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Stable changes in voltinism strategy and their implications

Leonard Winokur, Department of Biology, The University, Southampton U.K. ([1])

Summary

Several Lepidoptera can occur as either univoltine or multivoltine populations while some populations can be uni- or multivoltine depending on climate. Pupae of *Pararge aegeria tircis* Butler, 1867 were given cold shock. When their progeny were reared under summer daylength at 15°C, but without shock, a number underwent larval or pupal diapause. The effect of cold *per se* was also greater in the progeny of shocked compared to that of untreated animals. Cold shock did not increase mortality. Some shock phenotypes resembled known aberrants. The evolution of univoltinism from bivoltinism is modelled and the role of 'genetic assimilation' is considered. The ability of Lepidoptera to track climatic change is topical and is important in assessing conservation needs. It can also give insight into past demographic responses to long-term changes such as ice ages.

Introduction

On 13.9.1989, and timed to coincide with the 15th Symposium of the Royal Entomological Society of London — "The Conservation of Insects and Their Habitats", was held a discussion meeting — "Butterflies in a Changing Environment". This meeting largely addressed climatic changes, which have recently become a public concern on account of the 'Greenhouse Effect'. However, a cooling climate too can have far-reaching effects (Mikkola, 1991). But British butterflies have undergone major changes in range over periods little more than a century (Downes, 1948).

As climate becomes increasingly hostile, a species may seek suitable conditions elsewhere. Should there be no suitable habitat within access, it may be driven to extinction ; climate may also force changes in micro-habitat usage (Dennis, 1977). However, a species may need neither migrate nor die out if it can adjust. Moreover, it could also invade areas previously unsuited to it, but now amenable following adjustment. The alternatives are summarised in Figure 1.

([1]) Present address : Department of Pure & Applied Zoology, Reading University, P.O. Box 228, Reading RG6 2AJ, U.K.

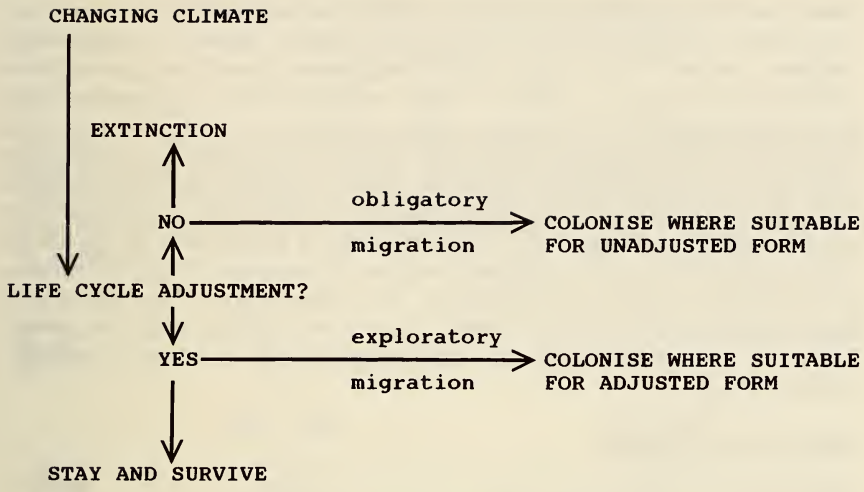

Fig. 1. Summary of possible responses to climatic change.

Some species have developmental options that enable them to cope with hostile climate, e.g. winter or summer diapause (Slansky, 1974 ; Masaki, 1980). Others are more flexible. In Sweden, *Pararge aegeria tircis* has bivoltine colonies in the South but is univoltine in the North. When switched between regions individuals from each locality undergo the strategy typical of the other, and these alternatives reflect differences in growth rate under each temperature (Wiklund *et al.*, 1983).

In the present article I shall address the question as to whether and how, under cooling climate, bivoltinism might give rise to univoltinism through a continuum of change in *Pararge aegeria* (Linnaeus, 1758). The first consideration is environmental and genetic controls. Temperature and daylength can influence growth rate and diapause ; in certain species this involves genetic components (Shapiro, 1984). Genetic variability in responses to environmental cues means selection may build up a genotype in which the response becomes increasingly and eventually wholly under genetic control, a process Waddington (1953) termed 'genetic assimilation'. Similar phenomena, some not requiring genetic variability or selection (Ho *et al.*, 1983) or involving other hereditary modes (see Winokur, 1989 for review), have since been demonstrated in a wide spectrum of organisms. The second consideration is the survival of immature and adult stages exposed to new conditions resulting from adjustments elsewhere in the life cycle (Porter, 1984).

The findings reported below consider the effects of cold shock on pupal duration and survival, and its influence on larval, pupal and life-cycle duration in the subsequent generation under different temperatures. Pupal cold shock can modify wing pattern in other species (Nijhout, 1984) when natural frosts

have also been implicated (Shapiro, 1975), but its effects on pupal and subsequent development have been largely ignored. Moreover, despite suggestions that the Waddington (1953) experiment should be repeated with Lepidoptera (Nijhout, 1984) in which assimilation-like phenomena have been demonstrated in at least two species (Harrison, 1928, Vuillaume & Berkaloff, 1974), and that genetically assimilated changes in development rate have been implicated in cicadas (Lloyd & White, 1976), genetic assimilation remains to be formally studied in Lepidoptera. *P. aegeria* is an ideal experimental subject as it can overwinter as either larva or pupa with the resulting brood thus comprising two parts (Goddard, 1962), its complex developmental options are influenced by temperature, photoperiod and genetic factors (Lees & Tilley 1980 ; Robertson, 1980b ; Shreeve, 1985 ; Nylin *et al.*, 1989), and it is one of the easiest species to culture in captivity (Cribb, 1983).

