Belg. J. Zool. - Volume 127 (1997) - issue 2 - pages 167-178 - Brussels 1997

SEASONAL PHENOTYPIC VARIATION IN THE SPECKLED WOOD BUTTERFLY (PARARGE AEGERIA L.): PATTERNS IN AND RELATIONSHIPS BETWEEN WING CHARACTERS

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Abstract. We studied the variation in dorsal wing colour, dorsal hindwing spotting and wing length within a population of the speckled wood butterfly *Pararge aegeria* (L.) in two successive years. Over time in the first generation, successively emerging butterflies were darker, larger and of higher spot type (a clearer fourth submarginal spot). In the second generation they remained on average rather dark and of median size, but variation in spotting repeated the pattern of the first generation. We interpret these patterns of variation in colour and size in relation to seasonal changes in the irradiance conditions within a temperate forest. We suggest that there is a larger genetic control of spotting than of wing colour and wing length. Females were darker, larger and of higher spot type than males. Darker males were on average larger and of higher spot type than pale males. These relationships can be interpreted in relation to the males' mate-locating strategies.

Key words: *Pararge aegeria*, butterflies, phenotypic variation, seasonal variation, wing colour, wing spots, mate-locating strategies.

INTRODUCTION

The reproductive success or fitness of an individual largely depends on how well its phenotype is adapted to the abiotic and biotic environment it experiences. Variation in phenotype may help individuals to better match the environment in time (seasonal) and/or space (geographic) (ENDLER, 1986). Phenotypes of co-occurring individuals may vary adaptively in relation to for instance their behaviour (VAN DYCK *et al.*, 1997a, 1997b). In butterflies, phenotypic variation in wing features (colour patterns, wing size and shape, etc.) is a popular object of study since the wings are essential for flight activity (*e.g.*, DUDLEY, 1990) and play significant roles in predator escape (BRAKEFIELD *et al.*, 1992), thermoregulation (WASSERTHAL, 1975; DENNIS, 1993), sexual communication and mate choice (SILBERGLIED, 1984). Here we report on the phenotypic variation in a population of a butterfly which emerge over a long period during the year. In order to interpret the relationships between phenotypic variation, environment and behaviour, we need to know how the phenotypes vary with time of emergence and how the different phenotypic features are linked to one another.

Alternative phenotypes (WEST-EBERHARD, 1989) may exist as genetic polymorphisms caused by different genes (FORD, 1975) or polyphenisms induced by the environment (SHAPIRO, 1976). Examples in butterflies are white-yellow polymorphism in *Colias* (WATT, 1968), dry-wet seasonal polyphenism in tropical Satyrinae (BRAKEFIELD & LARSEN, 1984; WINDIG *et al.*, 1994) and spring-summer polyphenism in the European map butterfly *Araschnia levana* (MÜLLER, 1955). Polyphenism does not exclude genetic variation (HAZEL *et al.*, 1987) and can be itself under genetic control (BRADSHAW, 1965; WINDIG, 1994). Sometimes what looks like a genetic polymorphism turns out to be under environmental control (OWEN & GOULSON, 1994).

In the speckled wood butterfly *Pararge aegeria* (L.) there is geographic variation in wing length (size), wing shape and the pattern of dorsal pale patches (BRAKEFIELD & SHREEVE, 1992). Since in northwestern Europe adults of this satyrine butterfly emerge over an extended period from April to September (BINK, 1992), different individuals experience very different larval conditions throughout the season which may influence their adult phenotype. The adaptive value of the phenotype probably also varies over the season. When reared outdoors, British speckled woods vary seasonally in forewing length and in the size of the dorsal pale patches (ROBERTSON, 1980), as well as in the number of submarginal spots on the hindwings and the brown dorsal wing colour (pale or dark) (PACKER, 1984).

We studied the variation in dorsal wing colour, dorsal hindwing spotting and wing size (forewing length) amongst individuals within a Belgian population of the speckled wood in two successive years. We analysed (1) the temporal pattern of these wing features in both generations, and (2) the relations between the features. Of the two biometric studies on (British) speckled woods one did not consider the variation in brown dorsal wing colour (ROBERTSON, 1980) and the other one (on collection specimens) did not consider size (PACKER, 1984).

