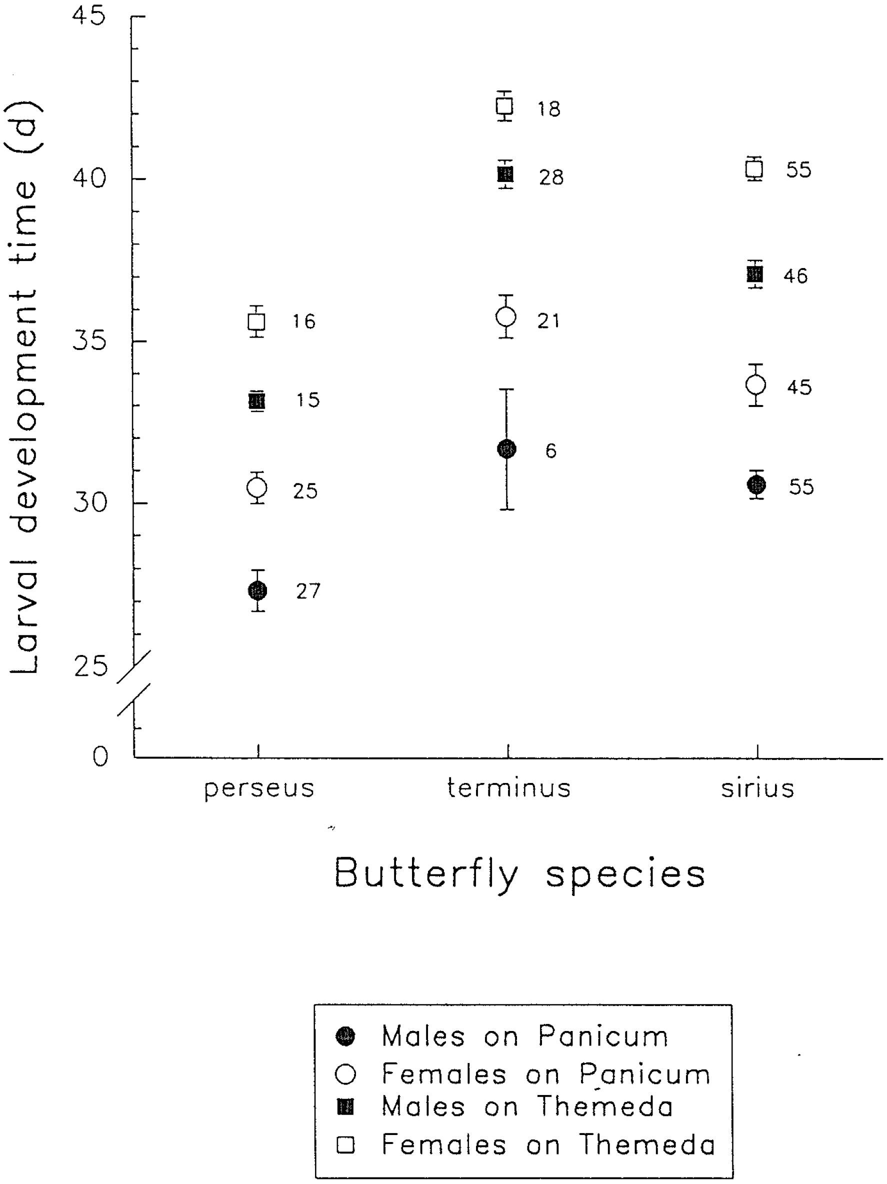

Fig. 6.4 Larval development times (mean ± S.E.) for males and females of each butterfly species reared on *Panicum maximum* and *Themeda triandra* at 25°C. Sample sizes are given next to each data point.

more slowly (Fig. 6.4). A three-way ANOVA examining the effects of HOSTPLANT species, butterfly SPECIES and SEX on larval development time revealed that all three factors were significant, especially HOSTPLANT (Table 6.3). Larvae developed faster on *Panicum* than on *Themeda*; males developed faster than females; and *M. perseus* developed most quickly, while *M. terminus* had the longest developmental time. A significant interaction also occurred between HOSTPLANT*SPECIES: the difference in developmental times on *Themeda* and *Panicum* was greater in *M. terminus* than in the other two species (Fig. 6.4).

Table 6.3 Three-way analysis of variance examining effects of hostplant, butterfly species and sex on larval developmental time (d).

Source of variation	*d.f.*	Larval development time (d)		
		MS	*F*	*P*
HOSTPLANT	1	1893.42	184.09	0.0001
SPECIES	2	534.37	51.95	0.0001
SEX	1	527.09	51.25	0.0001
Hostplant*Species	2	45.71	4.44	0.0124
Hostplant*Sex	2	10.08	0.98	NS
Species*Sex	2	2.29	0.22	NS
Hostplant*Species*Sex	2	8.76	0.85	NS
Error	343	**10.28**		

6.3.3 Instar number

Developmental times of each larval instar, for both sexes of each species reared on *Panicum* (Fig. 6.5), showed that development was generally shortest in instars

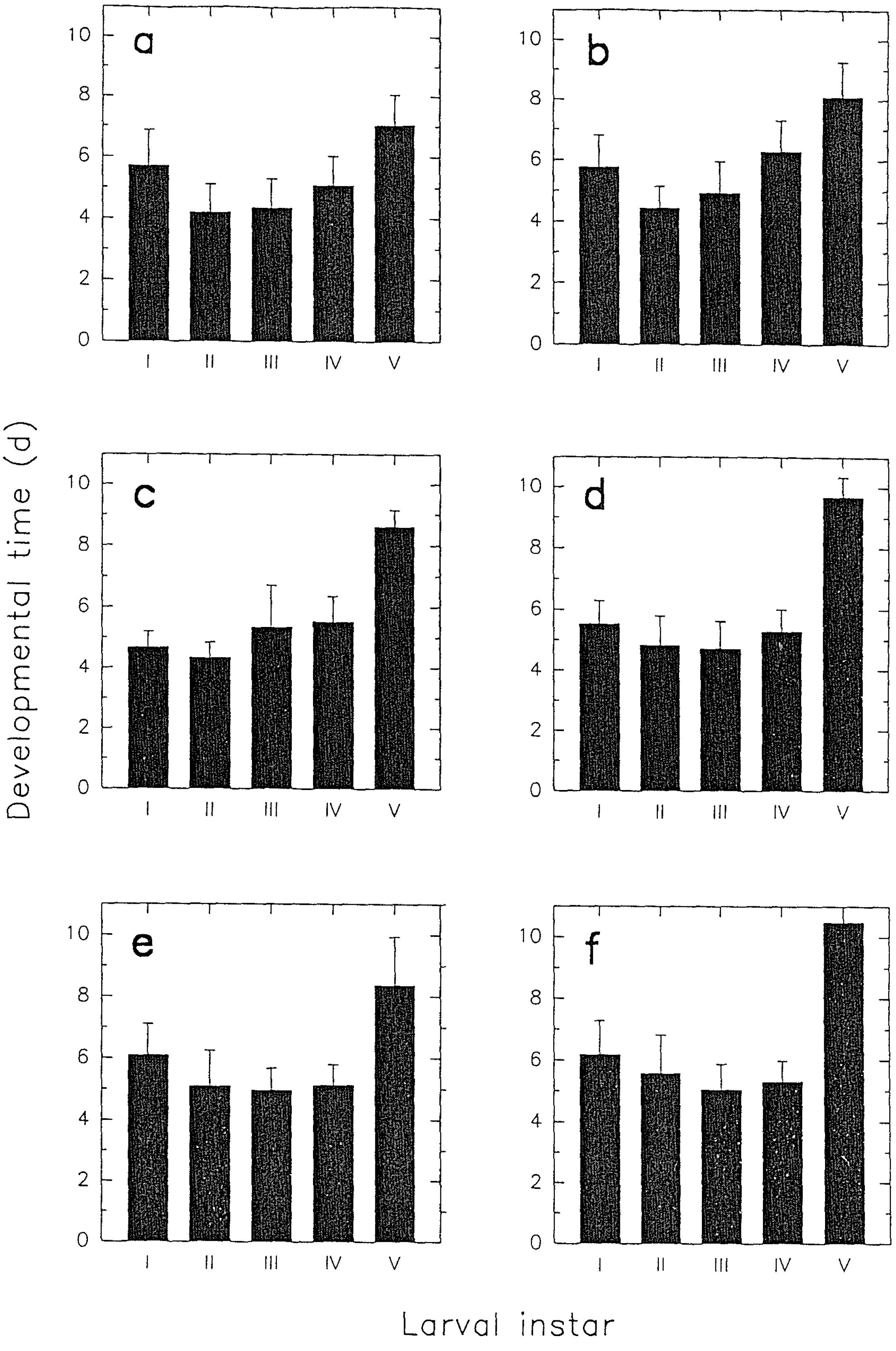

Fig. 6.5 Development times (mean ± sd) for each larval instar for (a) male *M. perseus*, (b) female *M. perseus*, (c) male *M. terminus*, (d) female *M. terminus*, (e) male *M. sirius* and (f) female *M. sirius* reared on *Panicum* at 25°C and 12:12 L:D.

II and III and longest in the final instar. The slower larval development in *M. terminus* and *M. sirius*, compared with *M. perseus*, was mainly attributable to a slightly longer final instar, though the other instars were also usually longer. All *M. perseus* and *M. terminus* larvae completed five instars but a few *M. sirius* larvae (15%) completed six instars. The developmental times of any six instars were pooled with instar five in Figure 6.5.

Comparison of mean total development time and mean pupal weight between the five and six larval instar phenotypes in *M. sirius*, for both males and females (Table 6.4), revealed significant differences in larval time but not in pupal weight. For larvae which completed six instars, larval development averaged 22% longer in both males (t=7.04, 53 *d.f.*, P<0.0001) and females (t=5.34, 43 *d.f.*, P<0.0001) than those larvae which completed only five. Although the mean pupal weights for six instar larvae were slightly higher than those for five instar larvae, by 4% in both sexes, differences in pupal weights between these groups were not significant for either males (t=1.13, P=0.265) or females (t=0.87, P=0.390) (Table 6.4).

6.3.4 Pupal developmental time

Pupal development times at 25°C differed between the three butterfly SPECIES and between the SEXES (Fig. 6.6, Table 6.5). Females developed faster than males; *M. perseus* developed more rapidly than *M. sirius* which in turn developed faster than *M. terminus*. Pupal time was not affected by HOSTPLANT. A significant interaction occurred between SPECIES*SEX: *M. terminus* showed greater sexual dimorphism with respect to development time, while *M. sirius* showed the least divergence between males and females.

Despite the slightly faster female pupal development time, males overall developed faster than females when the times taken for each life history stage were pooled (Table 6.6). Development on *Panicum* at 25°C ranged from 40.4 days in male *M. perseus* to 50.4 days in female *M. terminus*. The ratio between

Table 6.4 Larval development time and pupal weight in relation to number of larval instars of *Mycalesis sirius* reared on *Panicum* at 25°C, 12L:12D.

Sex	No. larval instars	N	Larval development time (d) mean ± s.d.	Larval development time (d) range	Pupal weight (mg) mean ± s.d.
Male	5	49	29.84 ± 2.26*	25-35	200.8 ± 17.58
Female	5	36	32.28 ± 2.84**	27-39	228.4 ± 24.27
Male	6	6	36.83 ± 2.64*	35-42	209.5 ± 21.05
Female	6	9	39.22 ± 5.50**	31-51	236.8 ± 31.11
		100			

[*] Two-tailed *t*-test, $P < 0.0001$.

[**] Two-tailed *t*-test, $P < 0.0001$.

Table 6.6 Mean total development time (egg to adult), protandry (the difference in mean development time between males and females), mean pupal weight and sexual size dimorphism (the ratio of female/male pupal weight) for each species reared on *Panicum* at 25°C, 12L:12D. Egg development times were calculated from the regressions in Table 6.2.

Species	Development time (d)		Protandry (d)	Pupal weight (mg)		Sex size dimorphism (♀/♂)
	Males	Females		Males	Females	
M. perseus	40.42	43.07	2.65	184.3	222.1	1.205
M. terminus	47.26	50.41	3.15	221.6	232.9	1.051
M. sirius	44.93	47.60	2.67	201.7	230.1	1.141

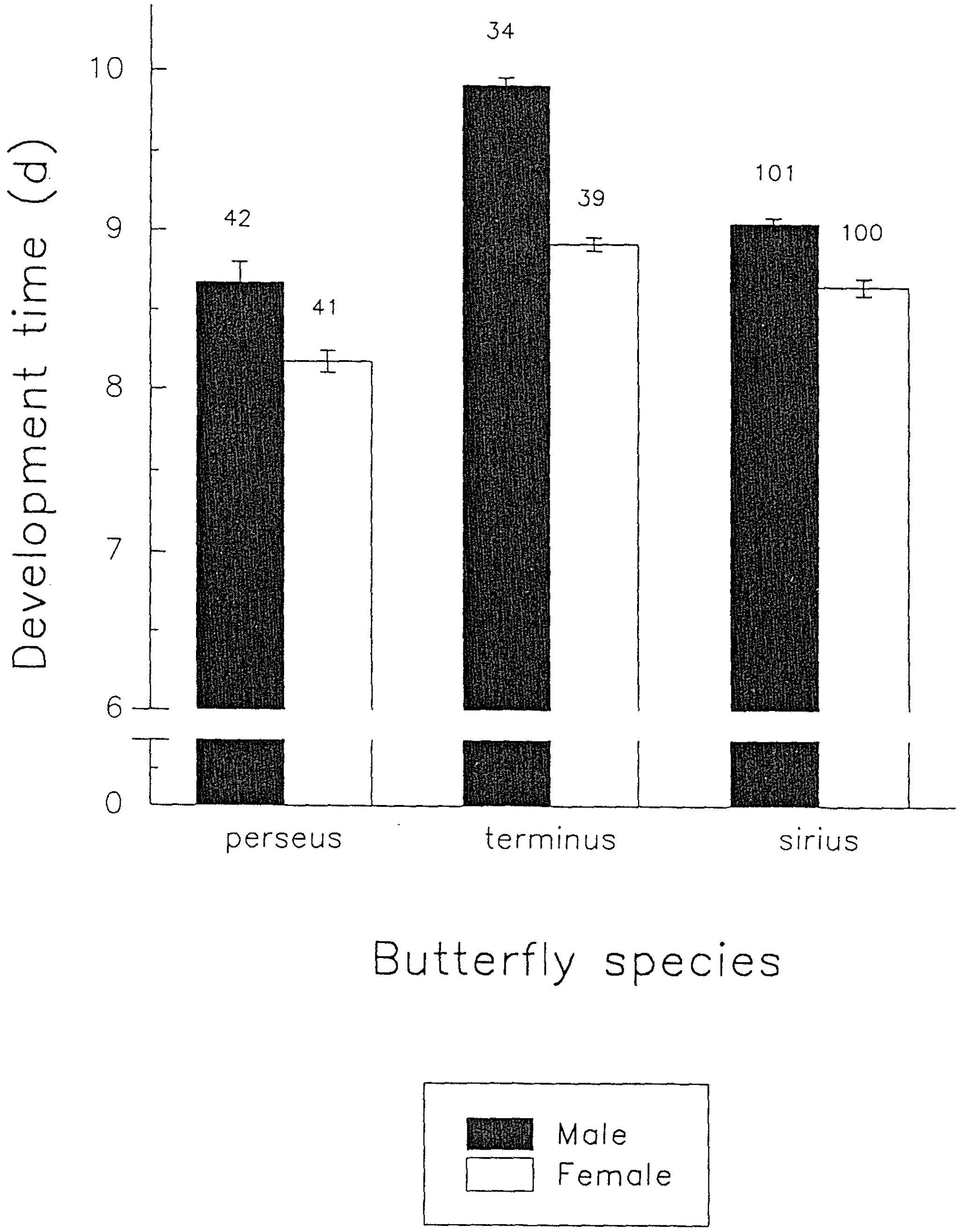

Fig. 6.6 Pupal development times (mean ± S.E.) for males and females of each *Mycalesis* species when reared at 25°C. Sample sizes given above each bar are pooled for the two hostplants.

Table 6.5 Three-way analysis of variance examining effects of hostplant, butterfly species and sex on pupal development time (d) and pupal weight (mg).

Source of variation	*d.f.*	Pupal development time (d)			Pupal weight (mg)		
		MS	*F*	*P*	MS	*F*	*P*
HOSTPLANT	1	0.5349	2.23	NS	70206.41	186.71	0.0001
SPECIES	2	19.3474	80.82	0.0001	6278.62	16.70	0.0001
SEX	1	28.8253	120.24	0.0001	22339.27	59.41	0.0001
Hostplant*Species	2	0.0349	0.15	NS	306.67	0.82	NS
Hostplant*Sex	2	0.0678	0.28	NS	2276.43	6.05	0.0026
Species*Sex	2	2.3443	9.78	0.0001	1449.61	3.86	0.0221
Hostplant*Species*Sex	2	0.0099	0.04	NS	261.02	0.69	NS
Error	343	0.2397			376.03		

male and female developmental times (i.e. sexual development dimorphism) was similar between the species.

6.3.5 Pupal weight and body size

Larvae of the three satyrines reared in captivity on *Themeda* produced smaller pupae than those reared on *Panicum* (Fig. 6.7). A three-way ANOVA examining the effects of HOSTPLANT species, butterfly SPECIES and SEX on pupal weight revealed that all three factors were highly significant, with HOSTPLANT having the greatest effect (Table 6.5). In addition to the hostplant effect, females were heavier than males, and *M. terminus* pupae were heavier than pupae of the other two species. Significant interactions also occurred between SEX*HOSTPLANT and between SEX*SPECIES indicating considerable divergence in pupal weights among the treatments. On *Panicum*, *M. perseus* pupae were lighter than those of *M. terminus* and *M. sirius*, but male *M. perseus* and *M. sirius* attained much lower pupal weights relative to females. Hence, sexual size dimorphism of pupae was greatest in *M. perseus* and smallest in *M. terminus* (Table 6.6). On *Themeda*, both sexes of *M. sirius* were very small, attaining extremely low pupal weights relative to the other two species (Fig. 6.7).

Relationships between pupal weight and the size of newly eclosed adults (measured by length of forewing) are shown in Figure 6.8. The significant positive curvilinear relationships confirm that heavier pupae indeed produce bigger adults in these tropical satyrines.

6.3.6 Correlation of life history traits

Regression analyses were performed on eight data sets (with sample sizes >20) to examine a possible trade-off between two life history traits, larval developmental time and body size. In four data sets these two attributes were correlated, but in only two of these (male *M. perseus* on *Panicum*, female *M.*

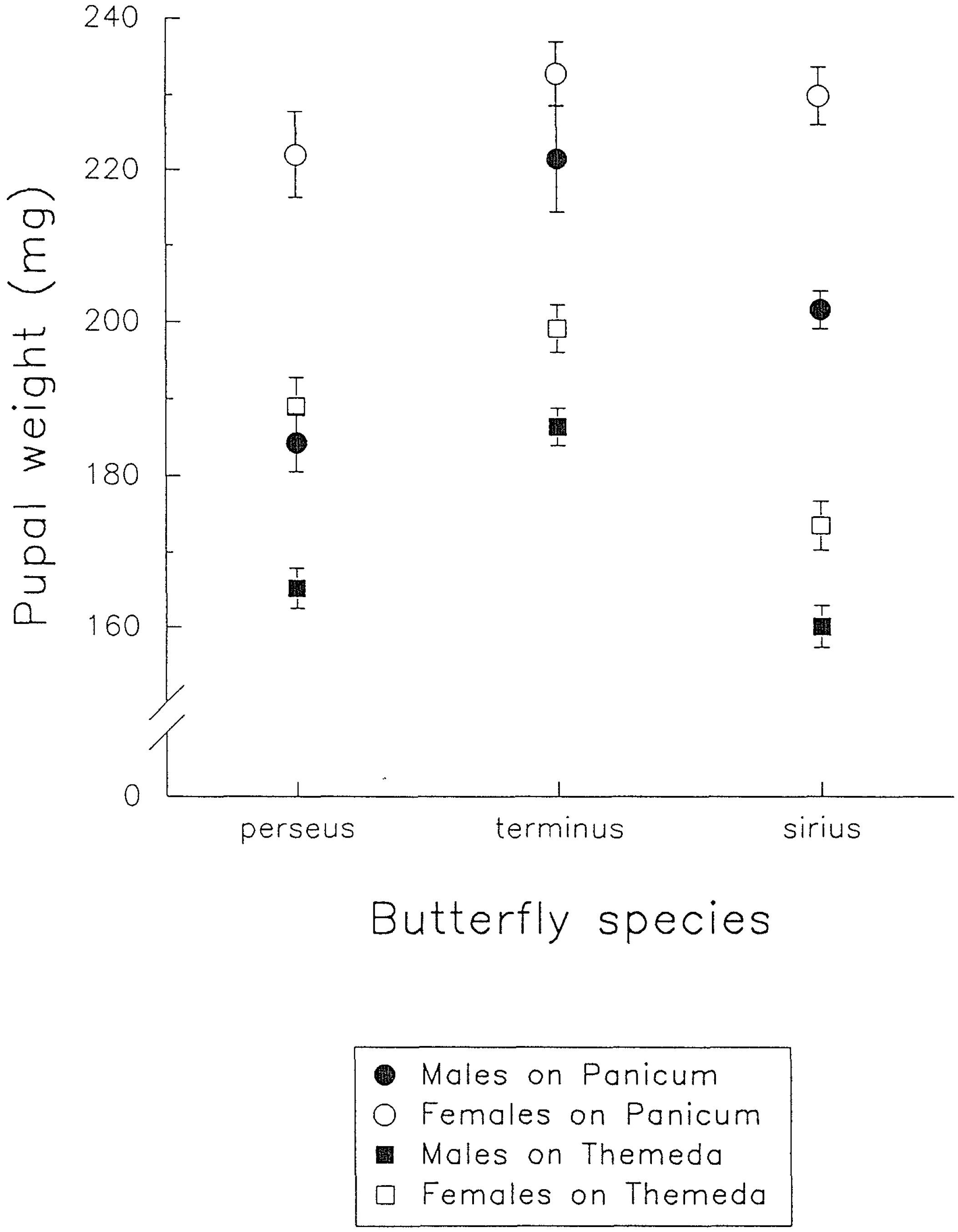

Fig. 6.7 Pupal weights (mean ± S.E.) attained for males and females of each butterfly species reared on *Panicum maximum* and *Themeda triandra* at 25°C. Sample sizes are same as those given in Fig. 6.3.

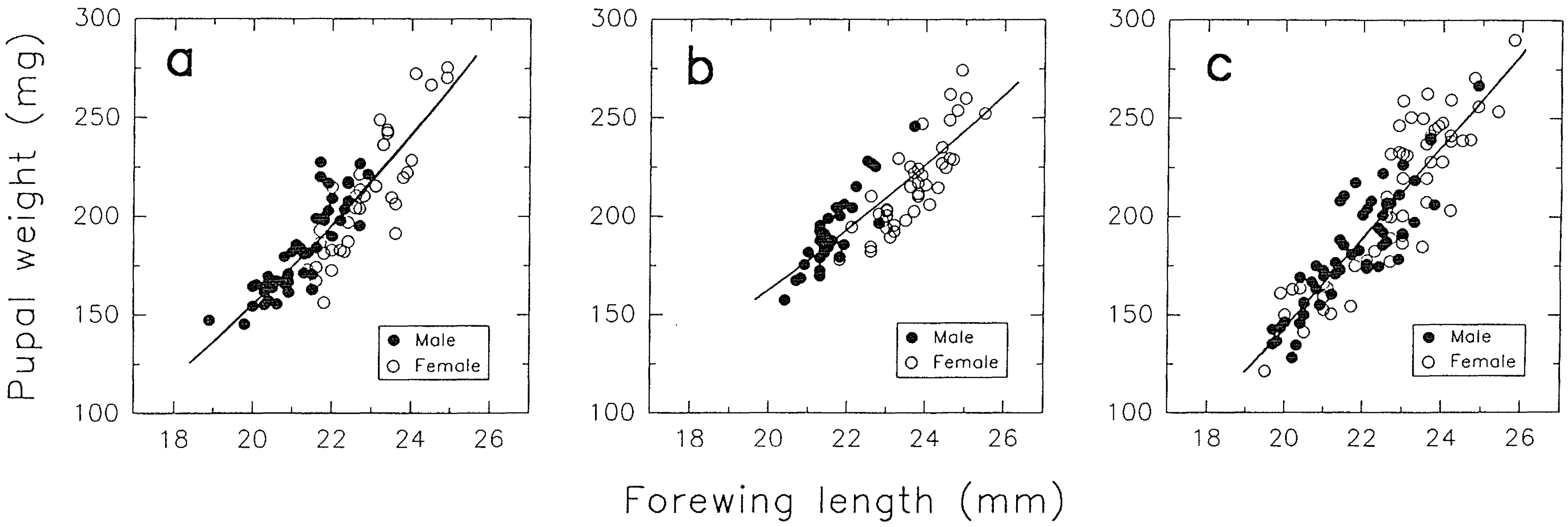

Fig. 6.8 Relationships between pupal weight and length of right forewing for (a) *M. perseus*, (b) *M. terminus* and (c) *M. sirius*. Regression equations: *M. perseus*, $y=0.1060x^{2.432}$ ($r=0.88$, n=84); *M. terminus*, $y=0.7470x^{1.798}$ ($r=0.87$, n=74); *M. sirius*, $y=0.0607x^{2.600}$ ($r=0.89$, n=112).

Table 6.7 Analysis of regression slopes of larval development time (d) on pupal weight (mg) for each species. Data sets of less than 20 points were excluded from the analyses.