Materials and Methods

a) Rearing

The study material concerns the F_1 and F_2 from two pairings of wild adults of generation 1 part i from Bassett, Southampton, U.K. (50° 53' N, 01° 25' W), all animals so derived constituting Stock 01. Lineages are shown in Figure 2. The F_1 were reared and subsequently paired indoors at 19°C (SD : ± 2.1°C) under natural summer daylength as described by Winokur (1988). The F_2 were reared in constant environment cabinets under a 16h photophase using fluorescent light of similar composition to daylight. Two rearing temperatures were used. One cabinet was maintained at 19°C (SD : ± 2.1°C) as for the F_1, the other at 15°C (SD : ± 6.7°C), to examine the effect of rearing temperature. Rearing was otherwise as for the F_1. In all cases individuals were segregated in plastic boxes and fed cut Cock's-Foot Grass, *Dactylis glomerata* L., grown from seed to ensure that foodplant composition was as uniform as possible. Pupae were left in their plastic boxes for experimental treatment.

b) Treatment

Three types of treatment were applied :

a) **Cold shock**. Pupae were placed in a refrigerator at – 1°C (F_1, SD : ± 0.8°C ; F_2, SD : ± 1.1°C) for 96h commencing within 12h post-pupation, although not if less than 5h old since such pupae may suffer mortality under cold shock (Nijhout, 1984). Boxes were wrapped in aluminium foil (to exclude spurious light when transferring others to and from the refrigerator) and labelled, then arranged so the pupae hung vertically. Following treatment, pupae were returned to rearing temperature.

b) **Foil control**. Pupae were held at rearing temperature throughout. The boxes were wrapped in aluminium foil for 96h commencing within 12h post-pupation to simulate the period of darkness experienced by the cold shock pupae.

c) **No-foil control**. Pupae were held under rearing temperature and daylength throughout.

c) Lineage

Of the F_1, only no-foil and cold shock animals were used for breeding and each pair used like-treated animals. For cold shock pairings the adults were as far as possible chosen with wing pattern visibly deviant from the wild-type, but not on the basis of pupal stage duration *per se*. Lineages are given in Figure 2.

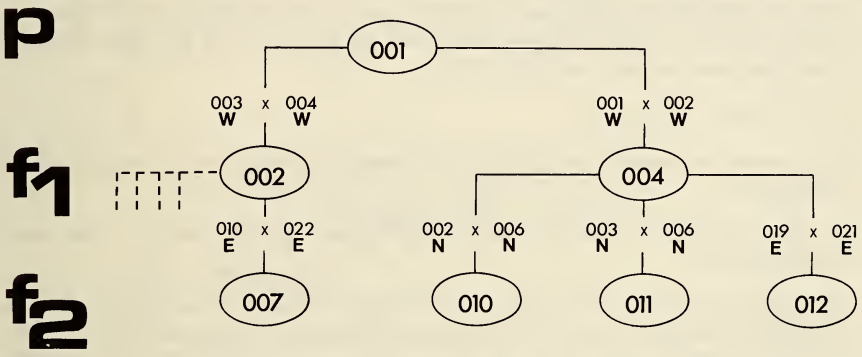

Fig. 2. Stock 01 F_1 and F_2 lineages. Families (encircled) numbered as shown for historic reasons. Pairings are given, females to the left, males to the right, with respective specimen numbers and pupal treatment : W = wild stock, N = no foil control, E = cold shock. Family 002 yielded four further families, but these do not concern the present study and so are not considered here.

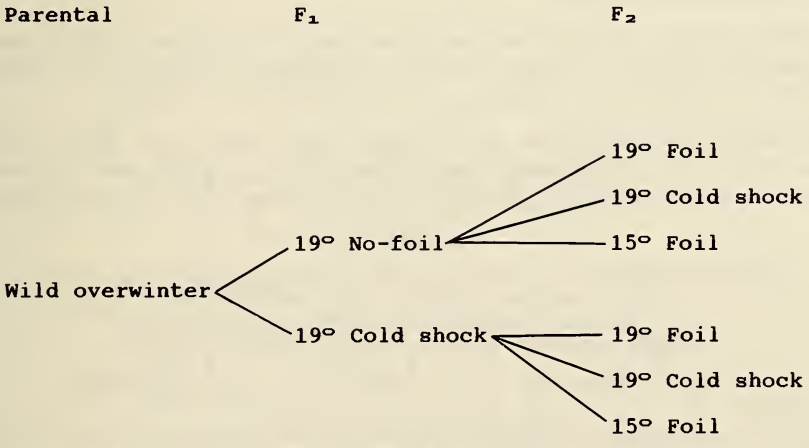

Fig. 3. Rationale of the breeding protocol. Rearing temperature (°C) and treatment are given.

The F_2 from each no-foil and cold shock pairings were given either foil or cold shock treatments. The F_2 reared at 15°C, however, were given only foil treatment on account of the small sample sizes. The protocol is summarised in Fig. 3.

Results

In the present study, larval duration refers to the interval between hatching and spinning up for pupation. Pupal duration refers to the interval between the pupal moult and eclosion, and comprises the 'prepharate' phase up to the onset of colouring up of the pupal wing cases, and the 'pharate' phase from the onset of colouring up to eclosion. Life cycle duration gives the interval between oviposition and eclosion. Pupal stage durations are given in Figure 4. Larval and life cycle durations are given in Figure 5. In all cases samples were compared pairwise with the t-test.

a) F_1 : Pupal treatment

There were no significant differences between families under corresponding treatments.

All stage durations were longer in foil than no-foil samples but none significantly so.

All stage durations were longer in cold shock than foil samples (Table 1). Pooling the families resulted in significant differences in prepharate ($t_{(9)} = 2.621$, $0.01 < P \leq 0.05$) and entire pupal duration ($t_{(7)} = 3.750$, $0.001 < P \leq 0.01$). The magnitudes of these differences were less than the duration (4 days) of treatment.

Prepharate and entire pupal duration were longer in cold shock than no-foil samples (Table 2). The magnitudes of these differences were less than the duration of treatment.