MATERIAL AND METHODS

The speckled wood occurs primarly in different types of woodland and the larvae feed on grasses (SHREEVE, 1986a). We studied the speckled wood (North-European subspecies *P. aegeria tircis* Butler 1867) in a set of deciduous woodland fragments («Lankem») in Herentals, northern Belgium (51°08' N, 4°49' E). As in the rest of Belgium, *P. aegeria* is a common species and has become even more abundant in recent decades (MAES & VAN DYCK, 1996). The study area consisted of five fragments of the same habitat type (<1 ha to >9 ha) which were close to one another and separated by maize fields and meadows. In all sites the dominant tree species was common oak, *Quercus robur*, with a mixture of other trees and shrubs. A large part of the forest floor was covered by brambles, *Rubus* sp. Larval hostplants (*e.g. Agropyron repens*, *Dactylis glomerata* and *Poa annua*) were abundant in and around the study areas.

We collected data on sex, dorsal wing colour, dorsal submarginal spot pattern and forewing length in two successive years: between 20 April and 18 September 1993 and 27 April and 25 August 1994. These periods covered the first and the second generation of each year, also called spring and summer generation respectively. In both years the first generation lasted until the end of June and the second generation started at the beginning of July. The end of the first generation was marked by a strong decrease in abundance and absence of newly emerged, *i.e.* undamaged and unmarked, butterflies. We captured butterflies with a hand net and marked them to avoid double measurements by writing a small number with a non-toxic black pen on the hindwings. We took all measurements at the first capture. We assigned the dorsal wing colour to one of four categories from pale brown (1) to dark brown (4). The dorsal pattern of black submarginal spots on the hindwings was scored in four categories: (1) three spots, (2) three spots and a vestigial fourth spot, (3) four spots but the fourth is small, and (4) four clear spots. Both indices have a high repeatability (VAN DYCK *et al.*, 1997a). Forewing length was measured from the wing joint to the wing tip and read to the nearest 0.05 mm with calipers. Date (April 20 being 1), time and exact location of the capture were also noted.

First, we compared the overall mean values of the different wing features between males and females. Further analyses were performed on the males only since we had insufficient data for the females. Second, we analysed the temporal variation of wing colour, wing length and spot pattern in relation to date, generation and year. In the graphs (Fig. 2) we expressed the mean values by four equally long periods in the first and second generation each, for convenience. In the second generation of 1993 we have data of a fifth period. Third, we analysed the relations between the wing features by calculating correlations and performing a principal component analysis. Smoothed values (moving averages) were used to plot the first principal component over time (cf. WINDIG *et al.*, 1994). These moving averages were calculated over a period extending immediately before and after each date so as to include a total of at least eleven individuals (five before and five after). Temporal variation in the wing features and the principal component was analysed by general linear regression models (GLM-procedure in SAS, with type III Sum of Squares). GLM handles discrete variables (*e.g.*, colour classes) and continuous variables (*e.g.*, wing length) (SAS, 1990). The full model for each wing feature or principal component started with the seasonal parameters (date, generation and year) and all interaction terms. Minimum adequate model selection was done by backward elimination of the least significant factors of the full model. We used the SAS-package for all the statistical analyses (SAS, 1990). Means are given ± SE.

RESULTS

Differences between sexes

In both years, males were the most commonly observed sex: 87.8% in 1993 (N=213) and 91.1% in 1994 (N=136). This is mainly caused by differences in activity, and thus apparency, of the sexes (cf. DAVIES, 1978). Considering all data, we found females on average to be larger ($F_{1,314}=35.81$, $P=0.0001$), darker ($F_{1,349}=5.09$, $P=0.024$) and of a high-