Species	Hostplant	Sex	N	Slope mean ± S.E.	*t*	*P*
M. perseus	*Panicum*	Male	27	3.031 ± 1.056	2.87	0.008
	"	Female	25	1.375 ± 2.509	0.55	NS
M. terminus	*Panicum*	Female	21	3.184 ± 1.236	2.58	0.019
	Themeda	Male	28	1.973 ± 1.045	1.89	NS
M. sirius	*Panicum*	Male	55	-0.566 ± 0.776	-0.73	NS
	"	Female	45	-0.100 ± 0.878	-0.11	NS
	Themeda	Male	45	-3.341 ± 0.882	-3.79	0.001
	"	Female	55	-3.765 ± 1.114	-3.38	0.001

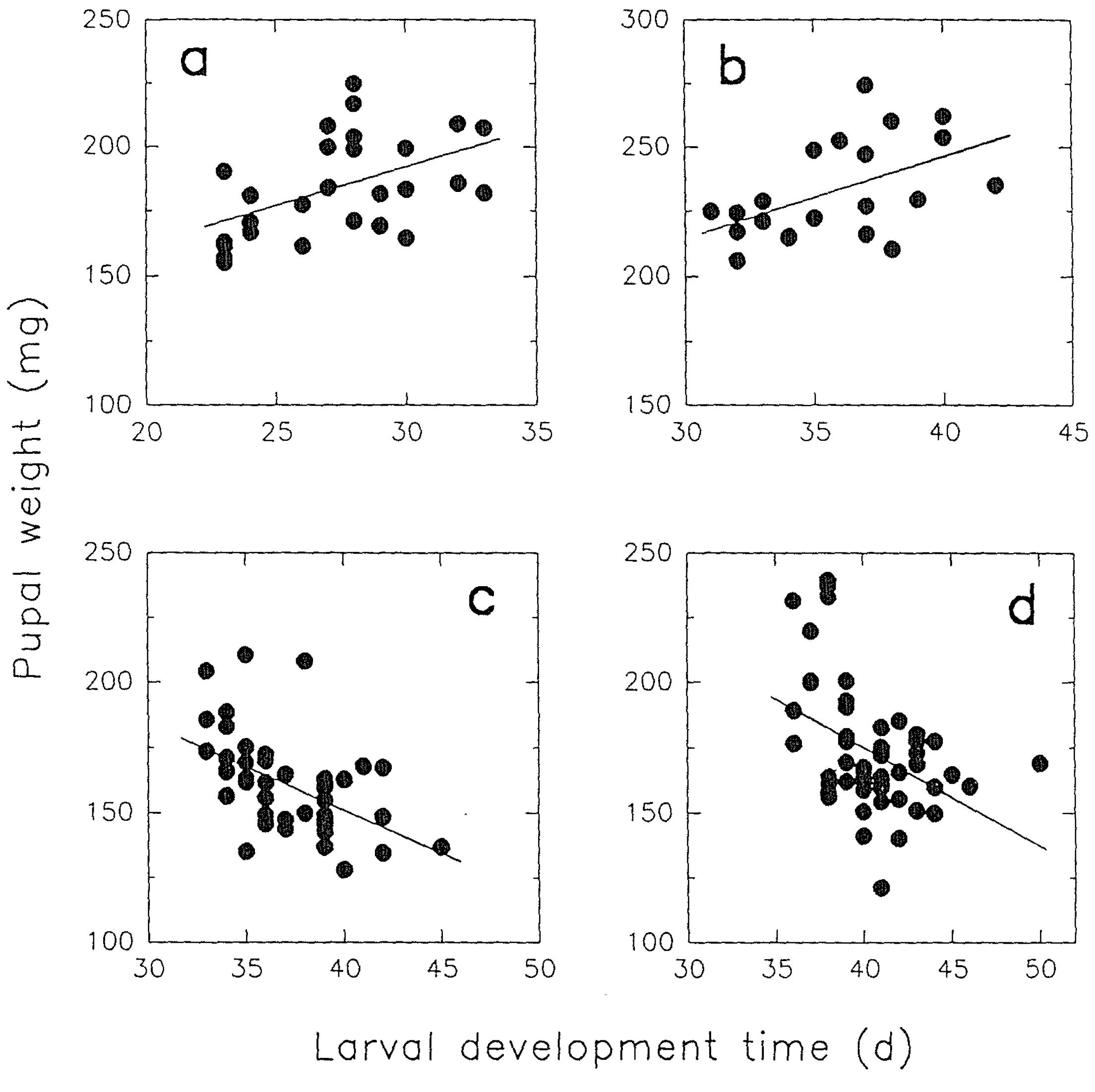

Fig. 6.9 Relationships between larval development time and body size attained, expressed as pupal weight, for (a) male *M. perseus* reared on *Panicum*, (b) female *M. terminus* reared on *Panicum*, (c) male *M. sirius* reared on *Themeda*, (d) female *M. sirius* reared on *Themeda*. Regression equations: male *M. perseus*, $y=3.031x+101.43$ ($r^2=0.248$, $n=27$); female *M. terminus* $y=3.184x+118.91$ ($r^2=0.259$, $n=21$); male *M. sirius*, $y=-3.341x+284.62$ ($r^2=0.250$, $n=45$); female *M. sirius*, $y=-3.765x+325.59$ ($r^2=0.177$, $n=55$).

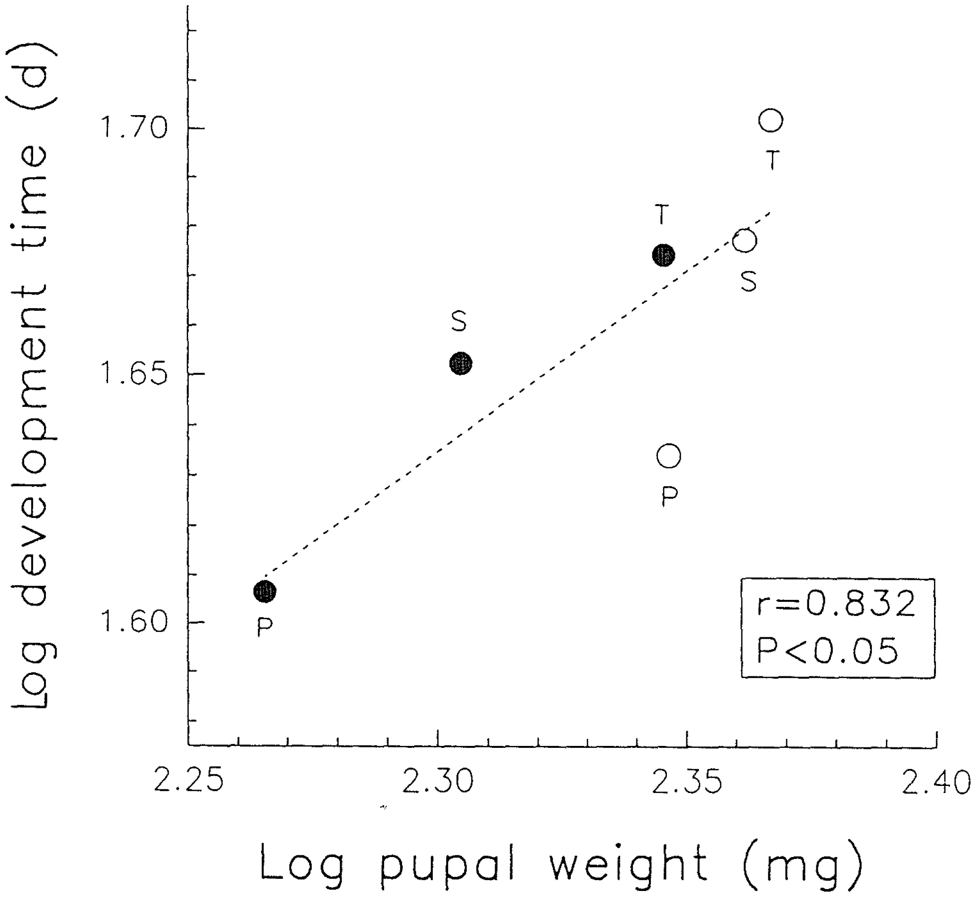

Fig. 6.10 Developmental time (from egg to adult) as a function of body size (pupal weight) in *Mycalesis* (P=*M. perseus*, T=*M. terminus*, S=*M. sirius*). Points represent the mean pupal weights attained and mean total development time for males (●) and females (○) reared on *Panicum* at 25°C, 12L:12D (from Table 6.6). If males and females are treated separately the correlations are not significant ($0.10 > P > 0.05$), but if the sexes are treated as independent units in the analysis the relationship is highly significant ($F=9.01$, 4 *d.f.*, $P=0.04$). Regression equation: $y=0.7275x-0.0385$.

terminus on *Panicum*) were the slopes positive (Table 6.7, Fig. 6.9). In these two species, larvae which matured early were comparatively smaller in size, indicating there is a size cost attached to any advantage gained in rapid development (or alternatively, any advantage in being a larger adult is offset by slower development). In the two other data sets (male and female *M. sirius* reared on *Themeda*), however, the relationships were negative, indicating that larvae which took longer to develop also attained the lowest pupal weights. This latter observation is in contrast to any expected trade-off, suggesting that *M. sirius* suffers resource limitation when reared on *Themeda*.

Within the genus there was a positive correlation between mean total development time and mean body size, expressed as pupal weight, for both males and females reared on *Panicum* (Fig. 6.10). Although the samples are small, if the sexes are treated as independent units in the analysis (i.e. as behaving as separate species) the relationship is highly significant ($r=0.832$, $P<0.05$). The correlation indicates that larger size is associated with longer development (*M. terminus*), while smaller size is associated with faster maturation (*M. perseus*).

6.4 Discussion

In contrast to most temperate satyrines in southern Australia which have a one year life cycle (Edwards 1973, Pearse 1978, Common and Waterhouse 1981, Braby and New 1988a, 1994), the juvenile stages of *Mycalesis* spp. completed development within 6-7 weeks when reared in the laboratory on *Panicum* at 25°C. Little comparative information is available on developmental times for other Australian tropical satyrines. Wood (1984, 1988) gives 49 d for *Elymnias agondas australiana* Fruhstorfer, 44 d for *Hypocysta angustata angustata* Waterhouse and Lyell and 38 d for *H. irius* (Fabricius). *H. adiante adiante* (Hübner) is recorded as taking 37 d (De Baar 1981, Valentine 1988), *Ypthima arctous arctous* (Fabricius) as about 40 d (De Baar 1981), and *Hypocysta metirius* Butler as 55 d and *Melanitis leda bankia* (Fabricius) about 50 d (Braby unpubl. data). Although the development times are not strictly comparable between the species, because of different temperatures and rearing conditions, the times are broadly similar to *Mycalesis* spp., that is 5-7 weeks. All of these species appear to breed throughout much of the year (*M. perseus* and *M. leda* are exceptions as they have a prolonged reproductive diapause during the dry season) and all are undoubtedly seasonally multivoltine. However, development in this group of butterflies is substantially longer than that recorded for other tropical groups which have been studied, for example, species of Pieridae:Coliadinae take 3-4 weeks at 25°C, and one species, *Catopsilia pomona pomona* (Fabricius), completes development in 16 d at 28°C (Jones *et al.* 1987).

6.4.1 Temperature effects

The first aim of the study was to establish if there were any consistent differences in the temperature requirements of each species. Although data derived from constant rearing temperatures should strictly be treated with caution when attempting to relate that information to a species' complex environment of fluctuating temperature (see Worner 1992 for review) the purpose of this work was to make adaptive inferences using a comparative approach. The marked differences observed in survival and development between these species suggest that at least two hypotheses can be proposed concerning the ecology of these animals.

First, *M. perseus* eggs survived better at the extremes tested (17, 35°C), suggesting that this species has a broader survival tolerance with respect to temperature variation, while the tolerance of *M. sirius* eggs appears much smaller. Secondly, comparison of maximum rate of egg development between the species showed that *M. perseus* achieved its highest developmental rate at 35°C compared with *M. terminus* and *M. sirius* (both 30°C). The results of Moore (1985) who also reared *M. perseus* and *M. terminus* eggs at two temperatures (21°C and 30°C) are consistent with the data obtained in the present study. Like the survival data, this result suggests a wider temperature niche exhibited by *M. perseus* compared to the other two species.

This wider niche may be associated with differences in the distribution and preferred habitat of the species. In north-eastern Queensland, populations of *M. terminus* and *M. sirius* are confined largely to a fairly narrow humid lowland strip stretching between the shoreline and the cool wetter tablelands, although *M. terminus* may intrude into the drier regions via cooler moist microhabitats which prevail along creeks and rivers (Chapter 2). *M. perseus* has a much broader range, extending into the hot drier regions both near the coast and slightly inland from the tablelands. Moreover, *M. perseus* is also more likely to occupy open environments less buffered from temperature extremes. Thus *M. perseus* eggs appear adapted to the environments which this species normally experiences. Poor embryonic survival at high temperatures in *M. terminus* and *M. sirius* may be one of the factors excluding these species from the more extreme environments, although it may well equally be argued that the habitats these species occupy has meant they do not experience any selective pressure for a higher temperature tolerance.

The magnitude of difference in temperature required for optimal egg survival and development between *M. perseus* (around 30-35°C) and *M. terminus/M. sirius* (around 25-30°C) was quite large, about 5°C, and this may reflect differences in breeding season. *M. perseus* has a much shorter breeding season compared with *M. terminus* and *M. sirius*, with egg-laying being confined to a few months during the wet season (Moore 1985, Chapter 4). Since the wet season normally occurs during summer, the hottest months of the year when mean maximum temperatures reach 31°C at Townsville, the higher temperature tolerance of *M. perseus* eggs may be more adapted to prevailing climatic conditions, or possibly a combination of seasonal climate and habitat (spatial distribution), rather than habitat distribution *per se*. In contrast, the lower optimal temperature range exhibited by *M. terminus* and *M. sirius* may reflect a more extended breeding season into the cooler dry season months, and/or preference for cooler microhabitats, particularly in *M. terminus* which occurs commonly in rainforest edge, both in coastal and upland areas.

At the lower temperatures, differences in egg survival may reflect differences in geographic distribution. The southern limits of *M. sirius* lie 950 km farther north-west than those of *M. perseus* and *M. terminus*. Therefore, the greater degree of embryonic survival at 17-20°C in *M. perseus* and *M. terminus* correspond with their extended southern latitudinal ranges. Conversely, the lower rate of egg survival in *M. sirius* may be a factor excluding this species from the cooler southern latitudes.

In summary, the smaller geographic range of *M. sirius* in north-eastern Australia may be partly explained by its apparently narrower ecological amplitude in embryonic success, whereas the greater survival plasticity in *M. perseus* eggs may be associated with a larger range and occupation of relatively harsher environments. The limited and patchy nature of coastal paperbark swamplands, the preferred habitat of *M. sirius* (Braby 1992, Chapter 3), is undoubtedly another factor contributing to the relatively small distribution of this species. The impact of temperature on the larval and pupal stages also require assessment in relation to the distributions of these species.

6.4.2 Hostplant effects

My second question was to determine whether or not *Mycalesis* development is affected by the hostplant on which it was reared, comparing a native grass (*Themeda triandra*) with an introduced grass (*Panicum maximum*), by examining three fitness components: larval survival, larval development time, pupal weight. The results suggest that neither of the grasses provide an optimal food source. Larvae of all three species survived better on *Themeda* but took longer to develop and attained lower pupal weights, indicating that this native grass is relatively poor in terms of nutrition. This was particularly pronounced in *M. sirius* in which adults attained very small sizes, compared with *M. perseus* and *M. terminus*, and in which pupal weight was negatively correlated with larv developmental time. In comparison, the poorer survival on *Panicum* suggests that *Mycalesis* are not yet well adapted to this host. Guinea grass is a relatively recent arrival to Australia. It was introduced from Africa late last century and was established in north-eastern Queensland sometime before 1931 (B.K. Simon, pers. comm.). It has invaded much of the natural habitat of *Mycalesis* and possibly displaced many of the native grasses on which larvae depend. However, once established on *Panicum maximum* larvae apparently can develop quite well and attain reasonable size.

The better larval development on soft growth of *Panicum* relative to *Themeda* may be due to differences in leaf nitrogen content. The total amount of nitrogen of soft leaves of *Panicum maximum* (1.56% ± 0.04 sd) is significantly higher than that of *Themeda triandra* (1.38% ± 0.03 sd) (t=5.82, 2 *d.f.* P=0.028; Chapter 8). Low nitrogen content of the native grass may therefore pose a limiting nutritional constraint to caterpillars.

Singer (1984) has suggested that when host quality is high, larvae should slow maturation thereby increasing storage reserves of nutritional resources so that adult fecundity is higher. Few studies have examined the relationship between larval development time and body size (as a measure of larval foraging efficiency in collecting storage reserves) with respect to variation in host quality (Nylin 1988). If host quality is poor, a larva could conceivably employ one of three strategies (cf. Stearns and Koella 1986): (1) increase development time to gather enough resources to attain 'normal' adult size by decreasing growth rate, (2) maintain 'normal' development time but attain smaller adult size with fewer reserves by decreasing growth rate, (3) decrease development time but become a smaller adult without changing growth rate. On *Themeda* it appears that *Mycalesis* larvae increase development time by decreasing growth rate (strategy 1), perhaps reflecting a trade-off in which survival is maximised, but individuals fail to achieve adults of normal size. A similar finding has been reported in the temperate nymphalid *Polygonia c-album*, a polyphagous species which on less preferred hosts such as *Corylus avellana* larval development time is almost doubled and pupae attain extremely low weights, however, larval survival is poor (Nylin 1988).

While the early stages of *Mycalesis* spp have been recorded on a range of grass species in the field (Chapter 2) Moore (1986) established that ovipositing females of *M. perseus* and *M. terminus* prefer different suites of potential hostplant species. *M. terminus* prefers the slower growing species and is less selective with respect to leaf quality, while *M. perseus* oviposits on faster growing hostplants and prefers nitrogen-rich plants and younger leaf tissue. Moreover, differences in host selectivity between these two satyrines is associated with the preferred habitats and with the length of time over which their larval resources may be expected to persist. *M. terminus* favours rainforest edge where grasses are more persistent and less prone to drying off, whereas *M. perseus* lives mainly in savanna woodland where grasses are generally more short-lived and less predictable, that is more prone to premature drying. Moore therefore concluded that the greater degree of intraspecific hostplant choice exhibited by female *M. perseus* may have resulted from strong pressures to choose plants which are likely to last longest (i.e. lay preferentially on young leaf tissue).

However, the relationship between such interspecific differences in host preference by female *Mycalesis* and subsequent larval success is not known. A poor correlation between adult host choice and various offspring fitness components has been noted in several, mostly oligophagous, species (e.g. Chew 1977, Courtney 1981, 1982, Kitching and Zalucki 1983, Singer 1984 and references therein), and it is generally believed that females are constrained by shortage of time to select optimal host(s) and oviposition sites (Courtney 1982). Conversely, Nylin (1988) found good agreement between larval performance, measured in terms of development time, and oviposition plant preferences in the polyphagous *Polygonia c-album*. The host selection strategy by female *M. perseus* suggests that variation in larval resource duration, that is the length of time over which a hostplant is expected to last relative to its generation time, may also be an important factor in determining adult host preferences.

Better information on host preferences, measured in terms of both species and nutritional quality selected by ovipositing females and subsequent success of offspring is needed, particularly for *M. sirius*.

6.4.3 Instar number

Variation in the number of larval instars occurred only in *M. sirius* in which 15% of all individuals reared on *Panicum* (N=100) completed six instars, compared with the normal five instars recorded for the other two species. Furthermore, larval development in *M. sirius* was significantly longer for larvae with six instars than those with five. Although the significance of this warrents further attention, an adaptive explanation may be sought in terms of environmental unpredictability by drawing an analogy with alpine lycaenid butterfly *Lycaena phlaeas hypophlaeas* (Boisduval) for which Ballmer and Pratt (1989) recorded larvae as having either four or five larval instars. Instar number in this species is apparently determined by prevailing environmental factors at oviposition (a formal homogeneity test of Ballmer and Pratt's frequency instar data for their two egg-laying conditions was significant: $X^2=14.39$, $P<0.001$) so that eggs laid during periods of short day lengths and low temperatures (i.e. in late autumn) are likely to promote larvae with five instars and longer developmental times. These larvae probably overwinter through the harsh snow periods and their protracted development may be adaptive for survival, reducing the likelihood of premature maturation during frequent periods of temperature extreme and unpredictability. Whether increased variation in the number of larval instars, and hence larval development, in *M. sirius* is correlated with similar habitat uncertainty, for example, during the dry season is not known.

6.4.4 Sexual size dimorphism and protandry

In the three *Mycalesis* spp. examined in this study, females are larger than adult males, especially in *M. perseus*, and development rate (from egg to adult) among males is faster than among females [by 2.6-3.1 d when the juvenile stages are reared on *Panicum* at 25°C (Table 6.6)]. Hence, the association of larger adult size with longer development time amongst females indicate that under field conditions populations of these multivoltine species are protandrous [i.e. males will tend to emerge a few days before the first females, Wiklund and Fagerström (1977)].

Protandry is common in temperate insects with discrete (non-overlapping) generations (Wiklund *et al.* 1992), and two main hypotheses have been advanced to explain its significance. The first is that sexual selection acts on either males to maximise the expected number of matings by eclosing before most females have emerged (Wiklund and Fagerström 1977, Iwasa *et al.* 1983, Parker and Courtney 1983), or on females to minimise the prereproductive period by eclosing when most males are available (Fagerström and Wiklund 1982). Indirect evidence in support of this adaptive explanation stems from experimental work on several temperate butterflies with different mating systems (Wiklund and Solbreck 1982, Forsberg and Wiklund 1988, Wiklund *et al.* 1991, 1992) where the sexes are shown to adjust their developmental period/pathway under different environmental conditions so that the degree of protandry is maintained. The second hypothesis argues that the difference in eclosion times is an incidental byproduct of selection acting on other life history traits, such as body size, and protandry is not selected for *per se*. For example, sexual selection acting on female body size may favour females to become larger, especially if female sexual size dimorphism is positively correlated with fecundity (Wiklund and Karlsson 1988, 1990). If growth rates are the same between the sexes, larger female size confers a longer developmental time for females than for males, so that males mature earlier at a smaller size. Since butterfly females allocate differently to males, because of their proportionally bigger reproductive reserves needed for egg-laying (Karlsson and Wickman 1990), a longer time may also be required in the larval stage by females to gather resources. Alternatively, selection may favour males to become smaller, particularly if small size carries advantages in terms of agility for mating success or for reduced predation (Thornhill and Alcock 1983, McLachlan 1986), although it is widely held that male size is positively correlated with reproductive success (see Wiklund and Karlsson 1988, Wiklund and Forsberg 1991 and references therein).

It has been argued that selection for protandry should lead to small male size with shorter developmental times (Singer 1982) and it should be strongest in species which maintain female monogamy (Wiklund and Forsberg 1991). In multivoltine species with overlapping generations, Singer (1982) predicts that protandry should be relaxed, because the competitive advantage of early male emergence is diminished, so that sexual differences in development time (and possibly size) should be reduced.

In tropical *Mycalesis*, however, populations are multivoltine but strongly protandrous. Moreover, the strong sexual size dimorphism from the laboratory trials is consistent with data obtained for wild caught specimens, and the pattern is also apparent amongst seven other Australian tropical satyrines (Table 6.8), all of which but one are presumed multivoltine [the exception is *T. helena* which is predominantly univoltine, Braby (1993)]. If it is assumed that these species also differ in their development times between males and females, Singer's hypothesis is clearly untrue for these butterflies.

Although protandry may be relevant for very seasonal tropical species such as *T. helena*, and perhaps *M. perseus* which breeds for only a restricted period of the year, it seems unlikely that for most of the other satyrines which fly and breed throughout most of the year (at least *M. terminus* and *M. sirius* do) selection should favour early male emergence. As Wiklund and Forsberg (1991) point out males may have to make a trade-off between size and development

Table 6.8 Mean body size, sexual size dimorphism (female forewing length divided by male forewing length) and carrying sex in copula in ten species of Australian tropical satyrine butterflies from north-eastern Queensland. Forewing length was measured from centre of thorax to apex of right forewing of field caught specimens curated at James Cook University and in the author's collection.

Species	Forewing length (mm)		Sexes pooled	N	Size	Carrying
	Female	Male	(mean ± s.d.)	(F/M)	dimorphism	sex
Ypthima arctous	19.76 ± 1.04	16.91 ± 0.75	18.34 ± 1.70	25/25	1.169	female
Hypocysta pseudirius	18.54 ± 0.78	17.45 ± 0.50	17.95 ± 0.84	11/13	1.062	not known
H. adiante	20.14 ± 1.23	17.22 ± 1.02	18.68 ± 1.85	25/25	1.169	not known
H. metirius	19.28 ± 0.92	18.97 ± 0.94	19.12 ± 0.93	25/25	1.016	female
H. irius	20.86 ± 1.38	20.81 ± 1.24	20.84 ± 1.30	25/25	1.002	not known
Mycalesis perseus	25.19 ± 1.05	22.20 ± 1.00	23.70 ± 1.82	25/25	1.135	female
M. sirius	26.11 ± 1.18	23.50 ± 1.11	24.81 ± 1.74	25/25	1.111	female
M. terminus	26.12 ± 1.04	23.48 ± 1.16	24.80 ± 1.72	25/25	1.112	female
Tisiphone helena	36.36 ± 1.36	30.56 ± 1.72	33.46 ± 3.31	20/20	1.190	not known
Melanitis leda	41.26 ± 2.10	38.63 ± 2.67	40.05 ± 2.69	19/16	1.068	not known

time (degree of protandry) according to the mating system favoured by females in order to achieve both optimal development time and adult size. They contend that selection should favour protandry under female monogamy and towards large male size (relative to that of females) under female polygamy. However, neither of these rules seem to apply to tropical Satyrinae; female *Mycalesis* are polyandrous (Chapter 4) and generations overlap, that is, are multivoltine. It is therefore concluded that protandry is almost wholy incidental (non-adaptive) in this group of butterflies.