Under foil and cold treatments pharate and entire pupal duration were negatively correlated (Pearson correlation coefficients : foil, $R_{(12)} = -0.6600$, $0.001 < P \leq 0.01$; cold shock ($R_{(25)} = -0.3366$, $0.01 < P \leq 0.05$).

Resulting adult phenotypes were normal except under cold shock : male no. 004.021 dorsal hindwings had missing s2-3 eye pupils and indistinct s4-5 markings ; female no. 004.016 dorsal forewings had a 'ripple' pattern.

Table 1

Differences between no-foil and cold shock treatment in mean pupal durations of F_1. Treatment yielding the longer duration (days) :
E = cold shock, magnitude of the difference in parentheses.
Significance levels : * $0.01 < P \leq 0.05$, ** $0.001 < P \leq 0.01$

Family	Prepharate	Pharate	Entire pupa
002	E (3.1) **	E (0.2) ns	E (3.3) *
004	E (2.7) *	E (0.7) ns	E (3.3) *

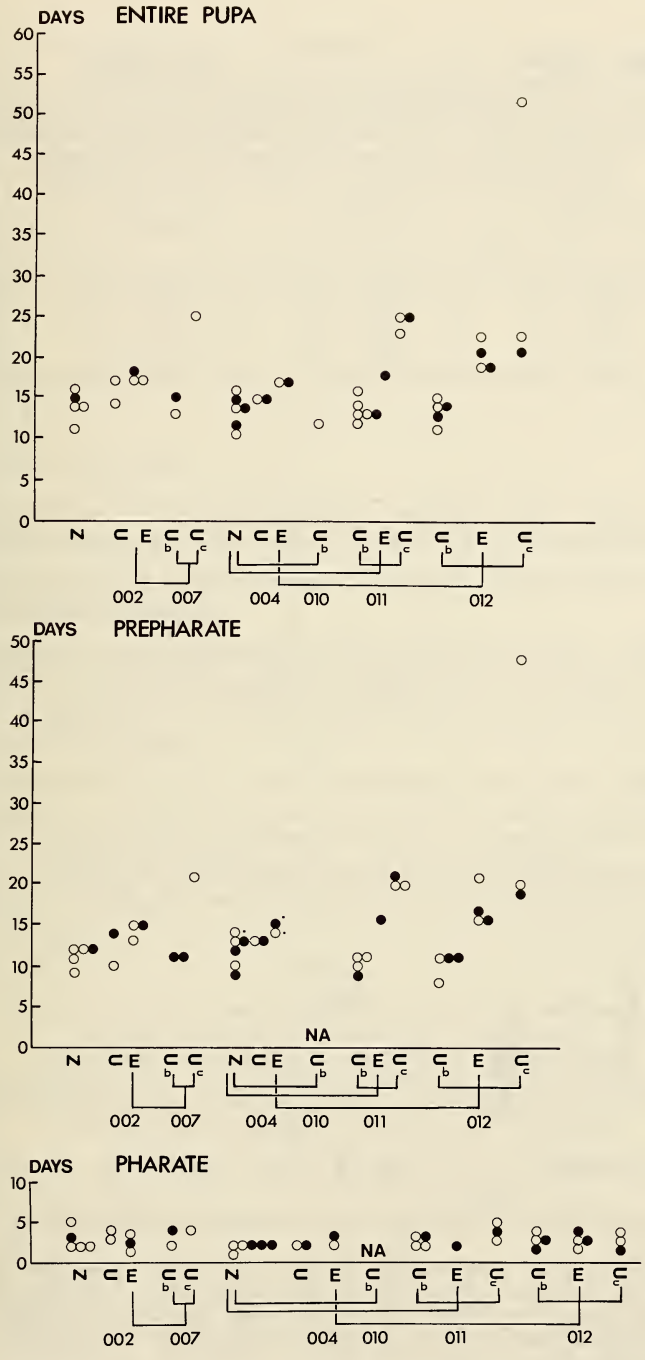

Fig. 4. Pupal stage durations of Stock F_1 and F_2 samples. Each point represents one individual, open circles = males, filled circles = females, dots = sex not ascertained. Lettering below each sample gives pupal treatment : N = no foil, C = foil, E = cold shock ; and respective families are identified. Lineage is shown to aid interpretation. Rearing temperature is shown where necessary to distinguish samples : b = 19°C, c = 15°C. NA = data unavailable.