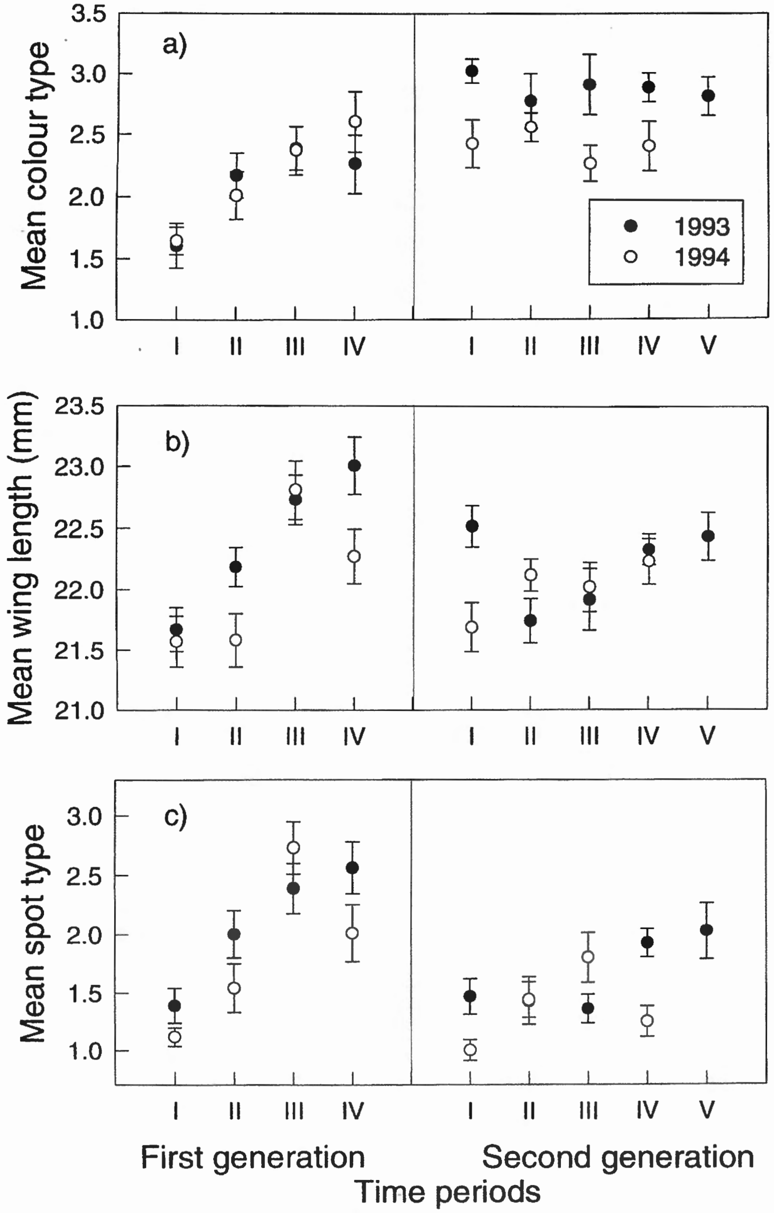

Fig. 2. – Seasonal pattern for (a) dorsal wing colour, (b) forewing length and (c) dorsal hindwing spot type in the males for 1993 and 1994. The mean values (±SE) are given for 4 equally long time periods of the first and second generation of 1993 and 1994. For the second generation of 1993 there is a fifth period. Generation 1: four periods between 20/4 - 25/6 and generation 2: five periods between 5/7-18/9.

er spot type ($F_{1,349}$=10.86, P=0.0011) than males. Male wing length varied between 19.4 and 24.8 mm with a mean of 22.17±0.01 mm, and female wing length between 21.5 and 25.4 mm with a mean of 23.41±0.04 mm. Fig. 1. shows the frequencies of the colour and spot types for both sexes.

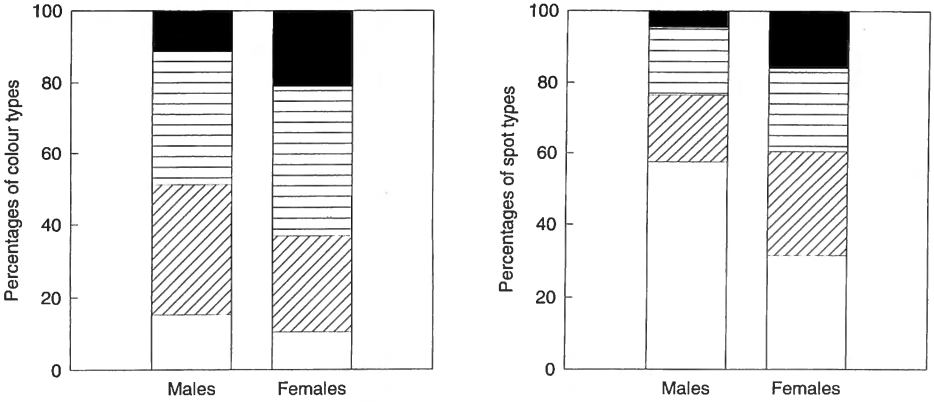

Fig. 1. – Total frequencies of (left) the different colour types (white=colour 1 or pale, shaded= colour 2, horizontal lines=colour 3 and black=colour 4 or dark) and (right) the different spot types (white=spot type 1, shaded=2, horizontal lines=3 and black=4) for males (N=311) and females (N=38) in the combined data set from 1993-94.