6.4.5 Size and development: comparison between species

When *M. perseus* eggs are reared at a range of temperatures they develop consistently faster than *M. terminus*. The same difference in developmental rate between the species also occurs in the larval and pupal stages when these are reared on either hostplant at 25°C. *M. perseus* evidently has evolved a much shorter developmental time period from egg to adult than *M. terminus* and *M. sirius* (Table 6.6).

The faster developmental rate of *M. perseus* may be an adaptation to the relative length of time over which its larval resources may be expected to persist. Rainfall in the wet-dry tropics is highly seasonal and unpredictable in nature, and in the savanna woodland habitats favoured by *M. perseus* grasses of suitable quality may persist for only several months. *M. terminus*, by contrast, lives in more predictable habitats where grasses are generally more persistent. Thus *M. terminus* seemingly can afford a much longer developmental phase, whereas shortage of time through habitat uncertainty or low durational stability (in the sense of Southwood 1988) may have been an important selective pressure in promoting rapid maturation in *M. perseus*.

Because of the relationship between developmental time and body size within the genus (at least when the sexes are treated independently, Fig. 6.10) differences in developmental rate may also be tied to differences in adult size.

Until more species are examined in the genus, to form a more general relationship as a basis for comparison (e.g. Clutton-Brock and Harvey 1979, Begon *et al.* 1990, Harvey and Pagel 1991), partitioning the effects of size from any size-independent effects is not possible. Smaller size (with its associated faster development) in *M. perseus* may have allowed this species a competitive edge over *M. terminus* and *M. sirius* to utilise more temporary habitats.

In conclusion, variation in rate of development and embryonic survival between these closely related satyrines are strongly tied with ecological differences in geographic distribution, habitat preference and resource durational stability (i.e. the relative length of time over which their larval resources may be expected to persist). *M. perseus* has faster rate of development in all juvenile stages, will tolerate a higher temperature range to achieve high egg survival and rapid development, and has a broader survival tolerance to temperature variation in the embryonic stage - attributes which are associated with a broader distribution and occupation of breeding environments in which larval resources are both seasonal and unpredictable. By contrast, *M. terminus* has a slower rate of development, requires a lower temperature range for maximum egg development, and its eggs survive poorly at higher temperatures - features which are appropriate to this species occupation of cooler and more predictable habitats. *M. sirius* has the narrowest ecological tolerance with respect to egg survival and has the smallest geographic range in north-eastern Australia.

CHAPTER 7: REPRODUCTIVE BIOLOGY

7.1 Introduction

The reproductive pattern of any insect species includes the number of eggs laid (realised fecundity), the total number of eggs and oocytes produced (potential fecundity), the distribution of eggs in space and time (oviposition pattern), the size of eggs, and the reproductive effort (the product of egg size and egg number as a proportion of total biomass). An understanding of these components, and the factors which influence them, is central to developing a more complete picture of a species life history strategy (Stearns 1976, Boggs 1981a, Sibly and Calow 1986). One important factor influencing the reproductive pattern is the amount and quality of resources collected and allocated by the female, since nutrient investment in eggs constitutes a major expenditure of energy. Accordingly, variation in resource acquisition and subsequent allocation may effect the fitness of a female's progeny and hence her reproductive success (Boggs 1981a, 1986, Parker and Begon 1986).

Nutritional resources allocated to egg production by butterfly females may be obtained in three ways: (1) during larval feeding, (2) by adult feeding or (3) from male contributions at mating. Nutrients accumulated in the larval stage are probably the primary source for egg production (Labine 1968, Dunlap-Pianka 1979, Chew and Robbins 1984, Boggs 1987, Svärd and Wiklund 1988), and several studies have shown that variation in larval diet may substantially affect realised fecundity in Lepidoptera (Danthanarayana 1975, Hough and Pimental 1978, Smith 1986). Essential nutrients, such as nitrogen (in the form of protein) assimilated during the larval feeding period and stored as fat body may be a limiting resource for reproductive output (Dunlap-Pianka *et al.* 1977, Mattson 1980, Boggs 1981a, Scriber and Slansky 1981, Karlsson and Wickman 1990). Species which feed as adults may enhance their nitrogen reserves or obtain other nutrients such as sugars or salts. These substances may be collected, either singly or in combination, from a variety of sources, including nectar (Baker and Baker 1973, 1982, Watt *et al.* 1974), pollen (Gilbert 1972, Dunlap-Pianka *et al.* 1977, Boggs *et al.* 1981), mud puddles (Downes 1973, Arms *et al.* 1974, Adler and Pearson 1982, Pivnick and McNeil 1987, Boggs and Jackson 1991), or possibly from carrion, animal excreta, sap and rotting fruit in some species. Acquisition of sugars by adult females greatly enhances fecundity and/or longevity in Lepidoptera (e.g. Stern and Smith 1960, Murphy *et al.* 1983, Leather 1984, Pivnick and McNeil 1985, Hill 1989, Hill and Pierce 1989, Karlsson and Wickman 1990) but the role of amino acids in the diet of nectar feeding species is uncertain as these often do not influence reproductive output or egg weight (Wickman and Karlsson 1987, Moore and Singer 1987, Hill 1989, Hill and Pierce 1989, but see Murphy *et al.* 1983). A third potential nutritional resource for Lepidoptera are spermatophores produced by males at mating, particularly in long-lived and/or polyandrous species (Svärd and Wiklund 1988, 1989, Wiklund *et al.* 1993). Spermatophores contain a variety of substances, including nitrogen (in the form of proteins and amino acids), and these male-derived nutrients may be absorbed by females and incorporated into eggs (Boggs and Gilbert 1979, Boggs and Watt 1981, Boggs 1981b, 1990). However, the role of male-donated nutrients in influencing reproductive output in butterflies varies: several studies have shown no effect (Greenfield 1982, Jones *et al.* 1986, Svärd and Wiklund 1988, 1991), while four studies have demonstrated a significant increase in fecundity (Rutowski *et al.* 1987, Watanabe 1988, Oberhauser 1989, Wiklund *et al.* 1993). The role of other adult dietary sources such as animal excreta, sap and rotting fruit on fecundity and longevity have not been investigated.

In tropical habitats, many species feed on rotting fruits (e.g. Owen 1971, Young 1979) and these items may represent an important nutritional resource. This study compares two tropical Australian satyrine butterflies in the genus *Mycalesis* to determine how availability of rotting fruits as opposed to sugar in the adult diet affects fecundity, longevity and egg weight. One of these species, *M. terminus*, regularly feeds on rotting fruits while the other (*M. sirius*) does not

(Chapter 2).

Many tropical butterflies, particularly in the families Pieridae and Nymphalidae, exhibit strong phenotypic seasonal variation with distinctive wet- and dry-season forms (Shapiro 1976, Brakefield and Larsen 1984, Chapter 5). A number of these species exhibit an adult reproductive diapause during which females contain extensive fat reserves with few or no eggs (Jones 1987). The diapause usually occurs in the dry-season form and allows the butterflies to survive the long harsh dry period when breeding conditions are unfavourable. One might expect the existance of an adult diapause to exact a cost in terms of their reproductive effort so that females of the dry-season form should have a reduced reproductive effort compared to those of the wet-season form, either as a result of passive resource depletion, or by diverting their resources more into longevity (somatic reserves) than reproduction (reproductive reserves) during juvenile growth and development (Karlsson and Wickman 1989). I compare the reproductive attributes of two seasonal phenotypes (wet- and dry-season forms) of a third tropical satyrine, *Mycalesis perseus*.

This study also allows a comparison of the reproductive patterns of wet-season forms of all three *Mycalesis* spp. when fed on rotting fruit.

7.2 Materials and methods

7.2.1 The species

Individuals used in this study were obtained from populations in Queensland at Townsville (19°05'S, 146°49'E) for *M. perseus* and *M. terminus*, and at Cardwell (18°16'S, 146°01'E) for *M. sirius*. The populations are multivoltine but egg-laying declines towards the end of the dry season when females may enter reproductive diapause (Chapter 4). Diapause is most pronounced in *M. perseus* in which whole populations may remain reproductively dormant for over six months. Reproductive dormancy appears to be terminated by the first pre-wet season rains in all three species. The satyrines also exhibit marked seasonal changes in adult morphology, with distinctive wet- and dry-season forms; again this is most pronounced in *M. perseus* (Chapter 5), and reproductive dormancy usually occurs in the dry-season form. Females of all species usually mate more than once (i.e. are polyandrous), although mating frequency varies seasonally, particularly in *M. perseus* in which diapausing females usually contain only one spermatophore during the dry season (Chapter 4).

Because of this complex pattern of breeding phenology and seasonal polyphenism, any information collected on reproductive traits must take into account the time of year and the adult form in order to make meaningful comparisons between the species. In this study these two factors were standardised by comparing traits of wet-season forms. The effect of two adult dietary sources (rotting fruit vs sugar as honey solution) on several reproductive traits (egg production, longevity, egg size) was examined in *M. terminus* and *M. sirius*. For *M. perseus*, data was collected on the dry-season form so that the two extreme phenotypes of this species could also be compared. Wet-season form adults of both *M. terminus* and *M. sirius* were obtained by rearing larvae in captivity, whereas for *M. perseus,* females of the two forms were obtained from the field as described below.

7.2.2 M. terminus and M. sirius

Eggs of *M. terminus* and *M. sirius* were obtained from captive stocks held in an outdoor shade house on the university campus. The eggs were placed individually into petri dishes and kept at 25°C ± 0.5°C and a 12:12 L:D cycle. The newly emerged larvae were transferred with a fine brush to freshly cut strips of soft leaves of the hostplant, *Panicum maximum*, and reared in the petri dish with moistened filter paper until pupation. The leaf strips and filter paper were changed every 1-3 days. Pupae were weighed 24 hrs after pupation on a Cahn 21 automatic electrobalance (0.1 mg accuracy).

On the day of eclosion the adults were sexed, and then transferred to 0.7 X 0.7 X 0.7 m flight cages which were kept in the shade house. Females were given access to at least two males for 1-2 days to ensure they had mated. [Since the males were also newly eclosed it was assumed that male quality at mating was high and relatively constant for each female]. The females were then transferred individually to small plastic cages (100 mm diam. X 100 mm height) lined with moistened filter paper and fitted with muslin cloth over the roof, and kept at 25°C ± 0.5°C and 12:12 L:D cycle. Freshly cut blades of soft grass were provided as an oviposition substrate, which were changed during the morning every 1-2 days. Females were given either fermenting banana-pear mixture, or a 25% honey solution, delivered as a small cotton pad. Honey contains sugars and amino acids, and is a derivative of nectar (White 1975). The food was changed during the morning every 1-2 days. Both *M. terminus* and *M. sirius* oviposit singly on the underside of leaves, usually during the mid afternoon, and at the end of each day all eggs laid were carefully removed from the cages with a fine pair of tweezers. For some females the eggs laid each day were weighed (if more than 20 eggs were laid, only 80% were weighed and the mean daily egg weight calculated) on the Cahn electrobalance (0.0001 mg accuracy). At the end of the experiment the females were dissected to ensure they had received only one spermatophore (multiply mated individuals were excluded from calculations).

7.2.3 M. perseus

The procedure for obtaining reproductive information for *M. perseus* was slightly different to that of the above two species in that adults were not reared from captivity. Difficulty was experienced in achieving successful matings from laboratory reared adults which emerged late in the season, and females did not readily oviposit in captivity at 25°C. Therefore, wild females were used for both wet- and dry-season forms.

A natural population in savanna woodland vegetation on the university campus was monitored for reproductive activity during 1990. Samples of dry-season form females were dissected every 2-3 weeks for examination of the ovaries. When they showed the first signs of ovarian development (i.e. termination of reproductive diapause), which occurred in mid December, live adults were collected and transferred to an outdoor shade house. They were placed individually into breeding cages, approx. 40 cm X 15 cm diam., fitted with potted tussocks of the grass *Themeda triandra*. The cages were watered twice daily. Only rotting fruit was supplied as an adult food source. Like *M. terminus* and *M. sirius*, egg-laying in *M. perseus* usually occurs in the afternoon. At the end of each day eggs were collected and weighed following the method outlined above for *M. terminus* and *M. sirius*.

In mid January 1991, the first wet-season form adults (i.e. the next generation, following the onset of egg-laying of the dry-season form) appeared on campus, and freshly emerged females (i.e. with no wing damage) were collected and transported to the breeding cages for egg-laying. Where possible, pairs in copula were taken to further minimise the risk of these females having laid in the field, and to ensure that they had mated. [Many of the early wet-season form females dissected in January were unmated and without developed eggs]. The procedure for obtaining eggs from these females was identical to that of the dry-season form. Again, only rotting fruit was used as an adult food source.

Figure 7.1 summarises the climatic data for the two *M. perseus* egg-laying trials. Mean daily (24 hrs) temperatures experienced for the dry- and wet-season forms were 28.6°C ± 4.2 (sd) and 28.8°C ± 3.4 (sd) respectively. Mean maximum daily temperatures were also similar, 33.3 and 33.2°C respectively. A pulse of heavy (cyclonic) rain occurred in late December-early January towards the end of the dry-season trial, and a similar pulse of rainfall occurred in early February near the end of the wet-season trial. Hence, climatic conditions were very similar between the two trials.

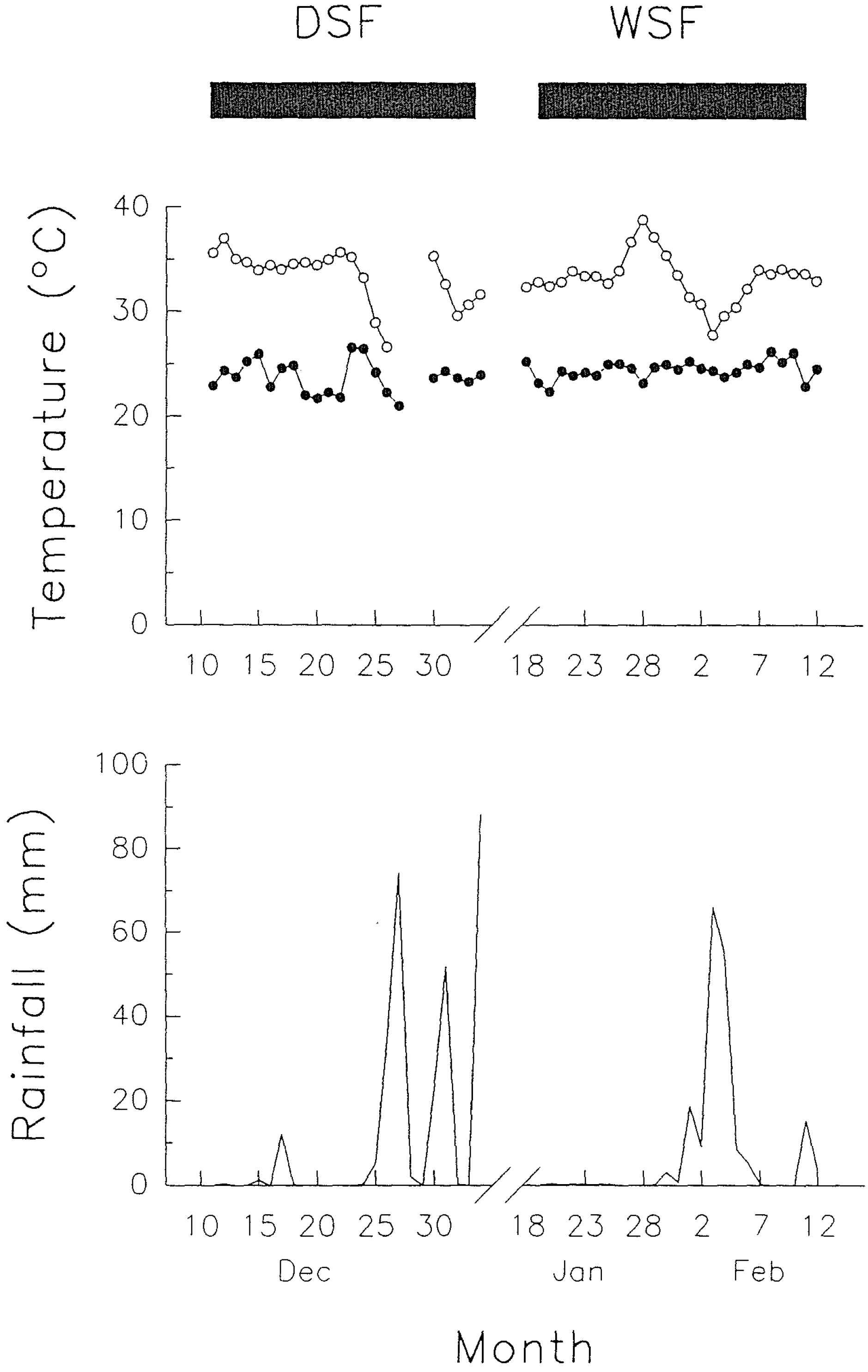

Fig. 7.1 Climatic data for *M. perseus* egg-laying field trials, 1991-92. Shown are daily minimum (●) and maximum (○) temperature, daily rainfall and the time period when the two phenotypes, wet-season form (WSF) and dry-season form (DSF), were used for each trial.

In both wet- and dry-season form females the developmental history and pupal weight of the individuals tested were unknown. Pupal weight was therefore inferred from wing length (measured from centre of thorax to apex of right forewing) using the relationship $y=0.1060x^{2.432}$, where y is the pupal weight (mg) and x represents forewing length (cm), based on laboratory reared specimens (Chapter 6), so that body size-fecundity relationships could be compared with those of *M. terminus* and *M. sirius*.

7.2.4 Reproductive traits

The following reproductive traits were measured for each individual female.

Oviposition pattern - Age-specific fecundities were determined from the number of eggs laid each day from when egg-laying commenced. The time taken to reach peak egg-laying, the size of the peak (measured as maximum daily fecundity), and the times taken to lay 50% and 90% of all eggs that were laid during the oviposition period were also measured.

Egg number and fecundity - Females were dissected at the end of adult life to ascertain the number of eggs and oocytes remaining in the body cavity. These counts were added to the total number of eggs laid (realised fecundity) to estimate the potential fecundity of each female. Estimates of realised fecundity and longevity were not made for females which died prematurely, which were unmated, or which had mated twice, as revealed by spermatophore counts. In addition, a number of laboratory reared females, not used for egg-laying trials, were dissected early in adulthood to assess the reproductive condition and to estimate potential fecundity.

Longevity - Adult captive lifespan was divided in two components: a preoviposition period, defined as the time interval from adult eclosion to the start of egg-laying, and a reproductive period, the interval from the start of egg-laying to death.

Egg size and reproductive effort - The total weight of all eggs produced by each female over her lifetime was used to determine the reproductive effort. The mean egg weight for each female was then determined by dividing the reproductive effort by the realised fecundity. Potential reproductive effort was calculated for each by dividing the product of mean egg weight and potential fecundity by the pupal weight.

7.2.5 Statistical analyses

The following reproductive traits were compared between the treatments for each species: maximum daily fecundity, realised fecundity, potential fecundity, proportion of eggs laid, preoviposition period, reproductive period, time to peak egg-laying, time to lay 50% of realised fecundity, egg weight and reproductive effort. For the two seasonal forms of *M. perseus* the effect of PHENOTYPE on each of these reproductive traits were analysed using one-way analyses of covariance with pupal weight as the covariate. Similarly, for both *M. terminus* and *M. sirius*, the effect of DIET on the traits were each analysed by one-way analyses of covariance, again with pupal weight as covariate to remove any variation which may be accounted for by body size. All analyses were performed using the generalised linear models procedure (SAS Institute Inc. 1988).

7.3 Results

7.3.1 Effect of adult diet

Oviposition pattern. Oviposition patterns showing the mean daily egg output during the reproductive period for *M. terminus* and *M. sirius* are presented in Figure 7.2. These essentially represent age-specific fecundity trends from which particular reproductive traits can be quantified and compared amongst the treatments (Table 7.1). The two species showed an initial rapid rise in egg-laying, followed by a short peak, a sharp decline and then a more gradual

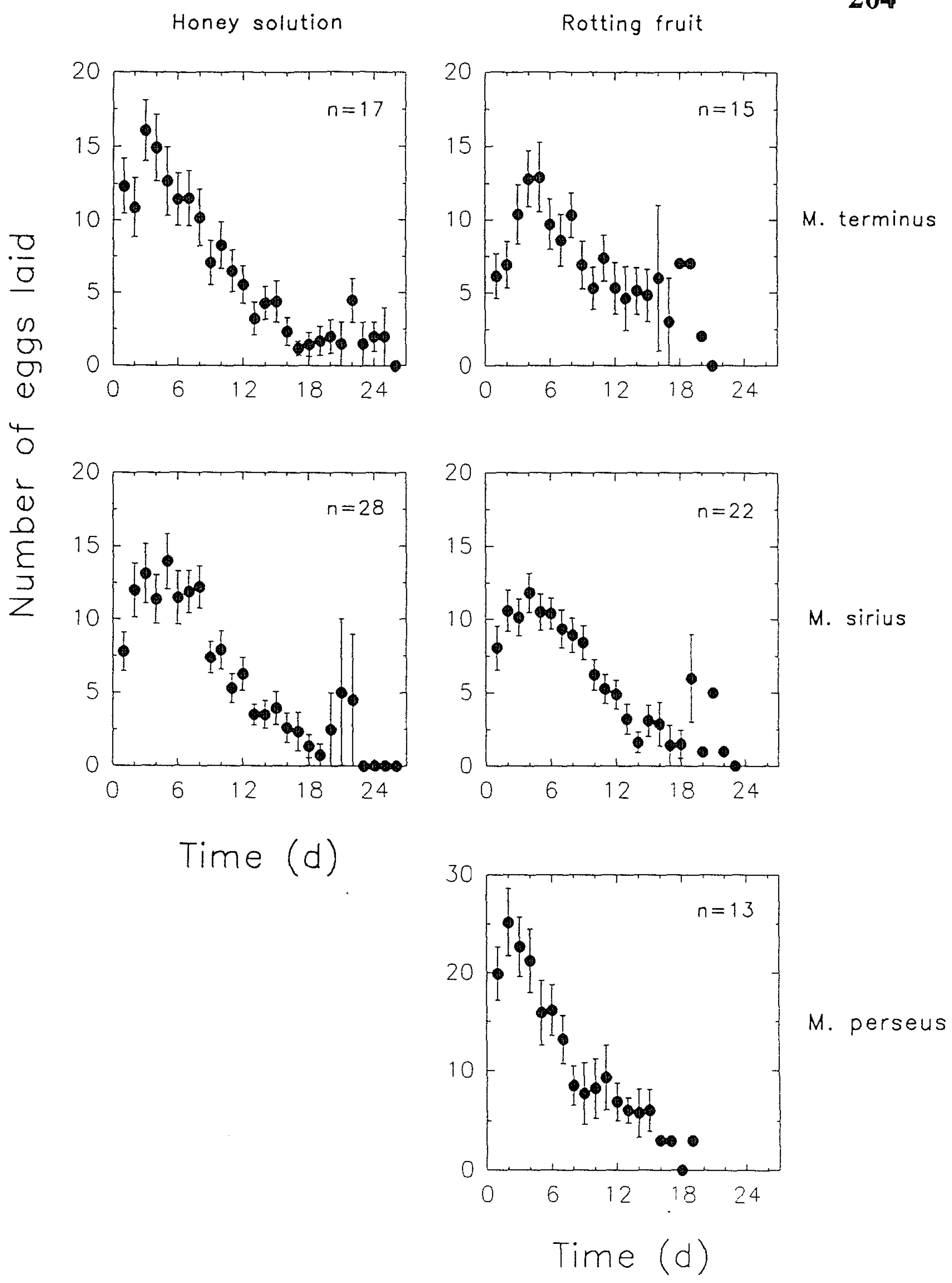

Fig. 7.2 Mean age-specific fecundities for *M. terminus* and *M. sirius* on honey solution and rotting fruit. Plotted are the number of eggs laid each day (mean ± S.E.) against female age given as time when egg-laying began. Sample sizes are the number of females tested. *M. perseus* wet-season form on rotting fruit is shown for comparison.

Table 7.1 The size of, and the time taken to reach peak egg-laying, given as maximum daily fecundity, and the times taken to lay 50% and 90% of realised fecundity for each species in relation to phenotype and adult diet. Times (days) represent mean ± s.d. Results of analysis of covariance are indicated below for each phenotype/diet treatment, with pupal weight as covariate.

Species	N	Max. daily fecundity	Time to peak	50% eggs laid	90% eggs laid
M. perseus dry-season f.	15	18.37 ± 7.77	4.60 ± 1.64	4.73 ± 1.16	7.47 ± 1.25
M. perseus wet-season f.	13	31.38 ± 6.05	2.62 ± 1.26	4.54 ± 1.56	9.46 ± 2.40
Phenotype		F=13.79, P=0.001	F=3.63, P=0.069	F=0.63, NS	
Covariate		F=0.96, NS	F=0.03, NS	F=1.59, NS	
M. terminus honey	17	24.76 ± 4.18	3.56 ± 1.79	5.56 ± 2.00	11.38 ± 3.91
M. terminus rotting fruit	15	20.80 ± 5.23	4.73 ± 2.34	6.33 ± 1.88	10.53 ± 3.02
Diet		F=1.56, NS	F=1.16, NS	F=0.69, NS	
Covariate		F=9.12, P=0.005	F=0.77, NS	F=0.17, NS	
M. sirius honey	28	23.32 ± 9.08	4.04 ± 2.01	5.92 ± 2.08	10.62 ± 3.29
M. sirius rotting fruit	22	18.73 ± 4.77	3.86 ± 2.17	5.82 ± 1.44	10.73 ± 2.51
Diet		F=1.79, NS	F=0.28, NS	F=0.15, NS	
Covariate		F=4.97, P=0.031	F=0.61, NS	F=0.37, NS	

decline. Diet had no affect on the magnitude of the peak, measured as maximum daily fecundity, or on the time taken to reach peak production in either of these species. The times taken to lay half (50%) and most (90%) eggs were also not affected by diet, and half of the eggs laid were deposited soon after peak production (Table 7.1). Pupal weight correlated positively with maximum daily fecundity in these two species (Fig. 7.3).