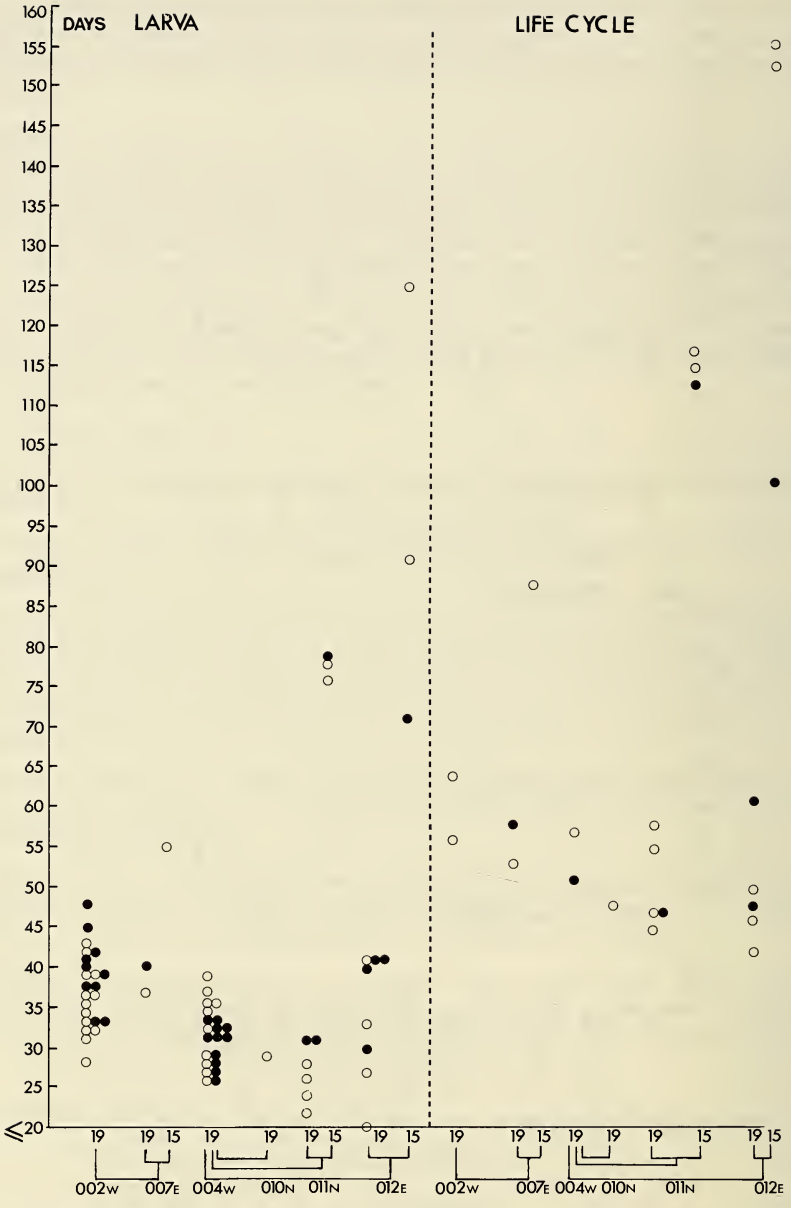

Fig. 5. Larval and life cycle durations of Stock 01 F_1 and F_2 samples. Each point represents one individual, open circles = males, filled circles = females. Numbering below gives rearing temperature (15° or 19°C) and families are identified. Lineage is shown to aid interpretation and parentage-type is indicated : W = 'wild', N = 'no-foil', E = 'cold shock'.

Table 2

Differences between foil and cold shock treatment in mean pupal durations of F_1 and F_2 (all individuals reared at 19°C). Treatment yielding longer duration (days) : C = foil, E = cold shock ; magnitude of the difference in parentheses. '=' no difference. Significance levels : * $0.01 < P \leq 0.05$, ** $0.001 < P \leq 0.01$

Family		Prepharate	Pharate	Entire pupa
F_1	002	E (2.3) ns	C (0.5) ns	E (1.8) ns
	004	E (1.8) ns	E (0.5) ns	E (2.0) ns
F_2	011	E (5.7) *	C (0.5) ns	E (4.5) ns
	012	E (7.2) **	= (0.0) ns	E (7.1) *

b) F_2 : Pupal treatment

F_2 families 007 and 010 furnished only foil controls : unless otherwise stated results below relate to families 011 and 012 (and their F_1 parent family 004).

Prepharate and entire pupal duration were again longer under cold shock than foil treatment (Table 2).

The magnitudes of these differences were greater than in the F_1 and exceeded the duration of treatment, especially in family 012 of 'cold shock' parentage. Here, the magnitudes of these differences were also greater than in family 011 of 'no-foil' parentage.

Under foil treatment, the F_2 of both parentage types showed similar pupal stage durations, and neither showed any duration significantly longer than in the F_1 (if anything they were shorter) (Table 3). A similar trend was found between F_2 families 010 ('no-foil' parentage) and 012 ('cold-shock' parentage), and between F_2 family 007 and F_1 family 002, both of 'cold shock' parentage.

Table 3

Differences between F_1 and F_2 and between F_2 of each parentage-type in mean pupal stages durations under each treatment (all animals reared at 19°C). Generation (F_1 = family 004) or parentage-type of longer duration (days) : N_p = 'no-foil' family 011, E_p = 'cold shock' family 012, magnitude of the difference in parentheses, '=' no difference. Significance level : ** $0.001 < P \leq 0.01$

Samples		Prepharate	Pharate	Entire pupa
Foil	004 v 011	F_1 (2.7) **	F_2 (0.5) ns	F_2 (1.5) ns
	004 v 012	F_1 (2.7) ns	F_2 (1.0) ns	F_1 (1.6) ns
	011 v 012	= (0.0) ns	E_p (0.5) ns	N_p (0.1) ns
Cold	004 v 011	F_2 (1.2) ns	F_1 (0.5) ns	F_2 (1.0) ns
	004 v 012	F_2 (2.7) ns	F_2 (0.5) ns	F_2 (3.5) ns
	011 v 012	E_p (1.5) ns	E_p (1.0) ns	E_p (2.5) ns

Under cold shock, prepharate and entire pupal duration especially were rather longer in the F_2 of 'cold shock' than 'no-foil' parentage although not significantly so. The F_2 of 'cold shock' parentage also showed consistently longer pupal stage durations than in cold shocked F_1 when their ranges of prepharate and entire pupal duration barely coincided (prepharate duration : F_1 = 14-16 days, F_2 = 16-21 days ; entire pupal duration : F_1 = 17 days, F_2 = 19-23 days). The non-significant differences in mean duration most likely reflect small sample size since there was significance at the $0.05 < P \leq 0.1$ level (prepharate duration, $t_{(6)} = 2.354$; entire pupal duration, $t_{(4)} = 2.630$).

c) F_2 : Pupal temperature

Prepharate and entire pupal duration were significantly longer at 15°C than 19°C (Table 4).

The magnitude of these differences was greater in samples of 'cold shock' than 'no-foil' parentage.

At 15°C prepharate and entire pupal duration especially were about a week longer in samples of 'cold shock' than 'no-foil' parentage when their ranges barely coincided (prepharate duration : 'no-foil' = 20-21 days, 'cold shock' = 19-48 days ; entire pupal duration : 'no-foil' = 23-25 days, 'cold-shock' = 21-52 days). Their non-significant differences in mean duration may reflect small sample size or large variability. Pharate duration was also longer at 15°C than 19°C but only in samples of 'no-foil' parentage.