Temporal variation in wing colour, wing length and hindwing spotting

Earlier emerging first-generation males were paler than late emerging first-generation and second-generation males (Fig. 2). The analysis of variation in the brown background colour in relation to date, generation and year, showed a significant generation-year (P= 0.021) and generation-date (P=0.0007) interaction. When analysing the generations separately, we found a strong effect of date and not of year in the first generation (F1,129= 13.55, P=0.0003) and a strong effect of year but not date in the second generation (F1,182=19.04, P=0.0001). The dorsal wing colour became darker with successive emergence within the first generation and remained dark during the second generation, but at a lower level in 1994 compared to 1993 (Fig. 2).

The largest individuals were found in the second half of the first generation (Fig. 2). The analysis of wing length variation showed a significant generation-date interaction (P= 0.0003). In the separate analysis by generation, we found date as well as year to be significant in the first generation ($F_{2,109}$=11.53, P=0.0001 : date F=20.13, P=0.0001 and year F=4.57, P=0.034) but we found no significant relation with any variable in the second generation ($F_{2,109}$=1.15, P=0.31). Successively emerging individuals were larger within the first generation of both years, but were smaller in 1994, which explains the year effect. In the second generation mean size was comparable to the first generation but with no seasonal trend.

Wing spotting increased with later emergence during the first generation, reaching the highest values in the late first-generation individuals (Fig. 2). The pattern of the second generation was more complicated and differed between years. The regression model kept date, year and the interaction between generation and date as significant terms ($F_{4,310}$= 11.76, P=0.0001: date F=28.84, P=0.0001, year F=6.60, P=0.0107, generation F=1.44, P=0.23, and generation x date F=5.98, P=0.015). Since there was a significant generation x date interaction, we split the further analysis for the first and second generation. In both cases, the only significant term retained by the final model was date (first generation: $F_{1,129}$=21.60, P=0.0001 and second generation: $F_{1,181}$=5.15, P=0.024). In contrast to seasonal variation of wing colour and wing length, we also found a significant, though weak, date effect in the second generation. The results suggest, as shown by fig. 2, that the temporal variation of spotting from the first generation is repeated in the second, although the values for the second generation were lower.

Relations between the wing features

Darker individuals were on average larger and of higher spot type (Fig. 3). The correlations between the wing features are: wing colour – spotting: r=0.41, P=0.0001; wing colour – wing length: r=0.35, P=0.0001 and spotting – wing length: r=0.32, P=0.0001. The first principal component (PC1) accounted for nearly 60 % of the total variation of the three features together. PC1 was equally loaded by wing colour, spotting and wing length (Table I). The second and third principal component (PC2 and PC3) had smaller eigenvalues (about 20 % each) and thus represented less variation than one trait separately. PC2 mainly contrasted a large size with a low spot type (or vice versa) and PC3 a dark wing colour with low spot type (or vice versa) (Table I).

TABLE I

Weightings and eigenvalues from principal component analysis of the three studied wing features (dorsal wing colour, dorsal hindwing spotting and wing length) for the 285 males (all data from 1993 and 1994)

	PC1	*PC2*	*PC3*
WING COLOUR	0.599	-0.236	-0.764
SPOTTING	0.581	-0.526	0.619
WING LENGTH	0.549	0.816	0.178
Eigenvalues	57.3%	23.1%	19.6%

PC1 values increased with date in the first generation in both years and in the second generation the values of 1994 were lower than those of 1993 (Fig. 4). The general seasonal pattern of the PC1 was similar between the years (Fig. 4). In the analysis of the PC1 with the temporal variables we found a significant date-generation interaction (P=0.0001). When splitting the further analysis for generation, there was a significant date effect in the first generation ($F_{1,110}$=30.55, P=0.0001) and a significant year effect in the second ($F_{1,173}$= 13.90, P=0.0003).