Fecundity. Diet did not affect the total number of eggs laid in either *M. terminus* or *M. sirius*. Potential fecundity was similar between the two species and was also not affected by diet (Table 7.2). Potential fecundity correlated significantly with pupal weight (Fig. 7.4). Overall, captive females laid about half of their potential fecundity (Table 7.2), although there was large variability in the total proportion of eggs laid within each species. Figure 7.5 summarises the relative changes in egg output, expressed as a proportion of the potential fecundity, laid in four-day intervals over time. In general, the proportion of eggs laid declined with female age, but there were noticeable differences between treatments. In *M. terminus* fed on honey solution, females laid proportionally more eggs initially ($F=9.04$, $P=0.006$), but those on rotting fruit maintained a higher relative egg output later in life. However, overall differences in the total proportions of eggs laid over female lifetime did not differ between the two diets (Table 7.2). Diet did not affect the proportion of eggs laid in *M. sirius*.

Longevity. *M. terminus* and *M. sirius* females both emerged with no eggs and took several days before their ovaries matured and egg-laying commenced. In captivity at 25°C, this preoviposition period was about 5 days in *M. terminus*, approximately twice as long for *M. sirius* (Table 7.3). Diet did not significantly affect the preoviposition period in either species. In *M. terminus* the reproductive period was longer for females fed on honey solution than for those fed on rotting fruit (Table 7.3, Fig. 7.6). However, diet did not effect longevity in *M. sirius*.

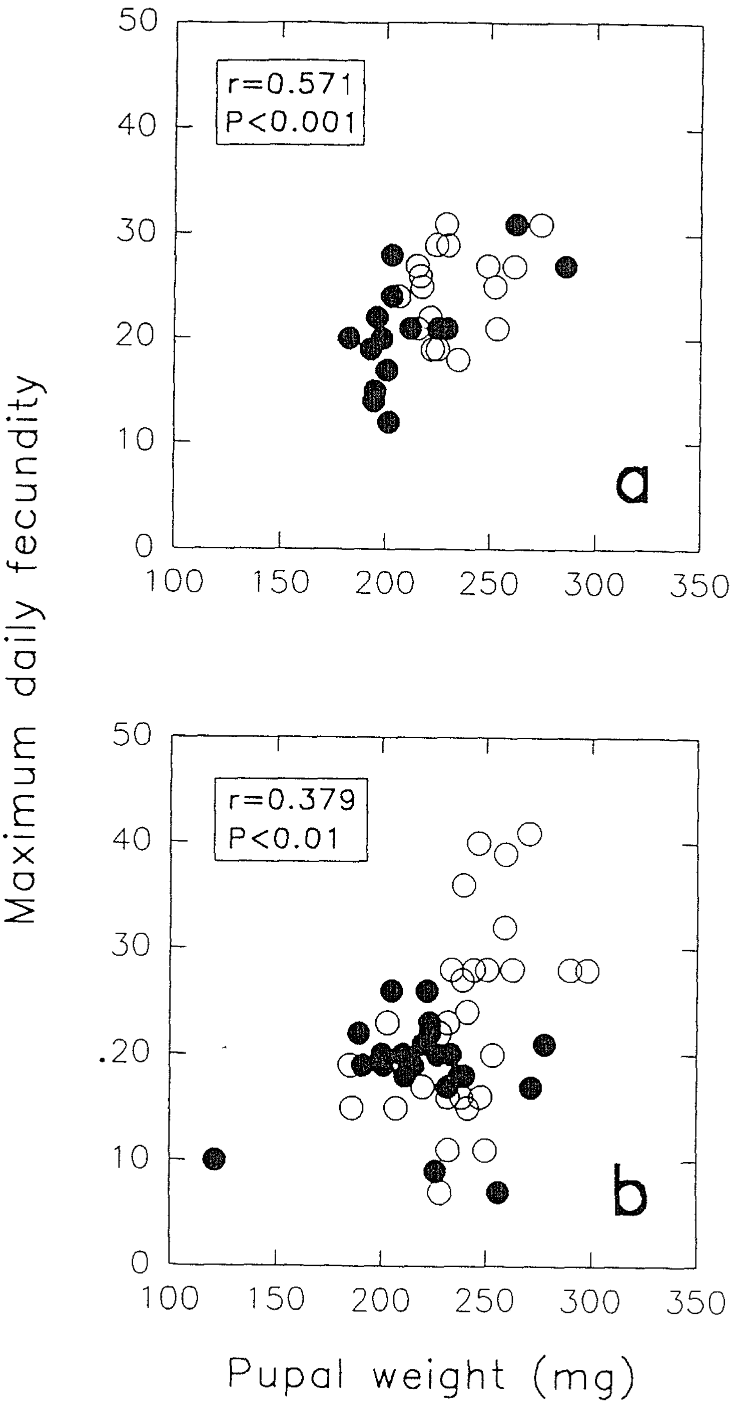

Fig. 7.3 Maximum daily fecundity as a function of pupal weight for (a) *M. terminus* and (b) *M. sirius*. Solid circles represent females fed on rotting fruit, open circles on honey solution. For *M. terminus* the relationship is not significant on honey solution (F=1.25, P=0.282), but is significant on rotting fruit (F=8.26, 13 *d.f.*, P=0.013). For *M. sirius* the relationship is significant on honey solution (F=7.54, 26 *d.f.*, P=0.011), but not significant on rotting fruit (F=0.07, P=0.797). When the diets are combined for each species the relationships are highly significant: *M. terminus*, y=0.112x-2.089 (F=14.50, 30 *d.f.*, P=0.0006); *M. sirius*, y=0.098x-1.371 (F=8.04, 48 *d.f.*, P=0.0070).

Table 7.2 Effect of phenotype and adult diet on realised and potential fecundity, and the proportion of eggs laid in the three satyrine species. Values represent mean ± s.d. with sample sizes in parentheses. Results of analysis of covariance are indicated below for each phenotype/diet treatment, with pupal weight as covariate.

Species	Realised fecundity	Potential fecundity	% eggs laid
M. perseus dry-season f.	72.0 ± 36.6 (15)	137.3 ± 43.3 (44)	45.9 ± 16.3
M. perseus wet-season f.	163.8 ± 62.5 (13)	230.3 ± 57.7 (44)	61.1 ± 14.8
Phenotype	F=24.87, P<0.0001	F=215.55, P<0.0001	
Covariate	F=5.57, P=0.0264	F=78.86, P<0.0001	
M. terminus honey	142.1 ± 58.8 (17)	242.1 ± 50.2 (17)	57.6 ± 16.4
M. terminus rotting fruit	110.1 ± 49.2 (15)	227.9 ± 44.6 (15)	47.2 ± 17.1
Diet	F=1.38, NS	F=2.40, NS	
Covariate	F=0.72, NS	F=38.86, P<0.0001	
M. sirius honey	126.9 ± 53.8 (28)	224.6 ± 44.4 (28)	55.7 ± 18.3
M. sirius rotting fruit	110.7 ± 46.3 (22)	193.8 ± 40.1 (22)	56.4 ± 18.8
Diet	F=0.40, NS	F=2.93, NS	
Covariate	F=1.53, NS	F=38.84, P<0.0001	

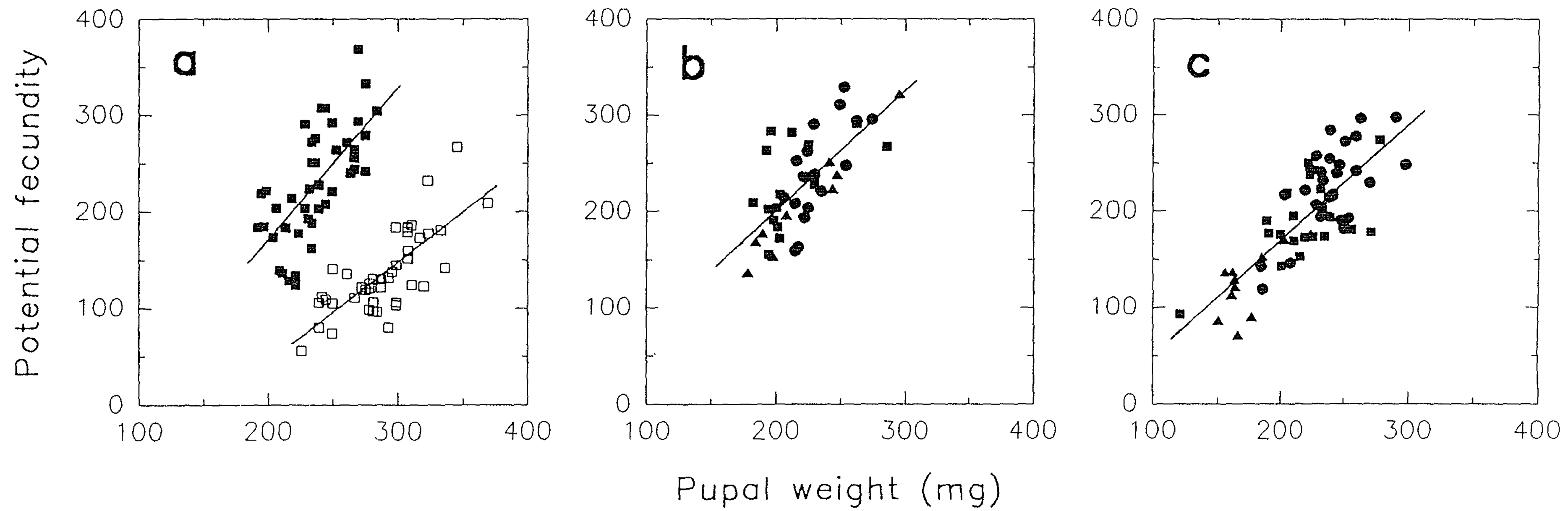

Fig. 7.4 Relationships between potential fecundity and pupal weight for (a) *M. perseus* (■ wet-season form, □ dry-season form), (b) *M. terminus* (● on honey solution, ■ on rotting fruit, ▲ dissected at eclosion), (c) *M. sirius* (● on honey solution, ■ on rotting fruit, ▲ dissected at eclosion). Regression equations: *M. perseus* wet-season form, $y=1.592x-148.02$ ($r^2=0.468$, $P<0.0001$); *M. perseus* dry-season form, $y=1.049x-166.67$ ($r^2=0.564$, $P<0.0001$); *M. terminus* (all points), $y=1.248x-48.21$ ($r^2=0.490$, $P<0.0001$); *M. sirius* (all points), $y=1.199x-69.34$ ($r^2=0.631$, $P<0.0001$).

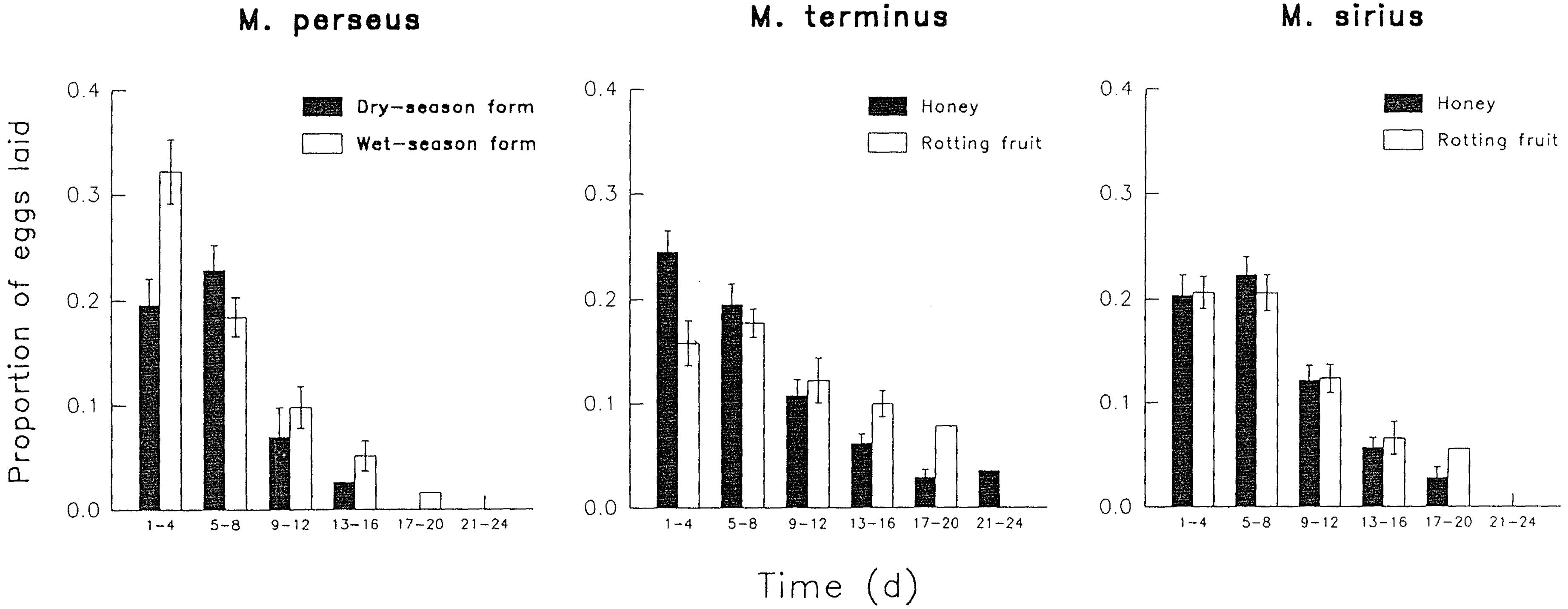

Fig. 7.5 Relative changes in egg output, expressed as a proportion of the potential fecundity, laid in four-day intervals over the egg-laying period for each species. Bars indicate means with error bars representing one standard error.

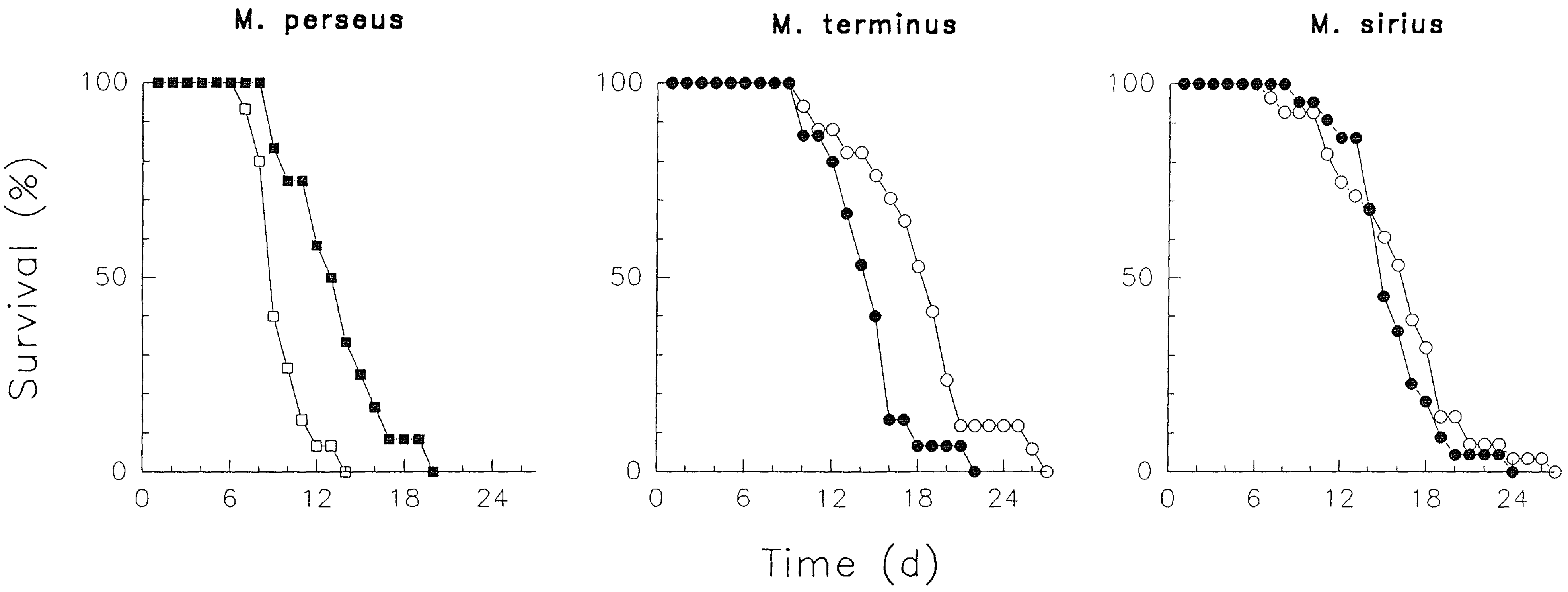

Fig. 7.6 Proportion of females surviving against time when egg-laying began for the three species: *M. perseus* (■ wet-season form, □ dry-season form), and *M. terminus* and *M. sirius* (● rotting fruit, ○ honey solution).

Table 7.3 Effect of phenotype and adult diet on female longevity, expressed as a preoviposition phase and a reproductive phase for each species. Values are mean ± s.d. Results of analysis of covariance are indicated below for each phenotype/diet treatment, with pupal weight as covariate.

Species	N	Preoviposition period (d)	Reproductive period (d)
M. perseus dry-season f.	15		9.27 ± 2.09
M. perseus wet-season f.	13		13.85 ± 3.76
Phenotype			$F=6.46$, $P=0.018$
Covariate			$F=0.06$, NS
M. terminus honey	17	5.56 ± 1.25	16.12 ± 4.91
M. terminus rotting fruit	15	5.14 ± 1.21	12.67 ± 3.04
Diet		$F=0.54$, NS	$F=4.55$, $P=0.041$
Covariate		$F=0.17$, NS	$F=0$, NS
M. sirius honey	28	2.71 ± 0.90	14.44 ± 4.41
M. sirius rotting fruit	22	2.70 ± 0.82	14.14 ± 3.07
Diet		$F=0.59$, NS	$F=0.01$, NS
Covariate		$F=1.44$, NS	$F=0.86$, NS

Egg size and reproductive effort. Mean daily egg weight decreased for *M. terminus* females fed on honey solution ($F=127.7$, $P<0.0001$), but those fed on rotting fruit maintained approximately equal weight over time (Fig. 7.7); the slope of the regression for this latter diet was not significantly different from zero ($t=-1.96$, 13 *d.f.*, $P=0.072$). Diet did not affect egg weight in *M. sirius*, females laid progressively smaller sized eggs with time when fed on either honey solution ($F=60.78$, $P<0.0001$) or rotting fruit ($F=91.07$, $P<0.0001$) (Fig. 7.7), and the slopes of the regressions were similar. Data obtained on the overall mean egg weight and reproductive effort for each species are summarised

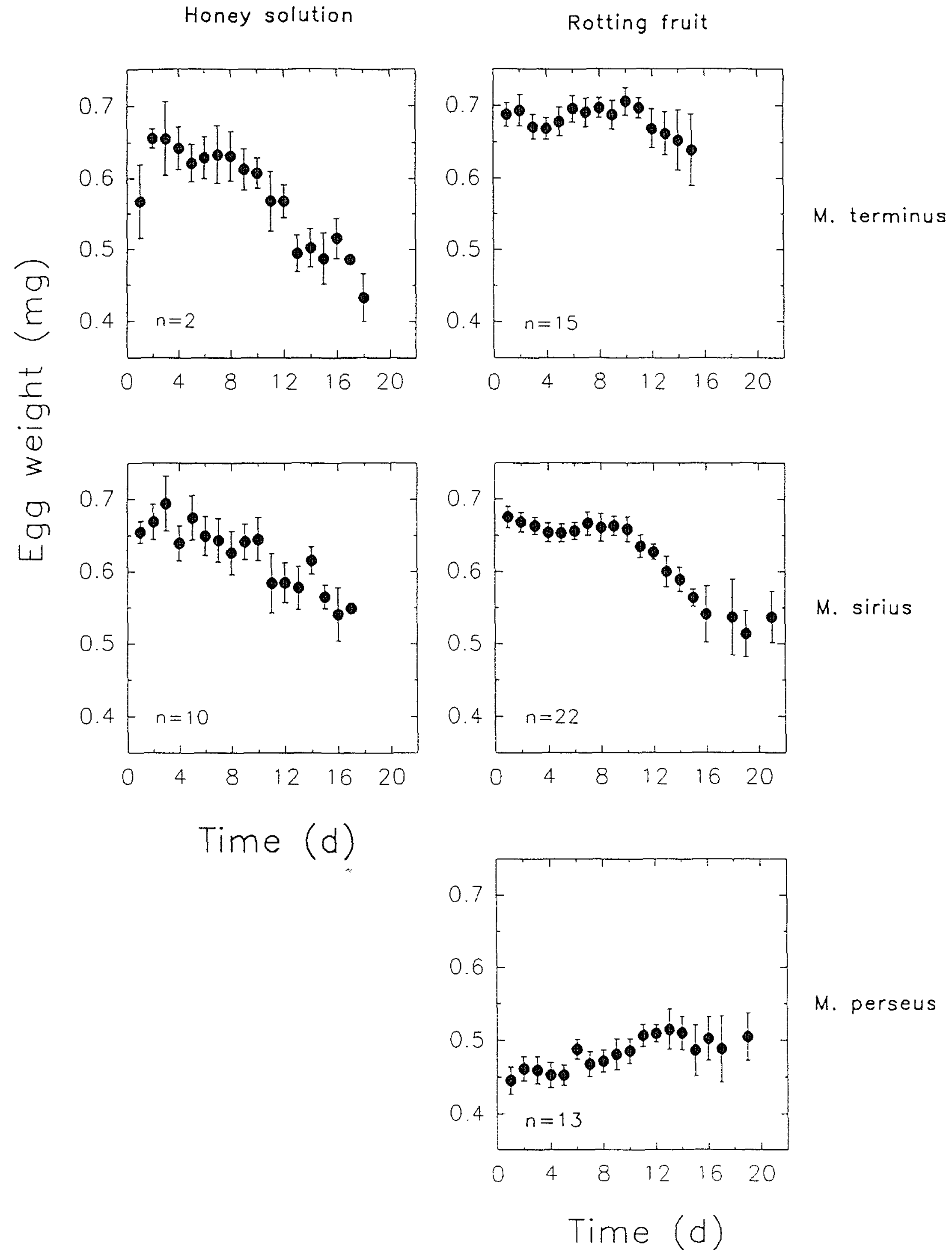

Fig. 7.7 Relationships between egg weight (mean ± S.E.) and female age given as time when egg-laying began for *M. terminus* and *M. sirius* on honey solution and rotting fruit. Sample sizes refer to number of females tested. Regression equations (of mean points): *M. terminus* on honey solution, $y=-0.0134x+0.707$ ($r^2=0.895$, $P<0.0001$, excluding day 1); *M. terminus* on rotting fruit, $y=-0.0020x+0.696$ ($r^2=0.227$, $P=0.072$); *M. sirius* on honey solution, $y=-0.0081x+0.694$ ($r^2=0.802$, $P<0.0001$); *M. sirius* on rotting fruit, $y=-0.0084x+0.705$ ($r^2=0.843$, $P<0.0001$). *M. perseus* wet-season form on rotting fruit is shown for comparison.

Table 7.4 Effect of phenotype and adult diet on egg weight, realised reproductive effort[1] and potential reproductive effort[2] for each *Mycalesis* species. Results of analysis of covariance are indicated below for each phenotype/diet treatment, with pupal weight as covariate.

Species	No. of females	Egg weight (mg)		Reproductive effort(mg)	Potential reproductive effort
		mean ± s.d.	range	mean± s.d.	mean ± s.d.
M. perseus dry-season f.	15	0.545 ± 0.073	0.330-0.877	38.68 ± 18.60	0.278 ± 0.065
M. perseus wet-season f.	13	0.462 ± 0.062	0.276-0.645	77.28 ± 34.87	0.483 ± 0.130
Phenotype		F=6.29, P=0.019		F=17.89, P<0.001	F=35.00, P<0.0001
Covariate		F=0.15, NS		F=5.34, P=0.029	F=8.24, P=0.008
M. terminus honey	2	0.608 ± 0.069	0.397-0.760		
M. terminus rotting fruit	15	0.679 ± 0.065	0.476-0.866	75.29 ± 36.41	0.733 ± 0.147
Diet		F=0.33, NS			
Covariate		F=0.45, NS			
M. sirius honey	10	0.647 ± 0.075	0.417-0.863	80.86 ± 33.21	0.578 ± 0.111
M. sirius rotting fruit	22	0.657 ± 0.089	0.376-0.913	71.84 ± 28.19	0.581 ± 0.087
Diet		F=0.61, NS		F=0.40, NS	F=0.04, NS
Covariate		F=1.38, NS		F=1.60, NS	F=1.01, NS

[1] measured as the product of mean egg weight and realised fecundity for each female.