Figure 4 shows (with respect to foil controls at 19°C) that cooler rearing temperature prolongs pupal development to a greater degree than immediate cold shock.

There was no clear-cut separation between the sexes in any of the pupal stage durations, except for one male of 'cold shock' parentage at 15°C (Fig. 4).

Resulting adult phenotypes were normal except at 15°C in family 012 of cold shock parentage : male no. 018 dorsal pattern was diffuse and ventral forewing s2-3 lacked markings ; male no. 019 lacked all dorsal eyespot pupils.

Table 4

Differences between temperatures for F_2 of each parentage-type, and between parentage-types at each temperature, in mean pupal stage durations. Temperature (°C) or parentage yielding the longer duration (days) : N_p = 'no-foil' family 011, E_p = 'cold shock' family 012, magnitude of the difference in parentheses, '=' no difference. Significance levels : * $0.01 < P \leq 0.05$, *** $P \leq 0.001$

Samples		Prepharate			Pharate			Entire pupa		
011	15° v 19°	15°	(10.0)	***	15°	(1.5)	*	15°	(10.8)	***
012	15° v 19°	15°	(18.7)	*	=	(0.0)	ns	15°	(18.6)	*
19°	011 v 012	=	(0.0)	ns	E_p	(0.5)	ns	N_p	(0.1)	ns
15°	011 v 012	E_p	(8.7)	ns	N_p	(1.0)	ns	E_p	(7.7)	ns

d) Larval development and the life cycle

For each family and temperature, larval durations are based on data pooled over subsequent pupal treatment classes and from eclosing adults only. Life cycle durations are based on data from foil treated animals only.

F_1 : Larval duration was 5 days longer in family 002 than family 004 ($t_{(42)} = 4.559$, $P \leq 0.001$). The life cycle too was 5 days longer in family 002 ; that this was not significant however may reflect small sample size ($n = 4$).

F_2 : In all cases (including family 007) larval and life cycle durations were considerably longer at 15°C than 19°C (Fig. 5).

In families 011 and 012 (of family 004 parentage) larval and life cycle durations at 15°C were respectively 7 and 9 weeks longer in samples of 'no-foil' parentage, but 9 and 12 weeks longer in samples of 'cold shock' parentage (Table 5). At 19°C there was no such difference between the parentages and similarly when family 010 (of 'no-foil' parentage) was considered.

In all cases (including family 007) larval and life cycle durations were considerably longer in F_2 reared at 15°C than in the F_1 (reared at 19°C) (Fig. 5). In the F_2 this difference was greatest for family 012 of 'cold shock' parentage (Table 5).

At 19°C there was no such difference between the generations.

There was no clear-cut separation between the sexes in larval or life cycle durations, except for two males of 'cold shock' parentage at 15°C (Fig. 5).

Table 5

Differences between F_1 and F_2 of each temperature class, between parentage-types at each temperature, and between temperatures for each parentage-type, in mean larval and life cycle durations. Generation (F_1 = family 004), parentage-type or temperature (°C) yielding the longer duration (days) : N_p = 'no-foil' parentage (family 011), E_p = 'cold shock' parentage (family 012), magnitude of the difference in parentheses. Significance levels : * $0.01 < P \leq 0.05$, *** $P \leq 0.001$

	Larva		Life cycle	
Samples F_1 v F_2				
19° 004 v 011	F_1 (4.2)	*	F_1 (3.6)	ns
19° 004 v 012	F_2 (2.1)	ns	F_1 (4.6)	ns
15° 004 v 012	F_2 (46.5)	***	F_2 (61.0)	***
15° 004 v 012	F_2 (64.8)	***	F_2 (82.7)	*
Samples F_2				
19° 011 v 012	E_p (6.3)	ns	N_p (0.8)	ns
15° 011 v 012	E_p (28.3)	ns	E_p (21.7)	ns
011 15° v 19°	15° (50.7)	***	15° (64.6)	***
012 15° v 19°	15° (62.7)	***	15° (87.3)	***

e) Survival

Pupal survival was estimated as *number eclosing/number pupating*. Survivals are given in Table 6.

Table 6

Pupal survivals in the F_1 and F_2 under respective treatments : N = no foil, C^{15} = foil at 15°C, C^{19} = foil at 19°C, E = cold shock. Sample sizes (n) give number pupating

F_1		F_2	
N	0.85 n = 13	C^{15}	0.86 n = 7
C	0.76 n = 17	C^{19}	0.66 n = 18
E	0.82 n = 28	E	0.62 n = 8

In the F_1 survival was slightly better under cold shock than foil treatment and similar to that of untreated pupae.

In the F_2 survivals under cold shock and foil treatment were more similar although both lower than in the F_1. Survival was rather better at 15°C than 19°C. This trend was similar for both parentage types whose overall survivals were identical (0.67).

f) Selection

Differential survival at each stage of the protocol was examined using the Chi-squared test of Manly *et al.* (1972) :

$$(P_1 - P_2)^2 / (Var_{p1} + Var_{p2})$$

where P_1 = mean of sample no. 1, P_2 = mean of sample no. 2, Var_{p1} = variance of sample no. 1, and Var_{p2} = variance of sample no. 2.

However, the means of all animals completing each respective stage were compared with those based only on data from animals eclosing after each pupal treatment rather than with those simply surviving to the next stage, in order to take into account possible non-random assignment of individuals to treatments, and to be more likely to detect the combined effects of factors which individually might pass undetected. In the cases of oval and larval selection treatment refers to that to which the respective animals were subsequently assigned. Selection could not be assessed for pharate or entire pupal durations whose measurement requires survival to eclosion. Results are given in Table 7.

However the differences were minimal and in no case significant.