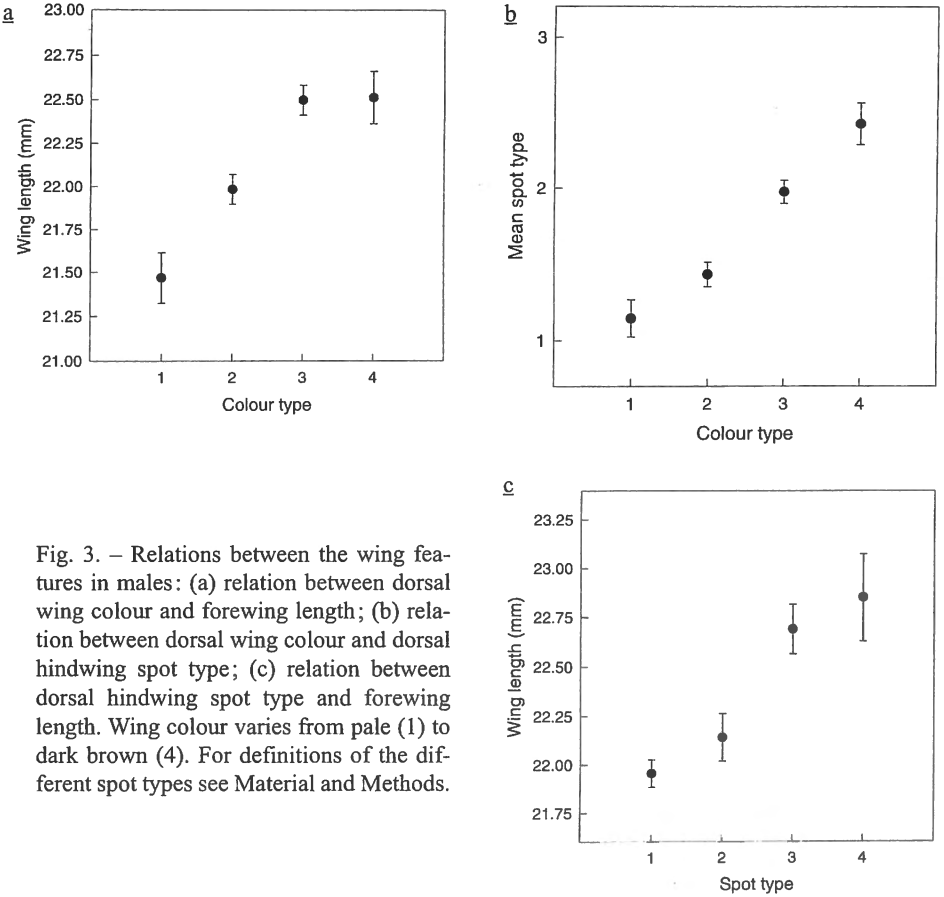

Fig. 3. – Relations between the wing features in males: (a) relation between dorsal wing colour and forewing length; (b) relation between dorsal wing colour and dorsal hindwing spot type; (c) relation between dorsal hindwing spot type and forewing length. Wing colour varies from pale (1) to dark brown (4). For definitions of the different spot types see Material and Methods.

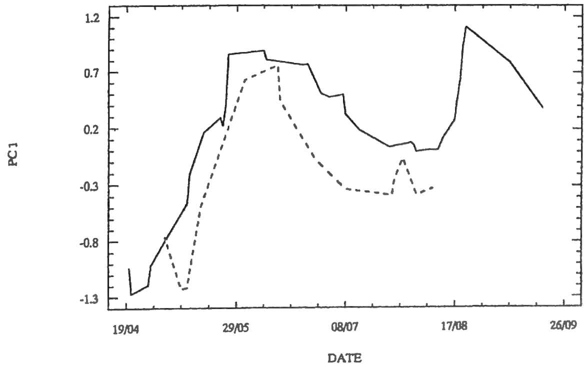

Fig. 4. – Seasonal variation of the PC1 of the principal component analysis in 1993 (black line) and 1994 (stippled line) expressed as moving averages (see Material and Methods).

DISCUSSION

We found that dorsal wing colour, spotting and wing length in the males of the speckled wood butterfly were interrelated and that these wing features varied seasonally but not necessarily in parallel. Darker males were on average larger and of higher spot type. In the first generation successively emerging males were darker, larger and of higher spot type. In the second generation they remained on average rather dark and of medium size, but the variation in spotting repeated the pattern of the first generation though less markedly. From the total dataset we showed that females were on average darker, larger and of higher spot type than the males.

The phenotypic composition of the population changed gradually over time, which implies that there were no discrete seasonal forms. The observed patterns of size and wing colour agree with the results on British speckled woods of ROBERTSON (1980) and PACKER (1984), respectively. The seasonal pattern of submarginal wingspots appeared to be different from the one of wing length and colour, since the trend of the first generation was more or less repeated in the second generation. PACKER (1984) also found a repetition of the seasonal pattern for wing spotting in the two generations. However, this was only the case when he analysed the specimens from a single location. In his entire dataset, with collected specimens from different localities, this trend was not detected and geographical differences for spot type within Great-Britain were found.