[2] measured as the product of mean egg weight and potential fecundity divided by pupal weight for each female.

in Table 7.4. In *M. terminus*, mean egg weight of females fed on rotting fruit was higher than for those fed on honey solution, however, as noted earlier, the difference occured only in older females since egg weights of honey-fed females declined whereas those of fruit-fed females did not. Diet did not affect the mean egg weight in *M. sirius*. Reproductive effort was not affected by diet in *M. sirius*, while the samples for *M. terminus* on honey solution were too small to test for dietary effects.

7.3.2 Effect of phenotype

Oviposition pattern. The distribution of eggs during the reproductive period in the dry-season form of *M. perseus* was far more variable than the wet-season form (Fig. 7.8). Peak daily fecundity was smaller in the dry-season form, and the time to peak was slightly longer, though the difference in times was only weakly significant (Table 7.1). Phenotype did not affect the time taken to lay 50% of realised fecundity.

Fecundity. Realised and potential fecundity were significantly higher in *M. perseus* wet-season form than in the dry-season form (Table 7.2). Potential fecundity correlated significantly with pupal weight (Fig. 7.4). There were strong phenotypic differences in the relative proportion of eggs laid (Fig. 7.5). The dry-season form laid similar proportions during the first two time intervals, reflecting a longer sustained peak, whereas the wet-season form had a significantly higher initial output followed by a rapid decline. Overall, the wet-season form laid a higher percentage of its potential fecundity than the dry-season form (Table 7.2). Fertility of eggs was high and not significantly different between the two phenotypes ($X^2=0.186$, $P=0.666$) (Table 7.5).

Longevity. Although *M. perseus* females emerge with no mature eggs the preoviposition period could not be reliably estimated from field collected adults because their age was unknown. *M. perseus* has a complex reproductive diapause and the precise environmental factors which stimulate egg production

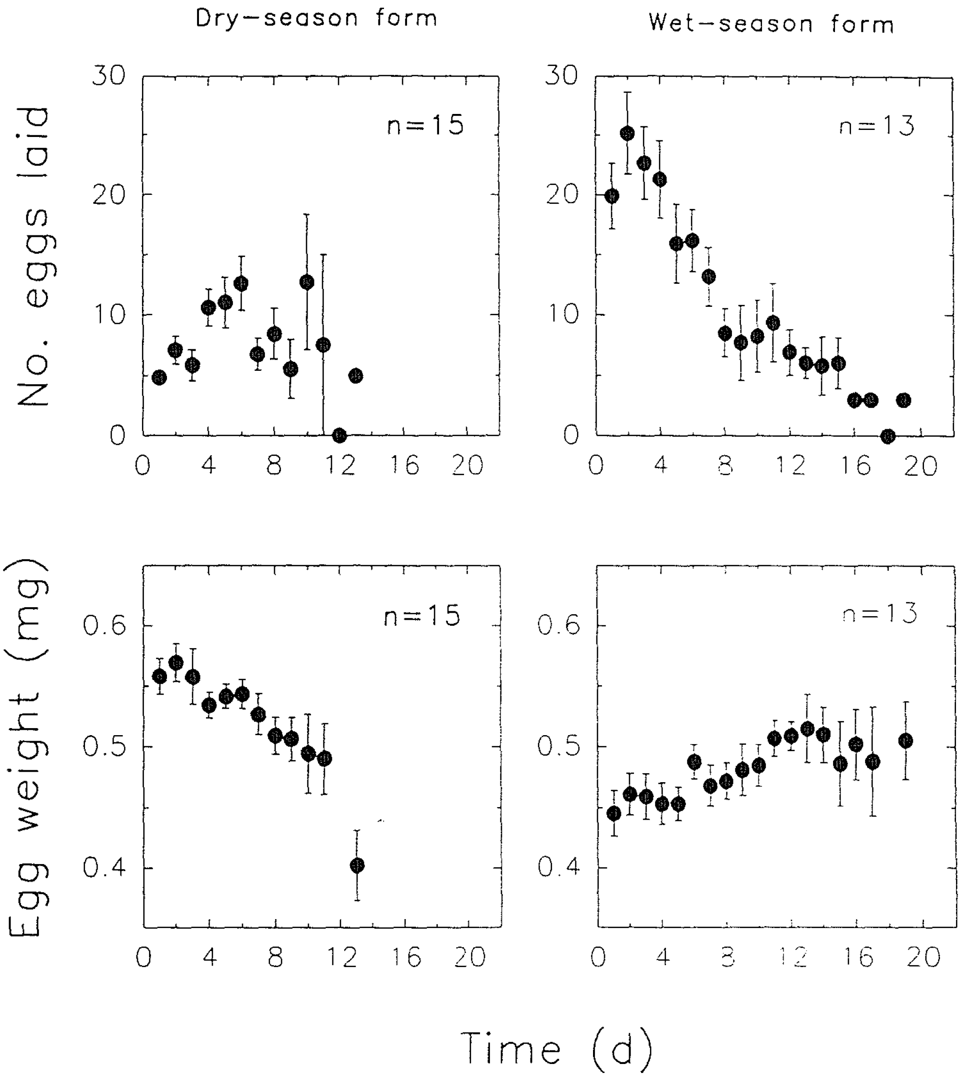

Fig. 7.8 Daily changes in the number of eggs laid (mean ± S.E.) and egg weight (mean ± S.E.) with female age for *M. perseus* dry- and wet-season forms. Sample sizes refer to the number of females tested. Regression equations for egg weight: dry-season form, $y=-0.0078x+0.577$ ($r^2=0.918$, $P<0.0001$, excluding day 13); wet-season form, $y=0.0034x+0.450$ ($r^2=0.675$, $P<0.0001$).

Table 7.5 Fertility of *M. perseus* eggs for wet- and dry-season forms.

Seasonal form	No. of females	No. of eggs laid	No. fertile eggs (%)
wet-season	5	820	775 (94.5)
dry-season	5	275	258 (93.8)

Table 7.6 Reproductive condition of *M. perseus* wet-season form females when kept at 25°C and 12L:12D.

Time after eclosion	No. females dissected	No. containing eggs (%)
4 hr	6	0
24 hr	2	0
48 hr	2	0
4 d	5	1 (20)
6 d	3	2 (67)
10 d	2	1 (50)
18 d	3	2 (67)

after eclosion in this species are not fully understood. Preliminary measurements made on laboratory reared wet-season form females held in captivity at 25°C after mating, and then dissected to assess their reproductive condition, suggest that individuals may take anywhere from 4-18 d or more before their ovaries mature and contain chorinated eggs (Table 7.6). Once egg-laying started, the reproductive period was significantly longer in the wet-season form than the dry-season form (Table 7.3 , Fig. 7.6).

Egg size and reproductive effort. Mean daily egg weight declined with female age in *M. perseus* dry-season form ($F=101.4$, $P<0.0001$), but increased slightly in the wet-season form ($F=33.21$, $P<0.0001$) (Fig. 7.8). There were also strong phenotypic differences in mean egg weight and reproductive effort (Table 7.4). The dry-season form laid heavier eggs than the wet-season form, despite the fact that egg weights of the wet-season form increased with female age. However, reproductive effort of the wet-season form was about twice that of the dry-season form.

7.3.3 Comparison of species

Reproductive traits. Although the reproductive period was not significantly different between the three species (i.e. wet-season form on rotting fruit) (Table 7.7), the oviposition peak (maximum daily fecundity) was earlier and higher in *M. perseus* compared with *M. terminus* and *M. sirius* (Fig. 7.2). The relative proportion of eggs laid during the first four days after egg-laying commenced was also higher in *M. perseus* (32%) than in *M. terminus* (16%) or *M. sirius* (20%) (Fig. 7.5). Although the potential fecundity (Table 7.2, Fig. 7.4) and the recorded maximum number of eggs laid by captive females were similar (*M. perseus* wet-season form, 246; *M. terminus*, 260; and *M. sirius*, 246), mean realised fecundity was substantially higher in *M. perseus* than in *M. terminus* and *M. sirius*, which were similar (Table 7.7). The species also laid different size eggs with *M. perseus* laying smaller eggs than those of *M. terminus* or *M. sirius* (Table 7.7, Fig. 7.7). Although realised reproductive effort was similar between the three species, the error terms were large, and the potential reproductive effort was higher in *M. terminus* (Table 7.7).

Egg colour. *M. perseus* (wet-season form) and *M. sirius* exhibited pronounced egg colour variation with female age (Fig. 7.9). Eggs laid early in the reproductive period were green but those laid later were yellow. During the colour transition from green to yellow an 'intermediate' colour between the two was evident. The colour switch occurred more quickly in *M. sirius* in which

Table 7.7 Comparison of reproductive traits between the three *Mycalesis* species (wet-season form on rotting fruit). Results of analysis of covariance are indicated below for each trait, with pupal weight as covariate.

Species	N	Reproductive period	Egg weight	Max. daily fecundity	Realised fecundity	Potential reproductive effort
M. perseus	13	13.85 ± 1.04	0.462 ± 0.062	31.38 ± 1.68	163.85 ± 17.33	0.48 ± 0.04
M. terminus	15	12.67 ± 0.78	0.679 ± 0.065	20.80 ± 1.35	110.07 ± 12.71	0.73 ± 0.04
M. sirius	22	14.14 ± 0.68	0.657 ± 0.089	18.73 ± 1.02	110.68 ± 9.88	0.58 ± 0.02
Species		F=1.01, NS	F=78.51, P=0.0001	F=16.81, P=0.0001	F=3.40, P=0.042	F=13.43, P=0.0001
Covariate		F=0.48, NS	F=0.47, NS	F=1.90, NS	F=0.18, NS	F=0.05, NS

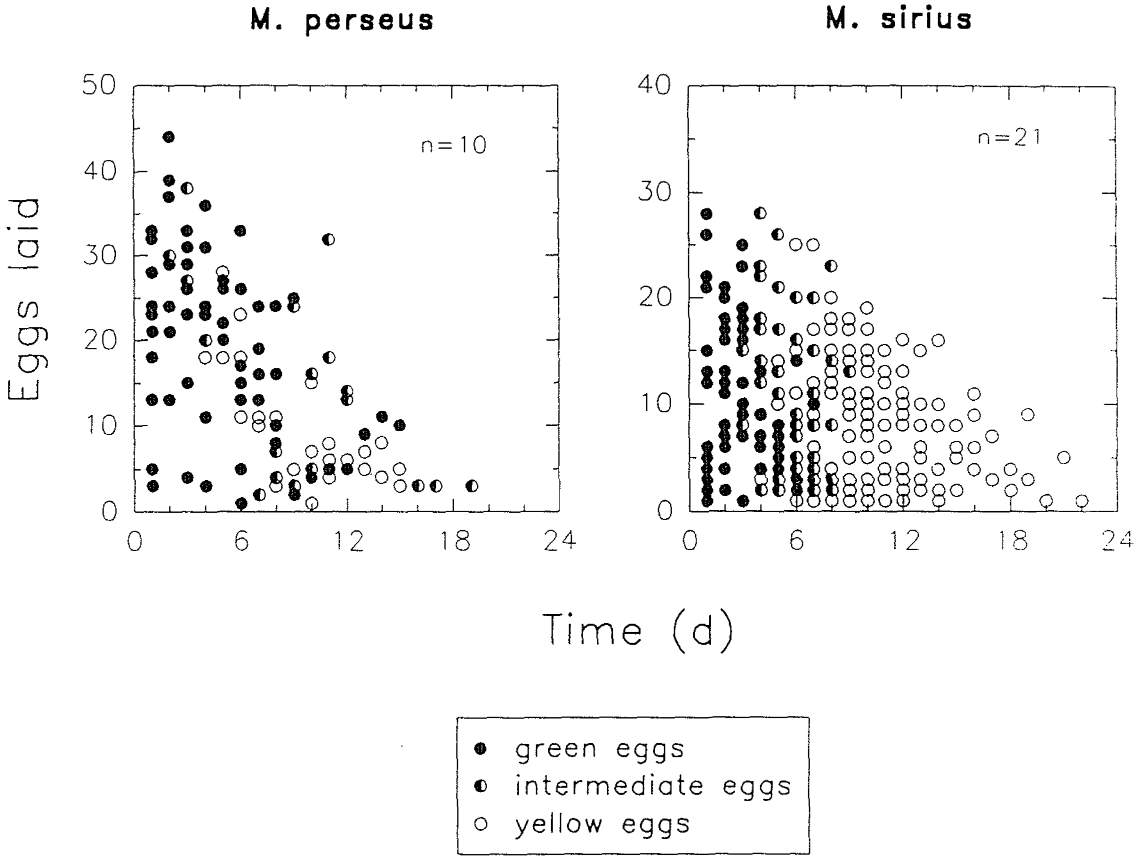

Fig. 7.9 Changes in egg colour with female age for *M. perseus* wet-season form and for *M. sirius*. Plotted are the number of eggs laid each day against time when egg-laying began. Sample sizes refer to the number of females examined.

only 29.2% of all eggs examined (n=2493) were green; in *M. perseus* green eggs represented 66.9% of all eggs (n=1845). *M. perseus* dry-season form also laid predominantly green eggs, though the colour change in this phenotype was not quantified. By contrast, *M. terminus* laid only yellow eggs, although those laid early in adult life tended to be of a richer golden-yellow.

Correlation of reproductive traits. Relationships amongst the reproductive traits were examined to determine the possible influence of factors on fecundity and egg size, other than the effects of phenotype and adult diet, and to establish if a trade-off exists between these two variables. Realised fecundity correlated strongly with daily maximum fecundity, but with the exception of *M. perseus* dry-season form, did not correlate significantly with pupal weight (Table 7.8). The number of eggs laid, however, did correlate positively with the reproductive period, that is, the length of time over which females laid their eggs, in four of the treatments. Also, in *M. terminus* and *M. sirius* fed on honey solution, realised fecundity was negatively correlated with the preoviposition period, indicating that females fed on this diet which delayed onset of egg-laying were likely to lay fewer eggs. Egg size correlated poorly with other reproductive traits and only one significant relationship was detected: a positive correlation between mean egg weight and realised fecundity in *M. perseus* wet-season form (Table 7.8). However, when data for the two seasonal forms of *M. perseus* were combined, a positive relationship between mean egg weight and pupal weight was evident (Fig. 7.10).

No inverse relationship was observed between mean egg weight and realised fecundity within treatments for any of the data sets. However, when mean egg weight and mean fecundity were analysed across species, a significant negative correlation was evident within the genus (r=-0.941, P<0.05) (Fig. 7.11). Neither mean egg weight nor mean fecundity was correlated with mean pupal weight (r=-0.597, P>0.10; r=0.730, P>0.10, respectively). Therefore, the relationship between egg weight and realised fecundity suggests a possible trade-off between egg size and egg number within *Mycalesis*, although the

Table 7.8 Correlation coefficients between realised fecundity and five reproductive traits for each species. Level of significance *=P<0.05, **=P<0.01, ***=P<0.001, NS=not significant.

Species	Preoviposition period	Reproductive period	Max. daily fecundity	Pupal weight	Mean egg weight
M. perseus dry-season f.		0.642^{**}	0.916^{***}	0.548^{*}	NS
M. perseus wet-season f.		NS	0.606^{*}	NS	0.652^{*}
M. terminus honey	-0.608^{**}	0.611^{**}	0.586^{*}	NS	
M. terminus rotting fruit	NS	0.577^{*}	NS	NS	NS
M. sirius honey	-0.523^{**}	NS	0.723^{***}	NS	NS
M. sirius rotting fruit	NS	0.642^{*}	0.814^{***}	NS	NS

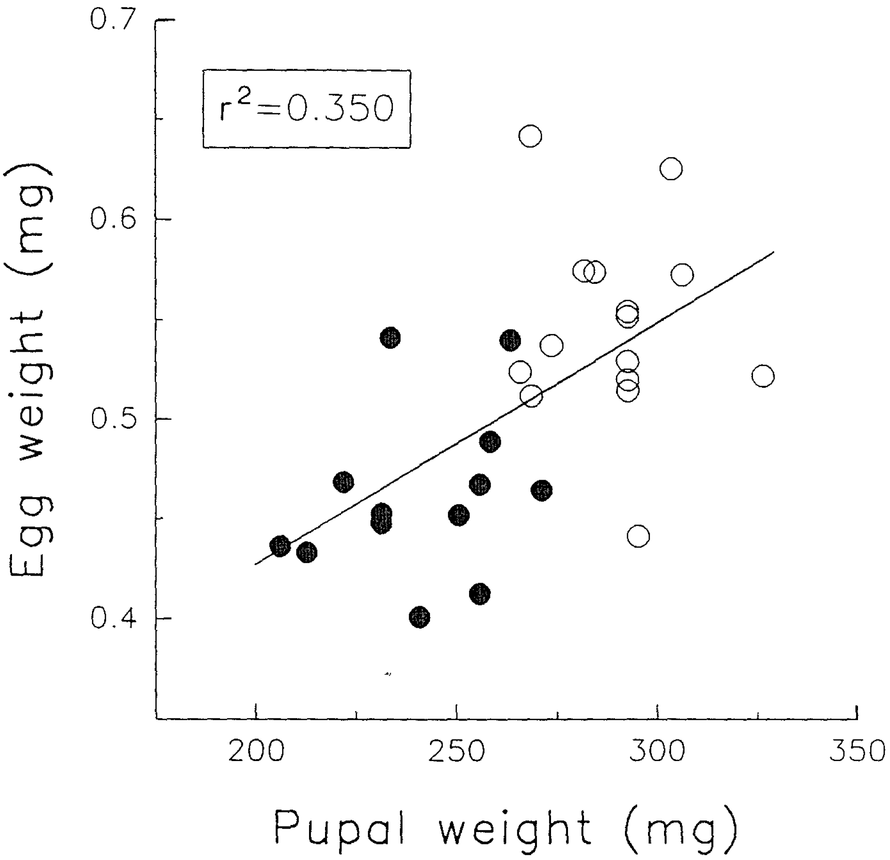

Fig. 7.10 Relationship between body size expressed as pupal weight and mean egg weight in *M. perseus* (○ dry-season form, ● wet-season form). The relationships are not significant for each seasonal form, but when data for the two forms are combined the relationship is highly significant. Regression equation: $y=0.00121x+0.185$ ($F=14.02$, 26 *d.f.*, $P=0.001$).

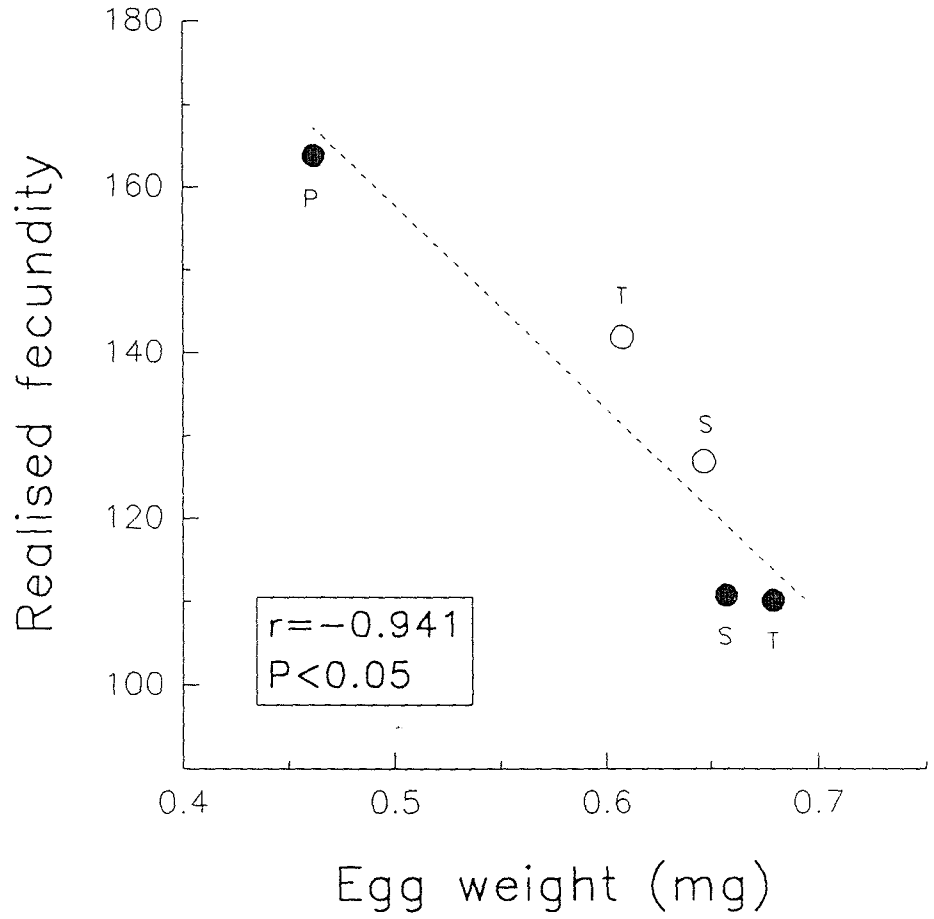

Fig. 7.11 Realised fecundity as a function of egg size in *Mycalesis* wet-season form (P=*M. perseus*, T=*M. terminus*, S=*M. sirius*). Points represent the mean number of eggs laid and the mean egg weight for each species. ● females fed on rotting fruit, ○ females fed on honey solution. Regression equation: $y=-245.43x+280.54$ ($F=22.97$, $P=0.0173$).

samples are small and the strength of the correlation is strongly influenced by an outlying point (*M. perseus*).

7.4 Discussion

7.4.1 Effect of adult diet

The adult diet of *M. sirius* had no affect on any of the traits measured. However, in *M. terminus* there were two main effects: (1) females fed on honey solution tended to live longer, and (2) females fed on rotting fruit maintained approximately equal egg weight over time while those fed honey solution laid smaller eggs with increasing age. Adult diet did not appear to affect fecundity in either *M. terminus* or *M. sirius*; although individuals fed on rotting fruit tended to lay fewer eggs, differences between the diets were not statistically significant.

Adult butterflies may feed from a wide range of substances, including nectar, mud puddles, carrion, animal excreta, sap and rotting fruit (Owen 1971, Downes 1973, Watt *et al.* 1974, Arms *et al.* 1974, Gilbert and Singer 1975, Pivnick and McNeil 1987, Boggs 1987, Boggs and Jackson 1991, Chapter 2). Such diverse feeding behaviour may have two functions: the replenishment of body fluids to avoid dehydration, and the assimilation of nutrients for reproductive functions (egg-laying/spermatophores) or soma (body maintenance, flight activity, longevity etc). For example, puddling behaviour in two male *Papilio* species is stimulated by sodium, an otherwise limited nutrient (Arms *et al.* 1974). The hesperiid *Thymelicius lineola* (Ochsenheimer) also acquires sodium by male puddling which is transferred to females at mating (Pivnick and McNeil 1987), and in male *Papilio glaucus* Linnaeus availability of nutrients (sodium and amino acids) in the adult diet may improve mating success and increase spermatophore size in subsequent matings (Lederhouse *et al.* 1990).

Many butterflies feed on nectar (e.g. Watt *et al.* 1974, Gilbert and Singer 1975, Boggs 1987, Chapter 2), and several studies have shown that the presence of sugars in the adult diet increases longevity and/or fecundity in Lepidoptera (Norris 1935, Stern and Smith 1960, David and Gardiner 1962, Cheng 1972, Murphy *et al.* 1983, Leather 1984, Pivnick and McNeil 1985, Hill and Pierce 1989, Hill 1989, 1992, Karlsson and Wickman 1990, Gu and Danthanarayana 1990, Boggs and Ross 1993). If essential nutrients are assimilated by adult feeding for reproductive functions, then females could potentially allocate these resources in several ways to maximise reproductive success: (1) increase reproductive output (realised fecundity), (2) increase egg size, or (3) enhance both fecundity and egg size, that is, increase reproductive effort. For instance, Hill and Pierce (1989) and Hill (1989) observed an increase in both realised fecundity and longevity, but not in egg weight, when females of two butterfly species were fed on medium (25%) diet of sugar concentration compared with a low (1%) sugar concentration. Boggs and Ross (1993) found that when individuals of the nymphalid *Speyeria mormonia* Edwards were allowed to feed on reduced amounts of 25% honey solution both realised and potential fecundity decreased whereas longevity remained unaffected.

In *M. terminus* and *M. perseus* wet-season form egg weight remained relatively constant or increased with age when females were fed on rotting fruit, even though oviposition rates declined in both species. This trend appears to be the first such instance recorded for Lepidoptera. In most species of Lepidoptera studied, females lay fewer and progressively smaller sized eggs during the oviposition period (e.g. Jones *et al.* 1982, Murphy *et al.* 1983, Wiklund and Persson 1983, Wiklund and Karlsson 1984, Karlsson and Wiklund 1984, 1985, Kimura and Tsubaki 1985, Boggs 1986, Wickman and Karlsson 1987, Karlsson 1987, Leather and Burnand 1987, Hill and Pierce 1989), a pattern which closely parallels that of *M. sirius*, and of *M. terminus* on honey solution. In nectar feeding species, this decline in egg production (i.e. egg number and egg size) occurs because females apparently cannot replace their reproductive reserves by extracting sufficient quantities of amino acids or other nutrients from nectar

(Wickman and Karlsson 1987, Karlsson 1987, Boggs 1987). The decline in egg size may be adaptive when egg size is positively correlated with offspring fitness (Chapter 8) since for insect species with relatively short and unpredictable life span the optimal strategy should be to lay their best eggs early in adulthood. By contrast, *M. terminus* and the wet-season form of *M. perseus* which feed primarily on rotting fruit are evidently able to mitigate this decline in egg weight over their life time so that older females can produce superior eggs, that is, lay higher quality offspring. Hence, availability of rotting fruit may play an important role in enhancing reproduction, especially in species with relatively longer adult lives, which may in turn contribute to the population dynamics.

These differences in egg weight patterns in *M. terminus* may reflect differences in the protein (nitrogen) content of the adult food resources. The protein content of ripe banana is 0.3 g per 100 g, about three times higher than that of 25% honey which is about 0.1 g per 100 g (Thomas and Corden 1977).