Table 7

Direction of selection in the ova, larva and prepharate pupa for each family and treatment. Sample subscripts denote rearing temperature where relevant, b = 19°C, c = 15°C. Treatments : C = foil [1] (no-foil in 002 and 004), E = cold shock. Direction of selection : > towards shorter duration, < towards longer duration, '=' no bias. Also pooled for each treatment and both together

	Ova	Larva	Prepharate pupa
Sample	C E	C E	C E
002[1]	> >	> >	= =
004[1]	> >	> >	> =
007b	<	<	=
007c	>	=	=
010b	>	=	= =
011b	>	<	= =
011c	<	>	=
012b	>	> <	= =
012c	=	=	=
Overall C and E	> > >	> > >	> = >

Discussion

a) Pupal treatment and temperature

That in the F_1 the difference in prepharate and entire pupal durations between cold shock and no-foil treatment was greater than between cold shock and foil treatment indicates that darkness does at least have some prolonging effect. F_1 pupae of all treatments eclosed 9-16d post-pupation, typical of non-diapause pupae (Lees & Tilley, 1980). The mediation of these effects is documented more fully elsewhere (Winokur, 1989), but is essentially as follows. Darkness diminishes early (< 12h) post-pupational ecdysone secretion characteristic of non-diapause lepidopteran pupae (Nijhout, 1980) ; cold slows metabolism directly but development is not suspended — which would explain the prolongation being less than the duration of cold application. Moreover, cold shock may inhibit ecdysone turnover but not production, so that the extracellular titre accumulates. With a rise in temperature, cellular uptake resumes and the abundance of ecdysone causes a surge in intracellular turnover. Such a 'pulse' may accelerate early post-shock development, explaining the inverse relationship between pharate and entire pupal durations. It might also explain the modified phenotype of specimen 004.021 and indeed cold shock phenotypes

in general, for phenotype reflects the pattern of scale-cell maturity whose development is triggered by ecdysone in the early pupa (Nijhout, 1980).

That in the F_2 cold shock prolonged prepharate and entire pupal duration to a greater extent in samples of 'cold shock' than 'no-foil' parentage, indicates that cold shock in the F_1 increases the degree to which it prolongs these stages in the subsequent generation. Cooler rearing temperature (15°C) also prolonged prepharate and entire pupal duration when the prolongation was similarly greater in samples of 'cold shock' parentage. It is proposed that cold shock induces a propensity for slowed development in the subsequent generation, although possibly requiring cold shock or cooler temperature to render it manifest. This propensity may interact synergistically with cold shock, accounting for the degree to which cold shock prolonged prepharate and entire pupal duration, i.e. exceeding the duration of treatment.

b) Inheritance

That larval development was also slowed more by cooler temperature in samples of 'cold shock' parentage, indicates that the propensity for slowed development also manifests in the offspring at life cycle stages prior to pupation. This propensity may be transmitted via sperm-line DNA, as has been reported for resistance to LSD-induced inhibition of pupal diapause in *Pieris brassicae* Linnaeus, 1758 (Vuillaume & Berkaloff, 1974). Alternatively it may be transmitted via the oocyte (cf. Burns, 1966). Moreover, the timings of the various life cycle stages appear to be coordinated as a 'linear growth dynamic' where changes to one stage are accommodated by changes elsewhere (Winokur, 1989, see also Winokur, 1992). It is postulated that cold exerts a direct slowing of oocyte maturation that continues through the life cycle.

That developmental prolongation and aberrant phenotypes were most evident in males however, suggests that their manifestation may be related to an interaction between a particular male karyotype and egg cytoplasm, as has been implicated for anomalies of inheritance in *Drosophila* (Ho *et al.*, 1983). That larval duration differed between the F_1 families 002 and 004 under identical conditions implicates some heritable contribution to development rate at least at this stage. Cold shocked male 002.022 was visibly normal and its progeny at 15°C too were normal, while cold shocked male 004.021 was aberrant as were its male progeny at 15°C (with phenotype more extreme). While there might therefore be some genetic involvement in the transmission of cold shock effect, it is not possible to say whether this transmission represents early 'phase 1' of genetic assimilation — marked by increased expressivity (Waddington, 1953), or 'Dauermodifikation' where the effect is only temporary (Ho & Saunders, 1982). However, that in the F_2 of family 004 'cold shock' parentage both males were aberrant and that the third specimen, a female, too had a protracted larval stage (71 days), would strongly point to some direct effect not *per se* requiring genomic DNA or variability therein (cf. Ho *et al.*, 1983).

c) Survival and selection

Cold shock did not diminish pupal survival. Most deaths occurred before they would have commenced treatment (Winokur, 1988). Pupal survival was worse in the F_2 but was not a cumulative cold shock effect as the trend was similar for foil controls with both parentage types ; it was most likely due to the incubator regime (Winokur, 1989).

Indeed, over the larger breeding programme of which the present study forms a part, there was no difference between the treatments nor any trend with successive generations of cold shock ; although prepharate and entire pupal (but not pharate) survival of cold shock pupae did decrease with inbreeding (Spearman rank correlations : Prepharate survival, $R_{(15)} = -0.7150$, $P \leq 0.001$; entire pupal survival, $R_{(15)} = -0.6277$, $0.001 < P \leq 0.01$). Larval survival, however, increased with inbreeding ($R_{(15)} = -0.6277$, $0.001 < P \leq 0.01$). These points are taken up below.

The lack of directional selection shows that the causes of mortality over the protocol acted randomly. Adult longevity was a week shorter in cold shocked animals but still similar to that in nature — about three weeks (Goddard, 1962). Thus cold shock is a suitable experimental factor for use within viable breeding programmes.

d) Pupal summer diapause

Male 012.018 of 'cold shock' parentage and reared at 15°C spent 48 days as a prepharate pupa, i.e. was in diapause, as defined by Lees & Tilley (1980). Although *P. aegeria* can hibernate as either larva or pupa (Lees & Tilley, 1980), summer diapause has been reported only for larvae (Wiklund *et al.*, 1983). Nylin *et al.* (1989) do, however, report pupal diapause under 16L : 8D photoperiod at between 13°C and 21°C for a S. Swedish population, but this can be discounted in the present study since larval rearing under the 16h light regime did not induce pupal diapause at either 19°C (even with 'cold shock' parentage) or at 15°C with 'no-foil' parentage. For these reasons, the pupal diapause induced by short daylength even at 22°C in British stock (Lees & Tilley, 1980) can also be discounted. It is thus proposed that the above case represents an instance of pupal aestivation, a further developmental option available to British *P. a. tircis*, and that it resulted from an interaction of cool rearing temperature with 'cold shock' parentage.

Specimens 012.018 and 012.019 spent 92 days and 125 days respectively as larvae which could represent either true aestivation or simply slowed development. Nylin *et al.* (1989) found a positive correlation between larval and pupal development rates and diapause occurrence, although the association between larval aestivation and pupal diapause was not obligatory. Indeed pupal duration in specimen 012.019 was 23 days, indicating direct development. Thus, even if associated with larval aestivation, the pupal diapause in specimen 012.018 need not necessarily exemplify the winter-type.

These larval durations too appear to have depended on interaction of temperature with 'cold shock' parentage as they were noticeably longer than in samples of 'no-foil' parentage at 15°C or of 'cold shock' parentage at 19°C.

e) Cold shock in nature

A number of wild larvae pupate in late November early December (Shreeve, 1985). The mean January temperature in Britain is 2°C (Dennis, 1977) but temperatures experienced by the pupae could be considerably cooler. Cole (1962) found a number of pupae amongst short grass under a gap in a damp woodland canopy, when one pupa was fully exposed in winter. Such habitats experience frequent night frosts (Geiger, 1950) when larvae are active (Lees, 1962), yet larvae show no inclination to seek more sheltered locations (Cole, 1962). Indeed larvae will pupate at 3°C (Wiklund & Persson, 1983). Wild *P. aegeria* pupae can survive severe cold (Shreeve, 1985), and it is postulated that larvae may sequester cryoprotectants from the foodplant. Hence pupal cold shock simulates a microclimate the species is likely to encounter naturally.

f) Diapause options in nature

Specimen 012.018 resembled a male ab. *cockaynei* Goodson captured 5.xi.1932 (see Russwurm, 1978). The life cycle of specimen 012.018 spanned five months (153 days) of which three were spent as a larva. Assuming the above ab. *cockaynei* developed similarly it would have derived from generation 1 part ii (1.ii) which tails off in early June (Goddard, 1962). This would place it as a late emerger of generation 2 part ii (Winokur, 1988), pupating in early September (before the onset of daylengths short enough to induce pupal hibernation, Shreeve, 1985) to eclose after pupal aestivation. In specimen 012.019, the life cycle was similarly five months (156 days) : such a wild derivative therefore also eclosing as a late 2.ii individual.

g) Evolution of univoltinism

Frost might cause a part of generation 1 part i (1.i) to eclose later than April : under an imminent cool summer their larval progeny would develop more slowly than usual. Such larvae could have three options :

Firstly, as with specimen 012.019, the 'F_1' larva pupates four months later (August). Daylength still prevents winter diapause so the pupae develop directly, eclosing in September, typical of generation 2.ii (Goddard, 1962). Their resultant 'F_2' larvae would develop slowly and so overwinter as larvae, eclosing as generation 1.ii in late May or early June.

Secondly the F_1 larvae pupate later than September and so diapause as pupae. These might experience frost, which, with the prolonging influence of the cooler ambient temperature, would further prolong their development to produce an atypically late 'F_2' generation 1.i ; they might even eclose as generation 1.ii in late May or early June.

Thirdly, as with specimen 012.018, the 'F_1' larva pupates late August or early September as a summer diapause pupa. Since daylength still exceeds that critical for winter diapause, in the F_1 of frost-exposed parentage, cool ambient temperature overrides summer daylength thereby precluding direct development. However, as summer daylength is still sufficient to prevent full (winter-type) diapause, the pupae undergo a partial (summer-type) diapause to eclose in November. Indeed, certain natural populations vary in the intensity of pupal diapause (Nylin *et al.*, 1989).

The third option however, does not resemble any natural strategy. While it might arise during a transition to univoltinism, it is probably not successful enough to become established : Firstly, F_1 adults developing within the pupae might die should the temperature suddenly fall (cf. Goddard, 1962). Secondly, even if the F_1 adults did eclose and breed, the resulting ova, being laid so late, might also succumb to a sudden frost or simply perish in the late autumn or winter, since eggs do not hatch below 6.8°C (Shreeve, 1985) and oval diapause does not appear to be possible (Lees & Tilley, 1980). Thirdly, even if the eggs hatched before the temperature dropped below 7°C, the larvae would develop too slowly for winter pupation since 1st instar larvae do not feed below 8°C (Lees, 1962). Furthermore, even if the November and December temperatures did allow them to feed, the January temperature (2°C, Dennis, 1977) would probably kill them, for final instar larvae do not well tolerate cold (Lees & Tilley, 1980). Hence any surviving progeny of summer diapause pupae would almost certainly overwinter as larvae, possibly feeding intermittently up to their 3rd instar when they diapause (Lees & Tilley, 1980), then resume their development in early spring to eclose in late May or June as generation 2.ii.

Thus, under cooler climatic conditions (with more winter frosts) and summers, as with increasing altitude or latitude or glacial onsets, an increasing proportion of individuals overwinter as larvae to eclose as generation 1.ii, while those that overwinter as pupae, and especially if they experience frost, produce an unusually late generation 1.i. Over successive winters, 'Dauermodifikation' would result in this prospective generation 1 (both parts) eclosing increasingly late in spring, while over successive summers the resultant generation 2 (both parts) larvae would appear increasingly late in summer and autumn and so more likely also eclose increasingly late the following spring.

Once synchronised with the annual seasonal cycle, this univoltine strategy would be maintained directly by temperature (including frost) and photoperiod, possibly further stabilised through 'Dauermodifikation'.