The *M. perseus* dry-season form, however, was unable to maintain constant egg weight with maternal age even though these females were given access to rotting fruit. It is possible that post-diapausing females are so resource depleted by the time egg-laying commences that these individuals are unable to benefit from adult feeding. In this context it is noteworthy that the reproductive period of the dry-season form was considerably less than that of the wet-season form.

The observed differences in reproductive traits between *M. terminus* and *M. sirius* in response to adult diet correspond with their known behaviour in the field. *M. sirius* does not feed on rotting fruits, and only rarely has been observed attending flowers (Chapter 2). In contrast, *M. terminus* (and to a lesser extent *M. perseus*) readily feeds on rotting fruits when these resources are available. Thus, because *M. sirius* adults rarely feed, and since the reproductive pattern is not affected when given access to rotting fruit or sugar in the laboratory, nutrients acquired during the larval feeding stage must comprise the main source for egg production in this species. *M. sirius* females may also benefit from male nutrients at mating because the species is more polyandrous than *M. terminus* and *M. perseus* (Chapter 4). By contrast, *M. terminus* may benefit when given access to rotting fruit because egg size is conserved, even though egg number declines during the oviposition period. It is therefore tentatively concluded that availability of rotting fruit to *M. terminus* (and probably *M. perseus*) plays an important secondary role in supplementing resources accumulated during larval period by producing better quality offspring over female lifetime so that reproductive success is enhanced, at least during the wet season. The role of rotting fruit on longevity, however, requires further attention because females clearly do not live as long as they do when given access to honey solution.

7.4.2 Effect of phenotype

There were major differences between the two seasonal forms of *M. perseus* in the way each allocated resources to offspring. The wet-season form had a much higher realised and potential fecundity, a larger potential reproductive effort, and laid smaller eggs. The dry-season form also had a shorter egg-laying period, and took slightly longer to reach peak daily fecundity, which may reflect a slower rate of egg maturation by females once diapause has been broken.

Thus adult diapause in the dry-season form of this tropical satyrine clearly represents a substantial cost to reproduction, a finding which has also been demonstrated in several temperate Lepidoptera which hibernate over winter (e.g. Fujiie 1980, Akkawi and Scott 1984). This lower fecundity of post-diapausing individuals compared with wet-season form individuals that reproduce directly, may arise in one of two ways. First, oocytes may be progressively resorbed so that resources are re-allocated away from reproduction during adult diapause. Alternatively, the longer-lived diapausing individuals may put less energy into reproduction and allocate more into soma (storage reserves, body structure etc.) during the juvenile stages so that the dry-season form has less resources for producing offspring, as has recently been demonstrated in the adult stage of the temperate nymphalid butterfly *Polygonia c-album* (L.) which hibernates over winter (Karlsson and Wickman 1989). The latter strategy essentially represents a trade-off between longevity and reproduction, and Karlsson and Wickman (1989) imply that this may be widespread in nature, especially if it is assumed that the structures of organisms are adjusted to their life expectancies (Boggs 1981a).

I have not tested the reproductive capacity of *M. perseus* dry-season form prior to diapause, but estimates of potential fecundity made from 10 freshly emerged females collected early in the season [in May 1991 at Townsville, mean=122.7 ± 26.13 (s.d.)] were not significantly different from those estimates generated in post-diapausing females collected late in the season (in December 1990 at Townsville, mean=137.3 ± 43.26, Table 2) (t=0.81, 52 *d.f.*, P=0.425). However, the estimates should be treated with caution because difficulty was experienced in counting the oocytes in the diapausing adults as they were scarcely visible (<0.2mm wide) and surrounded by numerous fat bodies. Hence, it is tentatively concluded that the reduced reproductive effort of the dry-season form is the result of a trade-off between life expectancy (soma) and reproduction. Furthermore, dry-season females are considerably larger than wet-season forms (Chapter 5), which might indicate that these individuals assemble a proportionally larger soma to increase longevity.

7.4.3 Comparison of species

These three closely related species, superficially so similar in many aspects of their reproductive patterns, in fact have substantial differences in resource allocation. Moreover, as discussed above adult diet and phenotype (seasonal form) profoundly effect several reproductive traits.

Thus females of *Mycalesis* spp (i.e. wet-season forms fed on rotting fruit) are remarkably similar in oviposition pattern, reproductive period, potential fecundity, and the proportion of eggs laid, but differ quite markedly with respect to realised fecundity, egg size and potential reproductive effort. Perhaps the most interesting aspect is in the way each has evolved different allocation strategies with respect to egg size since the weight of the total egg mass produced is similar. *M. terminus* and *M. sirius* lay eggs similar in size and in number, whereas *M. perseus* lays many smaller eggs (Table 7.7). Moore (1985) found similar differences in egg size and realised fecundity between wild caught wet-season forms of *M. perseus* (mean egg weight 0.58 mg, n=30; mean number of eggs laid 168, n=4) and *M. terminus* (mean egg weight 0.76 mg, n=30; mean number of eggs laid 134, n=4). Moreover, *M. perseus* lays a relatively higher proportion of eggs early in adulthood once egg-laying starts, suggesting greater urgency in this species to oviposit and disseminate its egg load.

Wiklund and Karlsson (1988) pointed out that butterflies can effectively increase fecundity in two ways: by becoming larger, or by producing smaller eggs. Although body size in *Mycalesis* varies seasonally, *M. perseus* is the smallest of the three satyrines (Chapter 6). The relatively higher realised fecundity in *M. perseus*, compared with *M. terminus* and *M. sirius*, has therefore been achieved by the production of smaller eggs rather than by an increase in body size.

What then is the adaptive significance of these interspecific differences in reproductive strategies? The egg size and fecundity differences in these butterflies may be associated with differences in the environments in which they live. *M. perseus* frequents more seasonal and less predictable habitats, chiefly savanna woodland, where breeding is limited to a relatively short period during the favourable (wet) season. By contrast, *M. terminus* and *M. sirius* live in more predictable environments, particularly rainforest edge and paperbark swampland respectively, where hostplants are in general more persistent and breeding is more consistently favoured (Chapter 3). Hence, there may be strong selective pressures on *M. perseus* to increase populations rapidly during the good times to counter environmental uncertainty, that is, a classic 'r-selected' strategy (MacArthur and Wilson 1967, Pianka 1970, Stearns 1976, Southwood 1988).

Because egg weight and realised fecundity did not correlate with body size (pupal weight), such profound differences in reproductive strategies within *Mycalesis* may reflect an evolutionary trade-off between egg size and number (Fig. 7.11). Although more comparative data is really needed to form a more general relationship (see Pagel and Harvey (1988) for review of analytical techniques) an inverse relationship between egg number and egg size exists across many organisms (e.g. Smith and Fretwell 1974, Parker and Begon 1986, Lloyd 1987, Elgar 1990) so that for a given reproductive effort a female could produce many small eggs or a few large ones. In butterflies the extent of this relationship has not been examined rigorously, but the pattern appears in the data presented by Labine (1968) and Young (1972) and also in the northern hemisphere satyrines (see Wiklund *et al.* 1987).

7.4.4 General Discussion

Although the total number of eggs laid may be affected by temperature (e.g. Leather 1984, Smith 1986) I assume that the measured differences in realised fecundity between *Mycalesis* spp. are in fact real and not because *M. perseus* females were kept at higher ambient temperature. This line of reasoning is based on the fact that *M. perseus* has a higher temperature optimum for egg development and survival (30-35°C) than *M. terminus* and *M. sirius* (25-30°C) (Chapter 6) so it is probable that *M. perseus* also requires a higher temperature for optimal egg production, particularly since females lay very poorly at 25°C. Egg-laying rates for each species should have been close to their respective optimal temperatures.

In many insects realised or lifetime fecundity correlates with body size (see Honek 1993 for review). In Lepidoptera, body size has been measured as either pupal weight (Danthanarayana 1975, Jones *et al.* 1982, Miller *et al.* 1983, Gilbert 1984, Haukioja and Neuvonen 1985, Smith 1986), wing length (Suzuki 1978, Dunlap-Pianka 1979, Carroll and Quiring 1993) or female weight (Blau 1981, Marshall 1988, Hill and Pierce 1989, Berger 1989, Banno 1990, Karlsson and Wickman 1990). However, in contrast to these studies pupal weight in *Mycalesis* spp. correlated very poorly with realised fecundity, but did correlate well with potential fecundity (Fig. 7.4). Moreover, female *Mycalesis* lay only about half of their full reproductive potential. Although the mean longevity (i.e. preoviposition period plus reproductive period) for captive female *M. terminus* and *M. sirius* on rotting fruit was only 17 days, this figure is probably realistic because the mean lifespan of *M. terminus* females estimated from the field using mark-release-recapture is about 10 days (maximum 33 days) (Moore 1985). Since adult diet does not affect fecundity in these two species, the positive correlation between potential fecundity and pupal weight suggests that the total number of oocytes is fixed at eclosion. This indicates that body size gives a reasonable index of reproductive investment in terms of the amount (but not necessarily quality) of resources gathered in the larval stage, but this apparently does not relate to the actual number of eggs laid, at least in captivity.

This lack of a correlation between body size and realised fecundity has been noted in several other species (Fenemore 1977, Wiklund and Persson 1983, Karlsson and Wiklund 1984, Boggs 1986, Leather and Burnand 1987) and Carroll and Quiring (1993) have recently suggested that interactions between size and temperature may obscure body size-fecundity relationships when they exist. On the other hand, Leather (1988) suggests that longevity, and the factors which influence it, such as temperature and adult food, is probably more important in accounting for variation in the number of eggs laid than female size *per se*. In this regard *Mycalesis* spp. are in agreement with Leather's hypothesis because in four of the six treatments the reproductive period correlated significantly with reproductive output (Table 7.8). The relationship between longevity and fecundity is also apparent in other Lepidoptera (Proshold *et al.* 1982, Wiklund and Persson 1983, Leather 1984, Leather and Burnand 1987).

Finally, the age-dependent egg colour polymorphism in *M. perseus* and *M. sirius* has hitherto been recorded in only one other butterfly, the temperate satyrine *Coenonympha pamphilus* (Linnaeus) (Wickman and Karlsson 1987). In this species Wickman and Karlsson established that within females the colour transition depended on the number of eggs laid, and that within eggs the colour did not vary with developmental time, but they did not offer a mechanistic or ecological explanation. These findings are in accord with *M. perseus* and *M. sirius*. It is quite possible that females are eliminating a colour pigment, probably chlorophyll accumulated during larval feeding that is not lost through the larval or pupal stages (F. Douglas, pers. comm.). If this is so, then this would account for the progressive change from green to yellow with an 'intermediate' stage. By contrast, *M. terminus* does not lay green eggs which may imply that this species eliminates the green pigment before adult eclosion. Whether the egg colour polymorphism is adaptive, for example, in relation to substrate colour is difficult to determine, and it is curious that *M. perseus* and *M. sirius* should differ in the proportion of green eggs laid and hence in the rate at which the pigment is lost. Wickman and Karlsson (1987) found that egg colour did not match the colour of the substrate on which the female deposited the egg, and they suggested that green and yellow eggs may be equally well concealed on grass leaves when considering the general background so the trait may be nonadaptive.

CHAPTER 8: EGG SIZE VARIATION

8.1 Introduction

Egg size is a critical aspect of an insect's reproductive strategy in 'deciding' how limited resources should be best allocated to offspring. Variation in offspring size may influence the survival of the female parent's progeny, and therefore affect her overall reproductive success. Several studies have shown that captive females from a wide range of Lepidoptera lay progressively smaller eggs during the oviposition period (Telfer and Rutberg 1960, Campbell 1962, Harvey 1977, Richards and Myers 1980, Jones *et al.* 1982, Murphy *et al.* 1983, Karlsson and Wiklund 1985, Kimura and Tsubaki 1985, Boggs 1986, Leather and Burnand 1987, Hill and Pierce 1989, Chapter 7). Moore and Singer (1987) have questioned whether such declines in egg weight with maternal age may in fact be an artefact of captivity. However, strong circumstantial evidence from two butterfly species examined recently under field conditions (Wickman and Karlsson 1987, Karlsson 1987), together with the fact that so many Lepidoptera (at least 17 species reported in the literature) lay heavier eggs early in adulthood, suggest the pattern is widespread.

While both mechanistic and theoretical models (Wiklund and Karlsson 1984, Begon and Parker 1986) have been proposed to account for the inverse relationship between egg weight and female age, the adaptive significance of the egg size variation is not clear. Capinera *et al.* (1977) noted that larger eggs of *Lymantria dispar* (L.) had more yolk available for embryonic development than smaller eggs. Despite this apparent advantage to bigger eggs, a series of extensive experiments on five Swedish satyrid butterflies by Wiklund and co-workers (Wiklund and Persson 1983, Wiklund and Karlsson 1984, Karlsson and Wiklund 1984, 1985) failed to find any significant connection between egg weight and offspring fitness. Richards and Myers (1980) did find that larger eggs of *Tyria jacobaeae* (L.) had greater hatching success, but the correlation was weak ($r^2 = 18\%$) and occurred only when eggs were reared at low temperature. More recently, Wickman *et al.* (1990) and Rossiter (1991) reported significant relationships between offspring size and several fitness traits, but again the correlations were weak, particularly in the latter study in which the proportion of total variability in two traits (male hatch time, female pupal weight) explained by egg weight was only 6%.

An aspect which has received little detailed attention in previous work concerns the hostplant quality upon which newly emerged larvae must feed. Several workers (Labine 1968, Dunlap-Pianka 1979, Murphy *et al.* 1983) have suggested that larger eggs should give rise to larger first instar larvae with larger mandibles enabling them to cope better with tougher foliage. If this hypothesis is correct the relative coarseness of the hostplant may set a critical lower limit on egg size. Moreover, egg size should be positively related with leaf toughness: that is, species in which the larvae feed on tougher plants should lay larger eggs. Both these expectations were met in interspecific comparisons of hesperiids which feed as larvae on grasses (Nakasuji and Kimura 1984, Nakasuji 1987), but not in temperate grass-feeding satyrids (Wiklund and Karlsson 1984, Karlsson and Wiklund 1984). However, any increased fitness of larger eggs may only be detectable when leaf quality is poor (Jones *et al.* 1982). For instance, Nakasuji and Kimura (1984) clearly showed that first instar larvae from both smaller (first and second generation) and larger eggs (third generation) of the hesperiid *Parnara guttata guttata* Bremer et Grey survived equally well on the relatively soft grass *Oryza sativa* (L.), but on the coarser *Imperata cylindrica* (L.) only third generation larvae survived. In other words, the selective advantage to larger offspring may not occur when host conditions are optimal. The aim of this study is to determine whether variation in hostplant quality affects offspring fitness in three tropical satyrine butterflies.

The three *Mycalesis* spp. chosen lay eggs which are highly variable in size, ranging from 0.276 to 0.877 mg in *M. perseus*, 0.397-0.866 mg in *M.*

terminus and 0.376-0.913 mg in *M. sirius* (Chapter 7). Egg weight usually declines with female age, although the rate of decline is influenced by adult diet, particularly in *M. terminus* and *M. perseus* when females are fed on rotting fruit. [In *M. perseus*, egg weights may increase slightly with age]. *M. perseus* lays the smallest eggs on average, while the mean egg weights of *M. terminus* and *M. sirius* are similar, around 0.61-0.68 mg. Mean egg weights in *M. perseus* also vary seasonally according to phenotype, from 0.46 mg in the wet-season form to 0.55 mg in the dry-season form (Chapter 7).

8.2 Materials and methods

The three butterfly species in this study were collected as adult females from Cardwell (18°16'S, 146°01'E) and Townsville (19°05'S, 146°49'E) in north-eastern Queensland, Australia, during the late wet seasons of 1989 and 1990. The species are multivoltine but may enter reproductive dormancy during the dry season (Chapter 4). The habitats in which they live consists of lowland open forest, woodland and rainforest edge close to the coast. In common with most other Australian satyrines the life histories and larval host plant preferences are poorly documented. Larvae of *Mycalesis* spp. feed on a range of grasses, including *Themeda triandra* Forrskal and *Panicum maximum* Jacq. which occur abundantly where the butterflies frequent (Chapter 2). Eggs are laid singly on the underside of grass blades and egg-laying usually occurs during the afternoon. In captivity, *M. perseus* females preferentially select high quality 'young' growth, whereas *M. terminus* is relatively unselective with respect to host quality (Moore 1986). The host preferences of *M. sirius* are not known.

8.2.1 Larval rearing

On the day of capture the females were transferred to 0.7 X 0.7 X 0.7 m flight cages. These were kept outside in a shade house on the university campus and supplied with potted tussocks of *T. triandra* or *P. maximum* for oviposition. The cages were watered daily and supplied intermittently with rotting fruit as an adult food source. Every day, before 900 hrs, all eggs laid on the previous day (i.e. within 20 hrs) were carefully removed from the grass blade with a fine pair of tweezers, weighed on a Cahn 21 automatic electrobalance (0.0001 mg accuracy), and placed individually into plastic petri dishes with filter paper. The petri dishes were then transferred to 25°C ± 0.5°C and a 12:12 L:D cycle. Eggs were checked daily until hatching. On eclosion each newly emerged larva was transferred with a fine brush to a freshly cut strip of the hostplant and reared alone in a petri dish with moistened filter paper until pupation. Larvae were checked every day and the leaf strips and filter paper were changed every 1-3 days. Pupae were weighed 24 hrs after pupation on the Cahn electrobalance (0.1 mg accuracy).

Two grass species were tested, native *Themeda triandra* and introduced *Panicum maximum*. *T. triandra* typically occurs in the more open woodland/forest areas; the leaves are rather coarse but the blades are narrow (<10 mm across). *P. maximum* grows as relatively large tussocks, reaching 2 m high during the wet season, and its leaves may span 50 mm across; the species is widespread along moister gullies and creeks. The leaves of both species typically dry out, that is, wither and become brown in the dry season.

8.2.2 Hostplant quality

Three different leaf qualities of *P. maximum* were tested, *viz.* 'soft', 'intermediate' and 'tough', but only one leaf quality ('soft' foliage) was examined for *T. triandra*. The 'soft' category for *Panicum* comprised older growth arising at or near the apex of blades, while the 'tough' category comprised the basal portion of the blade. These categories were readily differentiated qualitatively and quantitatively according to leaf toughness, as measured by a portable penetrometer (Sands and Brancatini 1991) (Table 8.1). [Lucas and Pereira (1990) provided an alternative but more elaborate method for measuring fracture toughness of leaves, however, for the purposes of this study the leaf penetrometer was found to give quick, accurate and repeatable field

Table 8.1 Leaf toughness and nitrogen content of *Themeda triandra* and of three arbitrary leaf classes of *Panicum maximum*.

Leaf category	Toughness (gms) mean ± s.d.	n	% Nitrogen mean ± s.d.
Themeda	60.4 ± 7.91	40	1.38 ± 0.03
Panicum			
soft	42.9 ± 7.21	30	1.56 ± 0.04
intermediate	62.5 ± 11.53	30	1.46 ± 0.03
tough	71.0 ± 14.83	30	1.10 ± 0.01

measurements]. The total nitrogen content was also determined in the laboratory for each leaf category using the semi-micro Kjeldahl procedure (Alan 1989). Differences in leaf toughness and nitrogen content between these categories for *Panicum* were significant (F=46.03, 2,87 *d.f.*, $P<0.0001$ for leaf toughness; F=104.8, 2,3 *d.f.*, P=0.0017 for nitrogen content); the foliage of *Panicum*-tough contained the lowest amount of nitrogen (Table 8.1). The leaf toughness of *Panicum*-intermediate was similar to that of *Themeda* but the nitrogen content of *Themeda* was lower than that of *Panicum*-soft (t=5.82, 2 *d.f.*, P=0.028).

8.2.3 Egg volume and larval size

Egg volume and the size of newly emerged first instar larvae were determined from a sample of eggs of known weight for each butterfly species. Eggs of *Mycalesis* spp. are approximately spherical in shape, although from the point of attachment they are slightly smaller in height than in width. The size of each egg was estimated by measuring the height (h) and radius (r) using a dissecting microscope fitted with calibrated eyepiece micrometer (0.05 mm accuracy). The volume was then calculated using the formula $2/3\pi r^2 h$. Two attributes of larval size were also measured, head capsule width and body length, again using the dissecting microscope and micrometer.

8.2.4 Experimental design and statistical analysis

Three offspring fitness parameters were examined in this study, *viz.* larval survival, larval developmental time and pupal weight in relation to egg size. The experiment was performed across four hostplant treatments to determine how hostplant quality (particularly leaf toughness) affects larval performance, by comparing the fitness parameters for larvae derived from heavier eggs with those derived from lighter eggs. Table 8.2 summarises the experimental design and gives the number of eggs, and their respective weights, used for each treatment.

In previous studies fitness variables have been analysed by arranging the data set in ascending order of egg weight, dividing it in half and then comparing the variables amongst the two egg weight groups (i.e. light eggs versus heavy eggs). In the present study, however, the unbalanced data set and multiple hostplant qualities examined makes such an analysis less efficient. Moreover, egg weights differed slightly among treatments within species. The analytical techniques for this study were chosen to overcome these shortcomings.

For each butterfly species larval survival was analysed by two-way analyses of variance using egg weight as the dependent variable. The two factors examined on egg weight were larval SURVIVAL (3 levels) and HOSTPLANT quality (4 levels). The three SURVIVAL categories were (i) larvae which died at instar I, (ii) larvae which died at instar II, (iii) larvae which survived to instar III. If larvae from smaller eggs are less able to cope with tough leaves, we would predict that the average weight of eggs giving rise to first instar larvae which died, would be less than the average weight of eggs giving rise to larvae which survived. This procedure gives a more sensitive analysis than the comparisons of frequencies which might otherwise be used. Later instar larvae should be less affected by leaf toughness because of their comparatively larger head capsules. [Most larvae in fact which survived beyond

Table 8.2 Experimental design showing the number of eggs and the mean and range of egg weights tested on each hostplant treatment for each butterfly species.

Species	Hostplant	Egg weight (mg)		
		mean ± s.d.	range	N
M. perseus	*Panicum*-soft	0.54 ± 0.100	0.331-0.808	119
	Panicum-intermediate	0.59 ± 0.095	0.441-0.877	115
	Panicum-tough	0.48 ± 0.067	0.311-0.619	71
	Themeda-soft	0.52 ± 0.075	0.344-0.640	37
M. terminus	*Panicum*-soft	0.65 ± 0.083	0.517-0.850	77
	Panicum-intermediate	0.61 ± 0.075	0.414-0.784	92
	Panicum-tough	0.64 ± 0.078	0.492-0.839	78
	Themeda-soft	0.68 ± 0.067	0.540-0.794	51
M. sirius	*Panicum*-soft	0.70 ± 0.075	0.533-0.837	80
	Panicum-intermediate	0.70 ± 0.101	0.486-0.863	84
	Panicum-tough	0.69 ± 0.083	0.488-0.856	80
	Themeda-soft	0.66 ± 0.096	0.414-0.848	139
Total Eggs				1023

the third instar reached pupation, although some were lost through viral infection]. Hostplant treatments in which either all larvae died or all survived, were not included in these analyses.

The analyses of larval developmental time and pupal weight were more direct. Both variables were analysed as two-way analyses of covariance, classifying the data by HOSTPLANT quality and SEX, and using egg weight as the covariate. All statistical analyses were performed using the generalised linear models procedure (SAS Institute, Inc., 1988).

8.3 Results

8.3.1 Egg and larval size

In all three butterfly species egg volume varied positively with egg weight (Fig. 8.1). The relationships confirm an underlying assumption of previous studies that egg volume is in fact proportional to weight, and also illustrate the magnitude of divergence in slopes that may arise amongst closely related taxa. *M. perseus* eggs (Fig. 8.1d) appear to be less dense, being lighter for a given volume, than *M. terminus* or *M. sirius* eggs. Alternatively, the shape of *M. perseus* eggs may be slightly different so that the estimated volume was not as accurate as that measured for the two other species.

Egg size was positively correlated with both the diameter of the head capsule and the body length of newly eclosed first instar larvae (Fig. 8.2). Head capsule width was more tightly correlated with egg size than body length for all species. *Mycalesis* larvae from the heavier eggs probably, therefore, had bigger mandibles.

8.3.2 Larval survival

The proportion of larvae which survived to the third instar or beyond varied

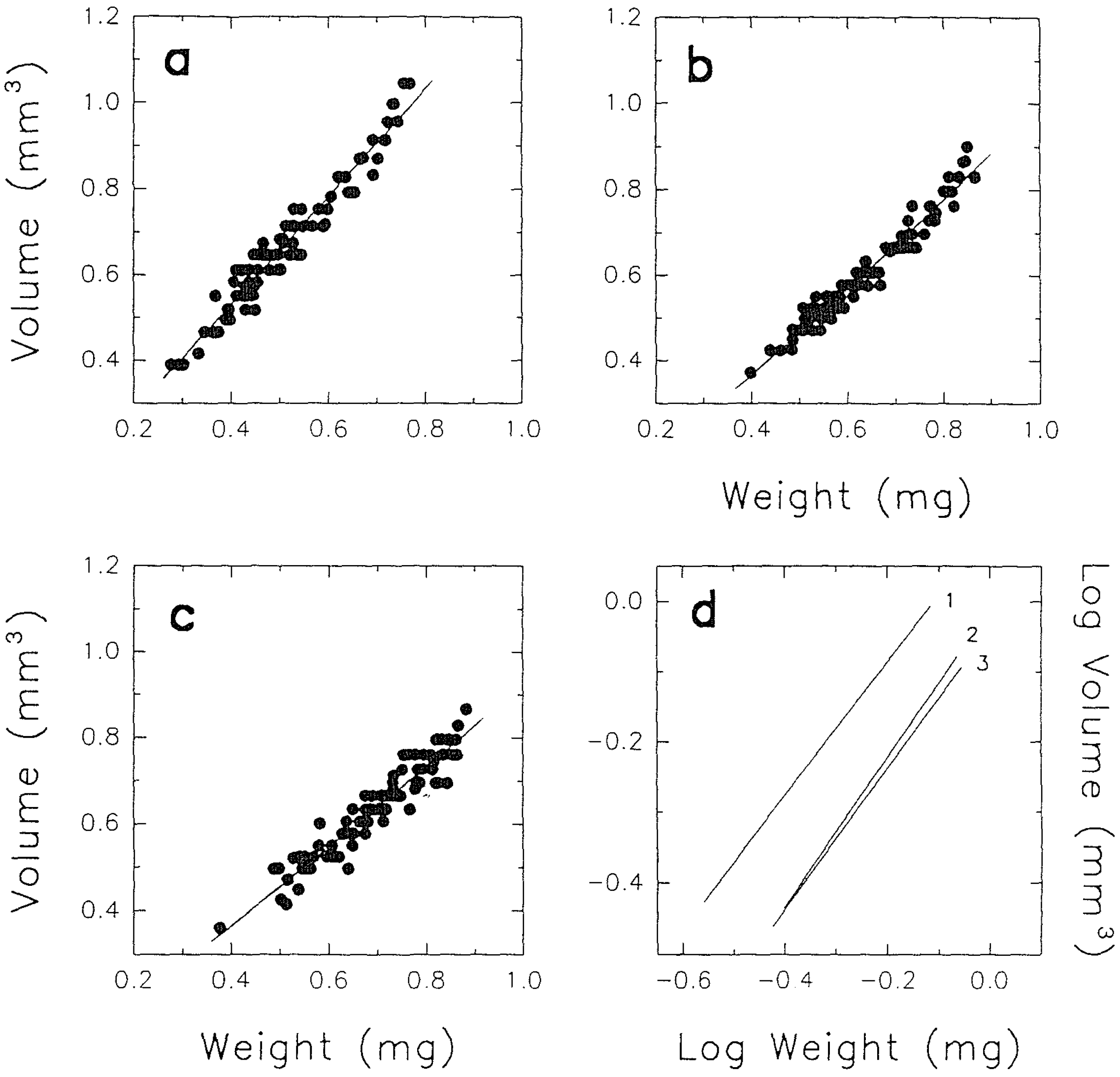

Fig. 8.1 Relationships between egg size and egg weight for (a) *M. perseus*, (b) *M. terminus* and (c) *M. sirius*, and (d) comparison of the fitted linear regression lines after logarithmic transformation (1=*M. perseus*, 2=*M. terminus*, 3=*M. sirius*). Regression equations: *M. perseus*, $y=1.267x^{0.948}$ ($r=0.97$, n=93); *M. terminus*, $y=0.975x^{1.058}$ ($r=0.98$, n=95); *M. sirius*, $y=0.914x^{0.994}$ ($r=0.95$, n=94).

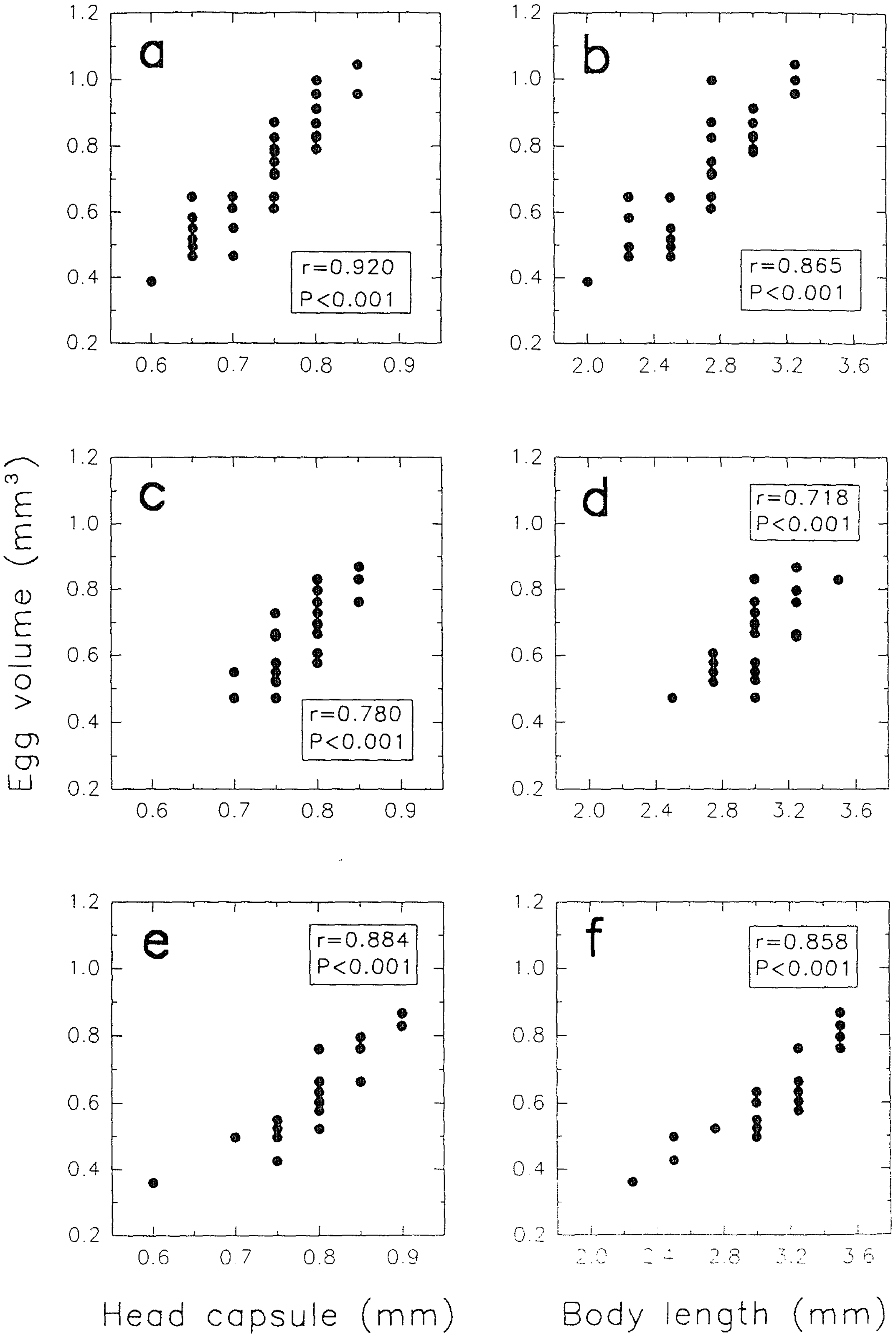

Fig. 8.2 Relationship between egg size and the size of newly emerged first instar larva, measured in terms of head capsule diameter and body length, for (a,b) *M. perseus*, (c,d) *M. terminus* and (e,f) *M. sirius*. Sample sizes for the three species are 40, 31 and 32 respectively.

markedly between the four hostplant treatments (Fig. 8.3). Nearly all larvae survived on native *Themeda* (with *M. perseus* having 100% survival) but only five larvae (all *M. sirius*) survived on introduced *Panicum*-tough. Consequently larval survival on *Panicum*-soft was lower than that on *Themeda* in all three satyrines. On *Panicum*-intermediate, *M. perseus* survived better than *M. sirius*, while *M. terminus* did very poorly with only seven larvae (7.6%) surviving. Hence, larval survival decreased with increasing leaf toughness on *Panicum*. However, leaf toughness could not explain variation in survival between host species, since *Themeda* and *Panicum*-intermediate were of similar leaf toughness.

Results for the two-way ANOVA comparing egg weights among HOSTPLANT treatments-larval SURVIVAL categories are given in Table 8.3. Interaction effects were not significant. The cell means for each survival-hostplant category are presented in Figure 8.4. In each species, SURVIVAL was highly significant, with the weights of eggs being considerably higher for larvae which survived to the third instar than those which died at the first or second instar. HOSTPLANT quality was significant only in *M. perseus*, with higher egg weights being recorded on the coarser *Panicum*-intermediate than on *Panicum*-soft or *Themeda*. This is a sampling artefact which needs to be taken into account in interpreting other effects since the average egg weights tested on *Panicum*-intermediate were higher than those on *Panicum*-soft by about 0.05 mg (Table 8.2). [In the other two species egg weights were more evenly distributed among treatments].

M. terminus and *M. sirius* showed a clear separation of egg weights between the survival categories on the tougher nitrogen-poor host *Panicum*-intermediate. A less marked separation occurred on *Themeda* and *Panicum*-tough for *M. sirius*, and for these two species there was almost no separation on the nitrogen-rich *Panicum*-soft (Fig. 8.4). *M. perseus* displayed a similar separation in egg weights between the survival categories to *M. terminus* and *M. sirius* on the coarser hosts, but heavier eggs also did better than lighter eggs on

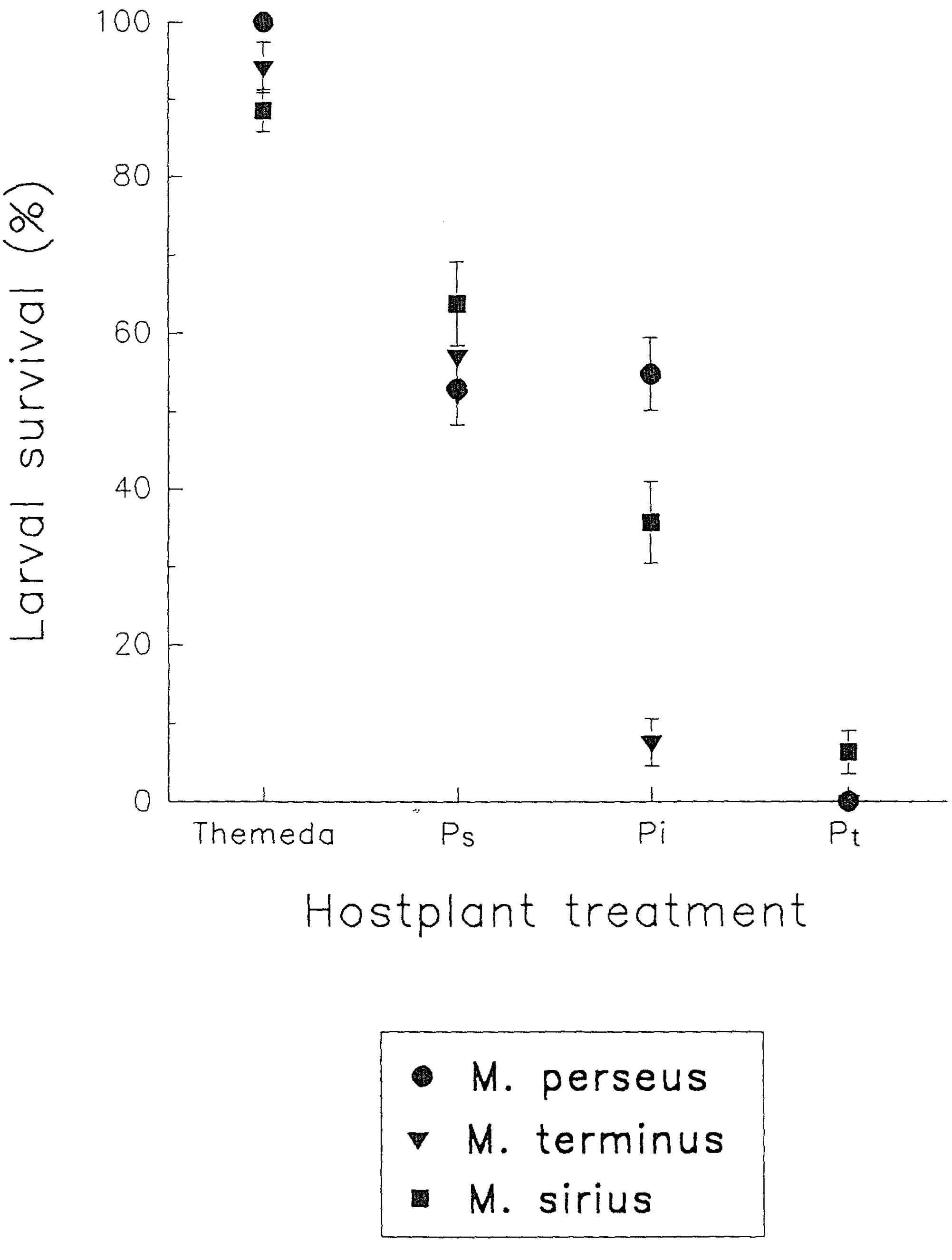

Fig. 8.3 Survival rates of larvae for the three satyrine species with respect to hostplant treatment: *Themeda*, *Panicum*-soft (Ps), *Panicum*-intermediate (Pi) and *Panicum*-tough (Pt).

Table 8.3 Two-way analysis of variance examining effects of larval survival and hostplant quality on egg weight for each butterfly species.

Source of variation	*d.f.*	MS	*F*	*P*
M. perseus				
SURVIVAL	2	0.1653	25.94	0.0001
HOSTPLANT	3	0.1140	17.88	0.0001
S x H	3	0.0147	2.31	NS
Error	333	0.0064		
M. terminus				
SURVIVAL	2	0.0237	4.29	0.0147
HOSTPLANT	3	0.0011	0.19	NS
S x H	4	0.0129	2.34	NS
Error	288	0.0055		
M. sirius				
SURVIVAL	2	0.0482	6.14	0.0024
HOSTPLANT	3	0.0172	2.19	NS
S x H	6	0.0094	1.20	NS
Error	371	0.0079		

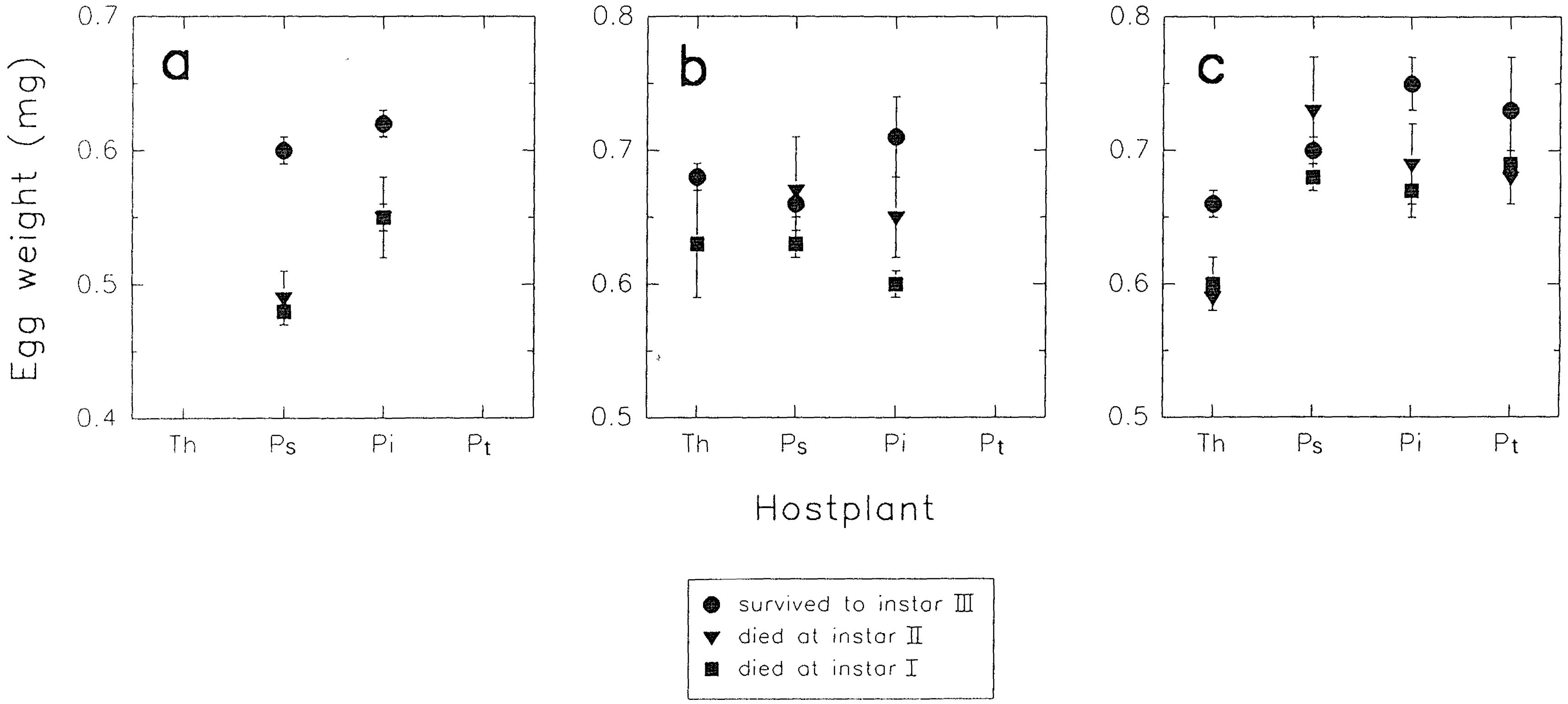

Fig. 8.4 Egg weights (mean ± S.E.) for each larval survival category in relation to hostplant treatment for the three butterfly species: (a) *M. perseus*, (b) *M. terminus*, (c) *M. sirius*. Th=*Themeda*, Ps=*Panicum*-soft, Pi=*Panicum*-intermediate, Pt=*Panicum*-tough. Egg weights are not shown for *M. perseus* on *Panicum*-soft, or *M. perseus* and *M. terminus* on *Panicum*-tough.

Panicum-soft. Egg weights for larvae which survived beyond third instar were higher on *Panicum*-intermediate than on *Panicum*-soft in all three species.

Hence, egg weight had a substantial effect on offspring fitness in terms of the survival categories tested in all three satyrines. Larvae derived from heavier eggs in *M. terminus* and *M. sirius* had a much better chance of reaching adulthood than lighter eggs when reared on the tougher hosts, whereas in *M. perseus*, heavier eggs had greater fitness when reared on either *Panicum*-soft or *Panicum*-intermediate. Leaf toughness could not, however, account for differences in survival between host species.

8.3.3 Larval developmental time

Egg weight affected larval developmental time for *M. sirius* but not for the two other species (Table 8.4). A negative correlation between developmental time and egg weight occurred in both males and females reared on *Themeda* (Fig. 8.5a) (analysis of the regressions give F=9.890, 43 *d.f.*, P=0.0030 for males, F=8.434, 53 *d.f.*, P=0.0053 for females), indicating that larvae from heavier eggs developed faster on this hostplant. Results for the two-way ANCOVA (Table 8.4) also showed that both SEX and HOSTPLANT had a profound effect on larval developmental time in all three butterfly species. Males developed faster than females, and both sexes took longer on *Themeda* than on *Panicum*. Interaction effects involving the covariate in the analysis were not significant.

8.3.4 Pupal weight

Egg weight affected subsequent pupal weight for *M. perseus* but not for the two other species (Table 8.5). A positive correlation between pupal weight and egg weight occurred in both sexes reared on *Panicum*-soft (Fig. 8.5b) (analysis of the regressions give F=12.05, 25 *d.f.*, P=0.0019 for males, F=6.315, 23 *d.f.*, P=0.0194 for females), indicating that larvae from heavier eggs produced larger pupae on this hostplant. A significant interaction occurred between the

Table 8.4 Two-way analysis of covariance of egg weight on larval developmental time for each butterfly species.

Source of variation	*d.f.*	MS	*F*	*P*
M. perseus				
SEX	1	189.35	21.43	0.0001
HOSTPLANT	2	255.70	28.94	0.0001
S x H	2	6.18	0.70	NS
EGG WEIGHT	1	1.96	0.22	NS
Error	125	8.84		
M. terminus				
SEX	1	125.96	17.54	0.0001
HOSTPLANT	2	366.43	51.01	0.0001
S x H	1	10.97	1.53	NS
EGG WEIGHT	1	5.83	0.81	NS
Error	68	7.18		
M. sirius				
SEX	1	324.01	28.37	0.0001
HOSTPLANT	1	843.22	73.84	0.0001
S x H	1	9.26	0.81	NS
EGG WEIGHT	1	220.57	19.31	0.0001
Error	139	11.42		

Table 8.5 Two-way analysis of covariance of egg weight on subsequent pupal weight for each butterfly species.

Source of variation	*d.f.*	MS	*F*	*P*
M. perseus				
SEX	1	29672.25	67.82	0.0001
HOSTPLANT	2	7605.35	17.38	0.0001
S x H	2	234.38	0.54	NS
EGG WEIGHT	1	4497.86	10.28	0.0017
Error	125	437.48		
M. terminus				
SEX	1	1902.21	7.92	0.0064
HOSTPLANT	2	7560.85	31.50	0.0001
S x H	1	4.97	0.02	NS
EGG WEIGHT	1	7.38	0.03	NS
Error	73	240.05		
M. sirius				
SEX	1	20394.54	43.96	0.0001
HOSTPLANT	1	75144.71	161.96	0.0001
S x H	1	4737.88	10.21	0.0017
EGG WEIGHT	1	179.02	0.39	NS
Error	143	463.96		

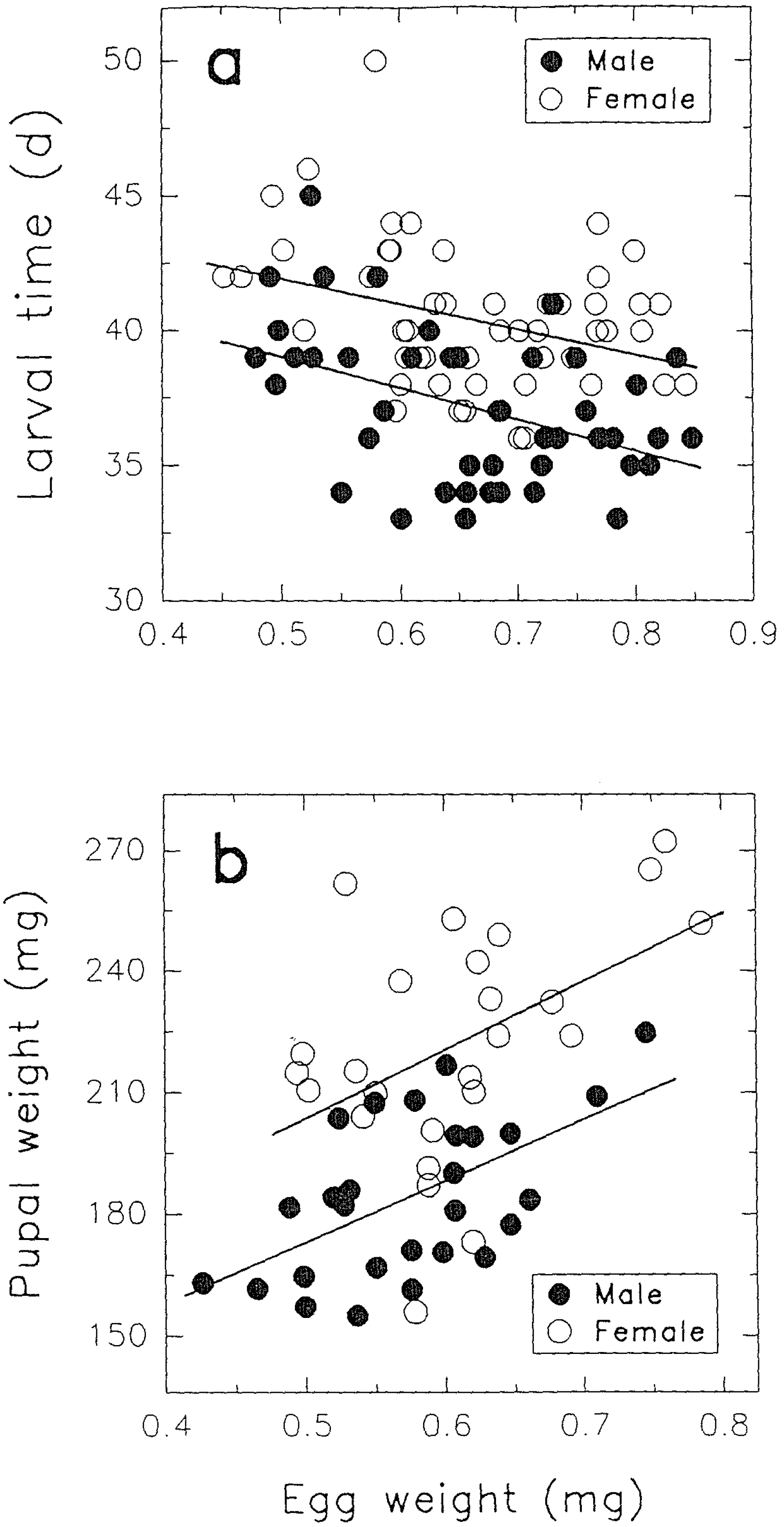

Fig. 8.5 Relationship between initial egg weight and two offspring fitness traits: (a) larval developmental time for *M. sirius* reared on *Themeda*, (b) pupal weight for *M. perseus* reared on *Panicum*-soft. Regression equations: male *M. sirius*, y=-11.57x+44.77 (r^2=0.184); female *M. sirius*, y=-10.63x+47.36 (r^2=0.135; male *M. perseus*, y=151.61x+97.14 (r^2=0.325); female *M. perseus*, y=168.91x+119.23 (r^2=0.215).

covariate EGG WEIGHT and HOSTPLANT ($F=4.52$, $P=0.0128$), although this may have reflected differences in weights between the treatments noted earlier. Results for the two-way ANCOVA (Table 8.5) also demonstrated that in all three satyrines both SEX and HOSTPLANT had a substantial effect on pupal weight. Females attained greater weight than males, and larvae of both sexes reared on *Themeda* produced smaller pupae than those reared on *Panicum*.