Such a shift in voltinism might account for the origin of univoltine races and subspecies. The Norwegian subspecies *pallida* Verity is the northernmost subspecies and its markings (cf. Henriksen & Kreutzer, 1982) resemble the laboratory phenotypes in the present study. Race *drumensis* flies in June above the tree line in Snowdonia (Thompson, 1952). Its large size suggests it overwinters as a larva (cf. Robertson, 1980a), and that it has a pattern

typical of generation 1.i yet flies in June, suggests it may have arisen from a lineage of generation 1.i parentage whose progeny hatched increasingly late with altitude until the cooler temperatures necessitated their overwintering as larvae ; the race forms an altitudinal cline (Thompson, 1952). Field and laboratory studies might throw light on whether this is reflected in the life cycle. I propose that the capacity of S. Swedish *P. a. tircis* to undergo partial pupal diapause under 16h photophase even at 21°C (cf. Nylin *et al.*, 1989) may have arisen through successive exposure to the cooler winters or summers of its northerly latitude ; and represents a natural life cycle option intermediate to those of S. and N. Swedish populations, and possibly transitional in the evolution of stably univoltine *pallida*.

h) Voltinism changes and population structure

I shall now consider how the above model relates to the breeding structure of *P. aegeria*. The following considerations assume both that lineages must remain true to treatment, i.e. both parents experience frost, and that only a few individuals undergo a resultant developmental prolongation.

Populations might originate from single females (Barbour, 1986). Thus, given that both parents must experience frost for assimilation to ensue, then at least a pair must found the potentially univoltine population. Hence there may be a potential for a shift to univoltinism even when few pupae suffer frost exposure and show prolonged development.

Yet groups of pupae may suffer exposure in 'frost hollows' (Shapiro, 1975). Moreover, fully formed adults may rest in the pupa until the temperature becomes warm enough for adult activity (Goddard, 1962). Thus a spell of cool weather after the unexposed adults have emerged may cause affected pupae to further postpone their already delayed emergence, so that the 'brood' becomes divided into two groups. Over successive winters and cool summers, the two groups become increasingly disparate. The progressive 'deceleration' of the life cycle over successive cool seasons results in more and more individuals leaving the unmodified early 'brood' and entering the delayed 'brood' (cf. Lloyd & White, 1976). In this way, the transition to univoltinism might ensue. Indeed temporal subspeciation has been implicated in *Maniola jurtina* (Linnaeus, 1758) (Thomson, 1971).

The initially small delayed brood, however, may suffer high inbreeding, when survival under frost decreases. The author proposes that there are three classes of response : an 'unreactive' class unaffected by frost ; a 'viable reactive' class whose development is prolonged to varying degrees ; and an 'inviable reactive' class killed by frost. As the transition ensues, some of the unreactive class shifts into the viable range so the proportion of unreactive individuals decreases, while the most reactive range of the viable class shifts into the inviable range. This limits the magnitude of response displayed by reactive individuals ; and would explain the increased mortality under cold in the experimental F_2. As the inviable class does not contribute to the breeding

population, further cycles of cool seasons segregate out the lethal response, and this is much more likely if the stimulus is successive and the remaining response adaptive. Just such segregation has been reported for a laboratory *Drosophila* phenocopy, where selection operated simultaneously on associated yet opposing traits (Waddington, 1957). The net result is an increase in average prolongation which becomes less variable as the reactive range gets compressed ; the response may eventually come under full genetic control. Indeed, certain populations of *Papilio zelicaon* Boisduval have undergone a total transition, albeit to *bi*-voltinism, in under 200 generations (Shapiro, 1976).

The crucial question is how closely the above model reflects the natural biology of *P. aegeria*. The species quickly recovers when faced with new habitat and a drastic fall in numbers (Rimington, 1986). Moreover, inbreeding concerns more individual genic balance than population structure as a whole (Oliver, 1981), and might even facilitate the transition : larval survival improved with inbreeding even in samples of 'cold shock' parentage. The spread of the species from deciduous to coniferous woodland within north-east Scotland implicates a minor shift in its climatic tolerance (Barbour, 1986) since coniferous woods are cooler (Geiger, 1950). Although the life cycle here has yet to be examined, in 1977 the butterfly produced two broods for the first time on the Isle of Canna showing that intra-population voltinism shifts can occur (Campbell, 1978). A possible barrier to a voltinism shift would be a sex-specificity, but the present results revealed no protandry or protogyny ; nor did Nylin *et al.* (1989) report any related sex difference.

Although the cooling summers may be damper, *P. aegeria* fares better in wet seasons (Lees, 1962), while extreme winter cold may help pupae survive otherwise adverse conditions (Masaki, 1980). The species can develop at 11°C without mortality, and the lesser annual build up of number in univoltines is compensated by their larger egg batches (Wiklund & Persson, 1983).

Furthermore, the dark phenotype of frost-affected adults may enable them to survive in cooler habitats. The species thermoregulates by dorsal basking ; and darker summer brood adults actively seek and breed in dense woodland too cool for activity earlier in the season (Shreeve, 1985), which in turn influences their offspring (Shreeve, 1986). Thus the phenotype of subspecies *pallida* may have originated as frost-induced phenocopies which slowly crept northwards. Indeed studies of life cycle and phenotype responses to different climatic conditions in the various subspecies could help reconstruct the species' history.

j) Conservation

It is important to evaluate the conservation status of species not only in the light of what is known of direct climatic effects on the species in question, but also of secondary factors such as foodplant and parasite interactions and their responses to climate (cf. Porter, 1984), in moves towards implementing appropriate conservation measures. The extensive literature on *P. aegeria*

(see WINOKUR, 1989 for review) shows populations to differ in their individual responses to climate. In the case of small localised populations (such as with *P. a. tircis* in N. Turkey, P. Sigbert WAGENER, pers. comm.), the capacity to adjust the life cycle in the face of climatic stress (e.g. drought or cold) could prove crucial to their survival or extinction, when laboratory studies would be invaluable in assessing their requirements for site management and captive breeding programmes.

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