8.4 Discussion

There are three intriguing aspects concerning egg size variation in these satyrines (see Chapter 7): (1) within a females' lifetime (age effect), (2) between generations developing at different times of year (phenotypic effect), and (3) between species (species effect). Why should there be such large variation within and between species?

Begon and Parker (1986) proposed that the decline in egg size and/or reproductive output observed in many animals is an adaptive strategy by the female parent, particularly in invertebrates in which age-independent (random) mortality is strong relative to reproduction-dependent mortality. Support for the concept stemmed from a wide range of species, particularly in a number of studies on butterflies (e.g. Jones *et al.* 1982, Murphy *et al.* 1983, Karlsson and Wiklund 1985). Begon and Parker's model assumes that larger eggs have greater fitness than smaller eggs, since in the absence of a fitness advantage to large eggs, females should increase fecundity by producing smaller eggs. However, an extensive series of experiments examining the effects of egg size variation in temperate satyrid butterflies (Wiklund and Persson 1983, Wiklund and Karlsson 1984, Karlsson and Wiklund 1984, 1985) revealed no positive correlations between egg size and six offspring fitness traits. Wiklund and Karlsson (1984) therefore proposed a non-adaptive mechanistic explanation (the resource depletion hypothesis) in which the portion expended on each egg is considered to be an unchanging function of the remaining reproductive reserves.

In this study, however, larvae from larger eggs had greater survival prospects in all three *Mycalesis* species. In *M. perseus* and *M. sirius* there were also significant positive correlations between egg size and two life history traits, pupal weight and larval developmental rate, respectively. The correlations in *M. sirius* were rather weak in that the proportion of total variability in larval development explained by egg weight varied from only 13.5% (females) to 18.4% (males). In *M. perseus*, however, the correlations were much stronger (r^2=32.5% in males, r^2=21.5% in females).

Thus, the results show that under certain conditions there is an advantage attached to laying larger eggs so that patterns of egg size variation will be subject to natural selection. This supports Begon and Parker's idea that the decline in both egg number and size with maternal age observed in a wide range of organisms is an adaptive trait by the female parent. That is, in relatively short-lived animals with unpredictable lifespans females should lay larger and proportionally more eggs early in adulthood (but see Dixon *et al.* 1993 for aphids). Marshall (1988), however, demonstrated that large eggs may not always be more successful: egg size in the pyralid moth *Parapediasia teterrella* was negatively correlated with hatching success. However, larger females were more fecund, laid bigger eggs and had greater egg hatching success, so that fitness of larger individuals was higher than that of smaller individuals despite the disadvantage to large eggs. This perhaps represents an unusual reproductive strategy and implies that there is selection for increased female body size (and hence fecundity) and this overrides any selection against large egg size.

One may therefore ask why no advantage for larger eggs could be demonstrated in the laboratory studies by Wiklund and co-workers. Wiklund *et al.* (1987) pointed out that two important selective pressures may influence egg size. First, selection to maximise fecundity should decrease egg size while keeping the reproductive effort unchanged. Second, a positive correlation between egg size and offspring fitness should select for an increase in egg size. If the strength of these selective forces is weak (or if the selection is absent) an allometric, or even phylogenetic, relationship should be expected between egg and female size. Both of these relationships were apparent among the Swedish temperate satyrids (Wiklund and Karlsson 1984, Wiklund *et al.* 1987), suggesting the selection on egg size in this group of butterflies is indeed very weak. [It should be noted that the data set examined by by Wiklund *et al.* (1987) was relatively poor; in 9 of the 17 (53%) satyrids less than 50 eggs were weighed and in 7 of these only 20 eggs or less were weighed. Given the extent of variation in egg size these species are known to display (e.g. Wiklund and Persson 1983) caution should therefore be given to the strength of the relationship between egg size and female size. However, if 6 of the species for which 20 eggs or less were weighed, plus the three species adapted to sun-exposed habitats, are removed from the analysis the relationship is still significant ($r=0.758$, 6 *d.f.*, $P=0.029$)].

Furthermore, the analyses in Wiklund's trials used comparisons of frequencies so that effects would need to be very large for the experimental design to detect them. Moreover, Wiklund's laboratory studies may not have tested the precise selective pressures which hatchlings are likely to experience in the field. At least four factors could select for a positive correlation between egg size and offspring fitness and therefore potentially influence egg size in butterflies:

(1) Predation. Dunlap-Pianka (1979) suggested that the degree of vulnerability of newly emerged larvae to predators, such as ants, may influence egg size: smaller offspring are probably more vulnerable to predation.

(2) Dispersal ability of larvae. In some species the ability of young hatchlings to spin silk and successfully disperse to hostplants may be correlated with egg size (Berger 1989).

(3) Climate. Richards and Myers (1980) found that at low temperatures of 15°C, egg size in *Tyria jacobaeae* (L.) was positively correlated with hatching

success (r=0.428), but at higher temperatures (22°C) the advantage to larger eggs diminished. Climatic variation, or climatic extreme, may therefore select for increased egg size. Overwintering (diapausing) eggs of the lymantrid moth, *Orgyia thyellina* Butler, for example, are larger than non-diapausing eggs (Kimura and Masaki 1977). If smaller eggs have reduced offspring fitness, this species could be used to examine how temperature variation may have selected for large egg size. Temperature may also influence offspring size in another way for species in which the larvae must be of certain size to survive (diapause) through harsh conditions (Labine 1968, Murphy *et al.* 1983). The best evidence for this comes from the study by Wickman *et al.* (1990) who found, in the satyrine *Coenonympha pamphilis* L., a positive correlation between the weight of newly emerged first instar larvae and the maximum weight of larvae before winter diapause. Heavier larvae at the start of diapause were also more likely to survive than lighter larvae, so that smaller offspring were less likely to survive the harsh winter, apparently constrained by shortage of time to attain sufficient weight. Wickman *et al.* did not validate their assumption that egg size was correlated with the size of first instar larvae, but evidence presented in this study (Fig. 8.2) together with results for another satyrine species (Fig. 2 in Karlsson and Wiklund 1984) indicate that this is a reasonable assumption.

(4) Hostplant quality. A fourth selective pressure which could shape egg size in butterflies is hostplant quality, especially leaf toughness, and it is this factor which I have evaluated here. When *M. terminus* and *M. sirius* were reared on the tougher hosts larvae from heavier eggs had substantially better survival prospects than lighter eggs. However, when these two species were reared on a softer host the advantage to larger eggs diminished. Nakasuji and Kimura (1984) observed an almost identical pattern when they reared larvae of the rice skipper, *Parnara guttata guttata* Bremer et Grey, on two grasses, the rice plant *Oryza sativa* L. and the much harder cogon grass *Imperata cylindrica* (L). [Although the leaf toughness of these two hosts was not quantified in their study, Nakasuji 1987 showed in a later study that the leaf toughness of *Imperata* was about twice that of *Oryza*]. Nakasuji and Kimura found that no larvae from

smaller eggs (first and second generation) survived on *I. cylindrica*, but over 90% of larger eggs (third generation) survived to second instar. Conversely, survival of first instar larvae was similar between first and third generation eggs on *O. sativa*, although the samples tested were very small (n = 10). The survival differences between these generations was attributed to differences in host leaf toughness and not to interspecific variation in plant chemistry (Masuzawa *et al.* 1983). By contrast, when Karlsson and Wiklund (1984) compared survival of *Lasiommata megera* L. larvae from heavy and lighter eggs on the coarser *Festuca rubra*, no significant survival differences were found. However, the relative coarseness of the host was not quantified, and their samples were so small that only very large effects would be detectable (only 15 heavy eggs, and 22 light eggs were tested).

Poor host quality in the field giving larger eggs an advantage could arise in several ways. For example, old plant age, adverse seasons, poor soil nutrient status, and low light regime may reduce leaf quality, and this may greatly affect performance of newly hatched larvae. The presence of physical defence structures such as hairs, lignin and silicon (especially in grasses) and the distribution of secondary plant compounds (Minson 1971, Feeny 1976, Mattson 1980, Scriber and Slansky 1981, Singer 1984) may also reduce survival of smaller offspring.

An adaptive hypothesis relating egg size to host quality may also explain why *M. perseus* and *M. sirius* showed correlations between egg size and two offspring fitness traits (pupal weight and larval developmental time) on only *one* host quality (Fig. 8.5), if these hosts are in some way inferior. This is probably correct for *M. sirius* because larvae develop very poorly and attain low pupal weights on *Themeda* (Chapter 6). In the noctuid pine beauty moth, *Panolis flammea* (D&S), females appear to adjust egg size according to host quality. On 'good' hosts many small eggs are laid, whereas on 'poor' hosts females lay fewer, larger eggs, presumably to maximise offspring survival in the expectation of a low nutritional food supply (Leather and Burnand 1987).

In the rice skipper, *Parnara guttata guttata*, females vary egg size according to generation and habitat/host plant (Nakasuji 1982, Nakasuji and Kimura 1984). The migratory (autumn) generation lays larger eggs on course grasses growing in dry uplands, whereas females of the other generations lay smaller eggs on softer grasses growing in wet lowlands.

Similarly, the tropical grass-feeding *Mycalesis perseus* varies egg size seasonally: wet-season form females lay many small eggs while dry-season forms lay fewer large eggs (Chapter 7). Moreover, the seasonal change in female phenotype (and hence egg size) corresponds with seasonal changes in grass moisture content (Fig. 8.6). The wet-season form of *M. perseus* flies for a short period during the wetter months when grasses are green and luxuriant, whereas dry-season form females diapause during the long dry period and they do not start egg-laying until the first pre-wet season rains, at which point little new growth is yet available (Chapters 3,4,5). If moisture content reflects hostplant quality, seasonal variation in egg size in this species may be an adaptation to the seasonal condition of the larval hostplant(s). That is, since young leaves are generally softer and of better nutritional quality than old leaves (Coley 1980, Scriber and Slansky 1981) the larger egg size seen in the dry-season form may have evolved to counter the likelihood that its larval host will be of relatively poor quality. In contrast, *M. perseus* wet-season form females oviposit on the young growth, especially nitrogen rich foliage and faster growing species such as *Themeda* (Moore 1986) where there would be little advantage in laying larger eggs.

However, seasonal variation in *M. perseus* egg size also reflects seasonal differences in body size: dry-season forms are larger than wet-season forms, and when the two forms are analysed together, egg size correlates positively with body size (Chapter 7). Therefore, it can not be conclusively established that seasonal and/or interspecific variation in hostplant quality (leaf toughness and/or other hostplant attributes) has played a major selective force in determining egg size in this species.

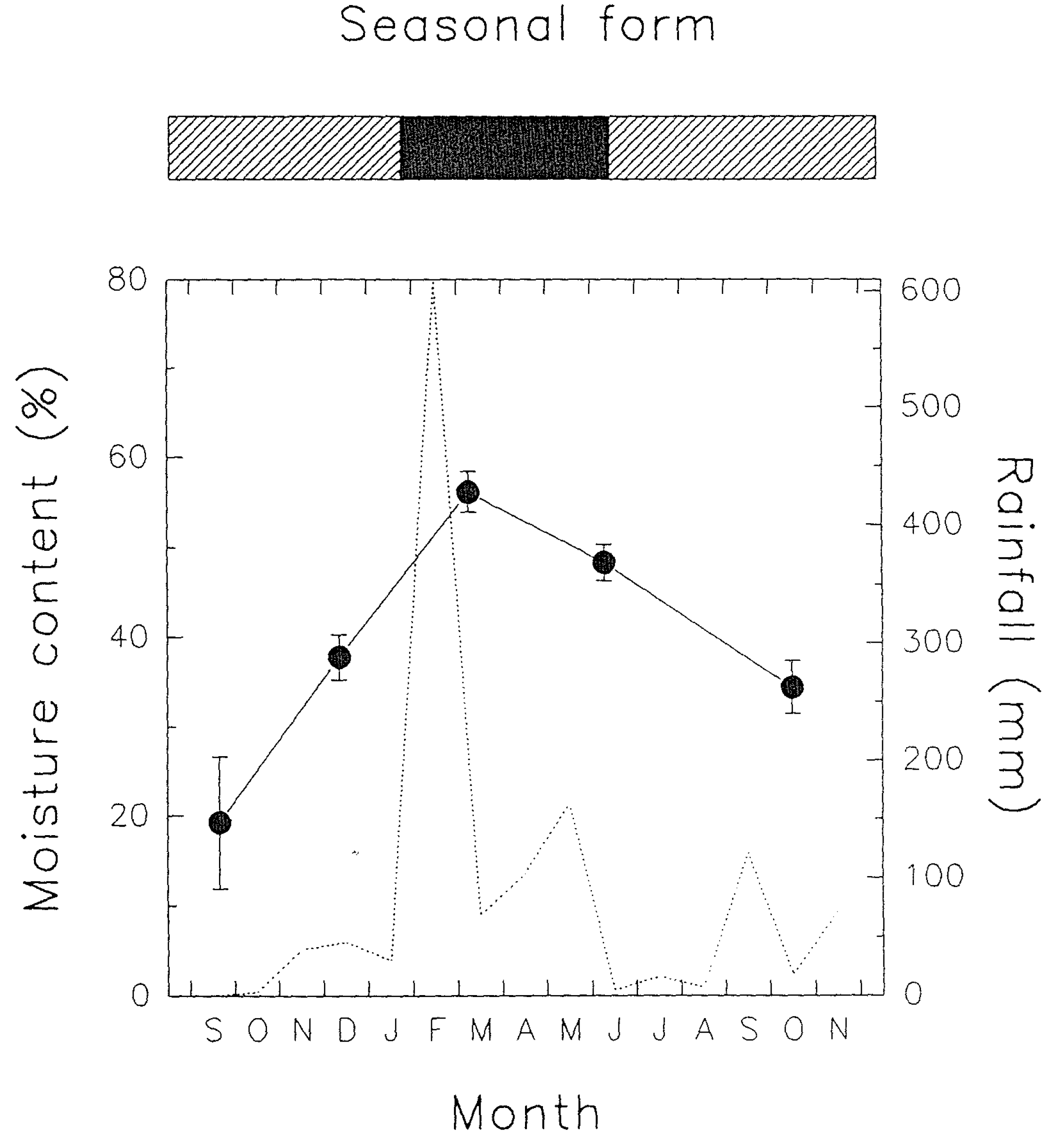

Fig. 8.6 Seasonal changes in grass moisture content (mean ± s.d.) for *Themeda triandra* at an open eucalypt forest site near Cardwell, north-eastern Australia, from September 1991 to November 1992. The site is typical habitat of *M. perseus*. Monthly rainfall is given (dashed line) and the approximate time when the two seasonal forms of *M. perseus* are present (stipple bar = dry-season form, solid bar = wet-season form).

Nevertheless, the positive correlation between head capsule diameter and egg size implies that larvae from larger eggs have larger mandibles. This provides indirect evidence in support of the mechanistic hypothesis that larger first instar larvae are better able to chew and survive on tougher foliage because of their bigger jaws. Nakasuji and Kimura (1984) also noted a similar relationship in *P.g. guttata* in that first instar larvae from larger eggs had greater head capsule width. If this hypothesis is correct leaf toughness may determine a critical minimum viable egg size within species. Moreover, egg size should be positively related to leaf toughness. Indeed, such an interspecific relationship has been shown for grass-feeding hesperiids: species in which larvae feed on tougher grasses tend to lay larger eggs (Nakasuji 1987). Nakasuji indicated that the relationship may also hold for Japanese Satyrids. Furthermore, hesperiids which lay larger eggs have a wider host range, indicating that those species which lay smaller eggs may be constrained by the number of hosts that they can potentially utilise. However, since Nakasuji also found that egg size correlated with body size (forewing length) it is very likely that body size also correlates with leaf toughness, that is, larger skippers probably feed on tougher plants. Hence, further analysis is needed to separate body size effects (and phylogentic effects) to establish that egg size variation is adaptive in hesperiids.

In conclusion, leaf toughness may pose a potential selective force in shaping egg size in butterflies. The strength of this force will depend on the relationship between egg size and the quality of the hostplant on which newly emerged larvae must eat under field conditions. For grass-feeding satyrines in tropical habitats where rainfall is highly seasonal and host quality may fluctuate dramatically (Fig. 8.6) the strength of this force could vary markedly in both space and time. Hence, production of larger eggs could be an adaptive strategy to counter unpredictable or adverse conditions - poor host quality or climatic uncertainty.

CHAPTER 9: GENERAL DISCUSSION

The main aims of this thesis were to establish how populations of relatively sedentary animals survive during the adverse dry season when food resources frequently become dormant, and to compare features of the ecology and life histories between these species. More specifically, the study aimed to determine how the influence of environmental variables, such as seasonality and predictability, may have moulded the species life histories. Three closely related satyrine butterflies of the genus *Mycalesis*, and several other related taxa, all of which specialise on grasses in the larval stage were chosen to examine these aims. The study was tackled by first establishing the spatial distributions, habitat preferences and host plant resources of the butterflies, and then by collecting information on how populations operate in the field during the wet- and dry-seasons. Key life history information on aspects of development and reproduction was also obtained from laboratory trials. Armed with this knowledge a detailed comparative analysis of the life histories of *Mycalesis* can then be placed in a broader context of life history theory.

It is clear from the preceding chapters (2-8) that although there are certain trait similarities between the three *Mycalesis* spp., there are several differences in their dry-season strategies, life histories and other aspects of their ecology such as distribution and habitat. Table 9.1 summarises the attributes of each species and highlights the important differences between them in terms of their population characteristics, life history traits and extent of phenotypic variability of the life cycle stages. All three species show the capacity to arrest reproductive activity in adverse conditions and this probably represents the primary survival strategy during the dry season (Chapter 4). That is, reproductive arrest (which may or may not be a true diapause) in *Mycalesis* is as an adaptation to minimise poor larval success when larval host plant quality is low. Other distinguishing dry season attributes that may enhance fitness, include pronounced egg size variability (Chapter 8), pupal polymorphism (Chapter 2), adult seasonal polyphenism (Chapter 5), and adult size variation (Chapter 5). Behavioural correlates include aggregation in moist refugia, and specialisation in moister microhabitats (especially *M. terminus* and *M. sirius*).

Life history theory attempts to explain trait differences between species in an ecological and evolutionary context, and a number of models have been proposed which predict which combination of traits will be favoured under various selection pressures (see Chapter 1 for review). These models propose that life history traits are shaped by selective forces generated by the habitat, the forces mediate their effects through individual fitness, and it is assumed that over evolutionary time fitness is maximised to produce the 'optimal' life history (Fig. 1.1, p.10).

The models most relevant to this work are the habitat templets developed by Grime (1977), Southwood (1977, 1988) and Taylor *et al.* (1990) among others. Although the life history models proposed by MacArthur and Wilson (1967), Pianka (1970) and MacArthur (1972), Schaffer (1974), Begon (1985), and Sibly and Calow (1985) make important predictions, these classificatory schemes demand a detailed working knowledge of population density or age-specific and size-specific mortality schedules. Such population characteristics are beyond the scope of the present work, hence it is more instructive to turn to the templet models as a basis for comparison.

The Southwood-Greenslade templet (Fig. 1.2, p.12) identifies two abiotic axes or selective forces: habitat favourability and habitat predictability. Favourability is the inverse of adversity, and in the wet-dry tropics of northern Australia the major form of adversity for many phytophagous insects is the dry season when larval resources either disappear or decline in quality. All three *Mycalesis* spp. face this seasonal problem of resource deficiency, but *M. perseus* generally occurs in habitats which are somewhat drier and harsher (in terms of lower grass moisture content) than those favoured by *M. terminus* and *M. sirius* (Chapters 2, 3). Predictability refers to the temporal availability of food resources as a function of the generation time of the species. Larval host plants (grasses) were the single most important food resource to *Mycalesis* (Chapters 2, 7), and their availability is regulated by rainfall. Because rainfall in the wet-dry tropics is highly unpredictable in timing and in extent (Chapter 3) it follows that the larval foods favoured by satyrines are also largely unpredictable, at least in the time frame of a single season. However, evidence collected on habitat distribution and breeding phenology (Chapters 2, 3, 4) suggests that *M. terminus* and *M. sirius* live in moister and more predictable environments where larval resources are in general more persistent and predictable, whereas *M. perseus* has a shorter breeding season, lives in drier habitats and larval resources are more temporary. These differences in habitat characteristics between *Mycalesis* species are summarised in Table 9.1.

Perhaps the most striking aspect of Table 9.1 is how well the habitat characteristics of these species correspond with their life history attributes. *M. perseus* shows many features reminiscent of an *'r'* selected species [in the broad sense of Pianka (1970) and Southwood (1977)], that is, faster development, smaller size, earlier maturation, higher fecundity, smaller egg size, and rapid population increase - attributes associated with temporary or unpredictable habitats. The only discrepancy is the apparently lower potential reproductive effort of *M. perseus*. However, this discrepancy could be explained in terms of 'cost' if it is assumed that some other trait is 'maximised' through an evolutionary trade-off. The most likely trade-off is be between potential reproductive effort and adult dispersal or dormancy, since wet-season forms show greater tendency to aggregate and enter reproductive diapause than *M. terminus* and *M. sirius* if conditions happen to become unfavourable during the breeding season.

Some degree of caution should, however, be used when interpreting adaptive trends from comparative data (Gould and Lewontin 1979, Harvey and Pagel 1991). For example, fast development rate in *M. perseus* could be associated more with body size than habitat effects (Chapter 6). Nevertheless, a

Table 9.1 Summary of habitat, population, life cycle and life history attributes for the three *Mycalesis* species.

Attribute	*M. perseus*	*M. terminus*	*M. sirius*
Habitat characteristics			
Favourability	low	medium	medium
Predictability	low	medium	medium
Population characteristics			
Geographic distribution	wide	medium	restricted
Reproductive seasonality and breeding strategy	short (opportunistic)	long (continuous-seasonal)	long (continuous-seasonal)
Length of diapause (dormancy during unfavourable period)	long	short	short
Tolerance to adversity (egg survival in relation to temperature variation)	good	medium	poor
Rate of increase (during favourable period)	high	medium	low
Dispersal ability (adult movement into refugia)	high	medium	low-medium
Phenotypic variation of life cycle stages			
Egg size variation	strong	strong	strong
No. of larval instars	constant	constant	variable
Larval polymorphism	present	absent	absent
Pupal polymorphism	strong	very strong	strong
Adult seasonal polyphenism	strong	medium	weak
Life history characteristics (wet-season form)			
Sex size dimorphism	high	low	medium
Body size	small	large	medium
Developmental rate	high	low	medium
Time to maturity	short	long	medium
Realised fecundity	high	low	low
Egg size	small	large	large
Potential reproductive effort	small	large	medium

suite of *M. perseus* traits are in good agreement with the templet model. Other correlates associated with the *M. perseus* life history tactic include presence of larval polymorphism (Chapter 2), stronger phenotypic variation in the adult (Chapter 5), better tolerance to adverse conditions (Chapter 6) and a more flexible breeding strategy that is correlated with rainfall (Chapter 4). Two of these attributes (larval polymorphism, adult phenotypic variation) may represent adaptations designed to enhance crypsis, and hence survival, during the dry season.

M. terminus and *M. sirius*, on the other hand, have many life history attributes and other characteristics in common, and both have a longer breeding season associated with more permanent habitats (primarily rainforest edge and paperbark woodland respectively). They do not conform entirely to the *'K'* strategists syndrome since the habitat properties of each are never completely favourable and/or predictable; both species show capacity for reproductive dormancy (Chapter 4) and populations fluctuate considerably (Chapter 3). Nevertheless, compared with *M. perseus*, they do exhibit many traits which link them closer to *K*- or *A*-type strategies (in the sense of Greenslade 1983, Southwood 1988) rather than the type associated with *r*-selection. A detailed study of the life histories of some Papuan New Guinea species of *Mycalesis* from other habitats, especially those frequenting more benign habitats than *M. terminus* and *M. sirius*, could generate more interesting data as a basis for further comparison. In particular, such studies would provide valuable theoretical insights into the usefulness of the habitat templet as a scheme for differentiating selection processes and its potential as an 'ecological periodic table' (Southwood 1977) in cataloguing and predicting life histories.

As a final comment it is perhaps worth reiterating the comments raised by several eminent scholars (Greenslade 1983, Southwood 1988, Partridge and Harvey 1988) who point out that there may be more than one adaptive solution to a particular environmental problem. Different species which may or may not differ in size and phylogenetic position may find the same environment (e.g.

food resources) favourable or unfavourable, predictable or unpredictable, constant or ephemeral depending on intrinsic factors such as length of life cycle, scale of trivial movement and extent of dormancy. In this sense, and by way of example, it is notable that two satyrines, *M. perseus* and *H. adiante*, reside in similar environments and occupy similar habitats throughout their geographic ranges in the wet-dry tropics of north-eastern Australia, with the latter species intruding much farther inland into the drier climatic zone (Chapter 2). Yet the two species have evolved quite different solutions to the same environmental problem (Chapter 3). *M. perseus* remains reproductively dormant throughout much of the dry season, breeding only during the rain periods when conditions are ephemerally favourable. Hence, it may broadly perceive the habitat as severe and unpredictable. By contrast, *H. adiante* breeds for most of the year, probably by specialising on grasses frequenting moister microhabitats, and may thus find its habitat more predictable and fairly favourable. In other words the habitat or food resource characteristics of a species, depend not only on the environmental properties, but on how those resources scale to the temporal and spatial features of the organism. The extent of trade-offs and life history pattern adopted by a species may ultimately depend on which adaptive solution has been favoured to exploit those resources.