Is there an adaptive explanation for being small and pale in early spring? In several insects, including butterflies, it has been shown that spring individuals were more melanized than summer individuals for thermoregulatory reasons (e.g., SHAPIRO, 1976; KINGSOLVER & WIERNASZ, 1991; HOLLOWAY, 1993), which is contrary to the pattern described here. In temperate regions ambient temperature and particularly irradiance is the highest in summer (June-July-August). However, irradiance conditions vary in a different way at the floor of a temperate deciduous forest since there is interference with the degree of foliation: irradiance at the forest floor peaks in the period April-May (LARCHER, 1995). Later in the season increasing fraction of the forest floor becomes shaded and other parts receive less radiation. In these conditions an individual may benefit from a larger size, which implies less influence of convective cooling when flying through the forest (HEINRICH, 1993), and from a darker dorsal colour, which means an increased heating rate (VAN DYCK & MATTHYSEN, in press). Speckled wood males emerging in early spring (April-May) could afford a smaller size and a paler dorsal surface. From the beginning of July radiation levels on the forest floor remain at a low constant level (LARCHER, 1995), which could explain why we did not find significant differences with date for either wing colour and wing length in the second generation.

Submarginal wing spotting is often considered as a predator deflecting device (BLEST, 1957), hence our results might suggest seasonal variation in predation pressure. Alternatively, predation pressure may vary with the activity of the butterfly (BRAKEFIELD, 1984). Brakefield showed that males of the meadow brown butterfly, *Maniola jurtina*, which are more active and therefore more visible for predators than females, had more wingspots. Frequencies of behavioural strategies (probably with different predation risks) vary seasonally in the speckled wood (WICKMAN & WIKLUND, 1983; VAN DYCK *et al.*, 1997a) and may vary geographically as well. DENNIS *et al.* (1986) showed that spotting differences were correlated with activity levels in *Coenonympha tullia* on a geographic scale (*i.e.* Britain).

In our population three-spot-individuals (classes 1 and 2) were more abundant (76.5 % from the total dataset of males) than four-spot-individuals (classes 3 and 4). This is in contrast with the results of British studies with 35.1 % and 33.8 % three-spot males only (PACKER, 1984 and SHREEVE, 1987, respectively). Our study population seems to be representative for the Belgian population since three-spots were also more abundant in a large collection of Belgian speckled woods (Royal Belgian Institute for Natural Sciences, Brussels): from 313 male specimens 73.2 % were three-spot-butterflies (VAN DYCK, unpublished). This suggests a spotting difference on a geographic scale.

The correlations between the studied wing features in the male speckled woods can be interpreted in relation to their mate-locating and possibly dispersal behaviour (VAN DYCK *et al.*, 1997a): darker, larger males mainly patrol, while paler ones mainly perch in a territorial way. A higher spot type (i.e. four in stead of three wing spots) may be more beneficial to a patroller and disperser since the fourth wing spot is visible when flying but is rarely shown when sitting (VAN DYCK *et al.*, 1997a). However, it remains unclear whether having a small, fourth wing spot has an adaptive value for predator deflection, and whether there is selection on that feature in our study population.

The results for the females (which were on average darker, larger and with more spots than the males) suggest that they have the wing features of a «patroller». Females, which are single egg-layers, spend a lot of time searching for suitable hostgrasses (within a range of 24-30 °C) and they often have to cross shaded parts of the forest floor or even deposit eggs in shaded locations (SHREEVE, 1986a). The adaptive value of the female's phenotype needs however further investigation since not only mobility matters for the females but other aspects such optimal thermoregulation in relation to egg maturation and crypsis may play significant roles as well.

The role of genes in the production of the studied wing features cannot be estimated directly from the results of this study. Nevertheless, it is likely that wing length and wing colour are examples of phenotypic plasticity. The second generation, descendents of the first generation, had a different average phenotype compared to their parental generation. Both wing length and colour are probably influenced by the duration or timing of the larval stage (development time) which varies at least seasonally. As in Britain (SHREEVE, 1986b) and southern Sweden (NYLIN *et al.*, 1989), *P. aegeria* is able to overwinter in Belgium in the pupal stage and in the larval stage as well (VAN DYCK, personal observation). As a result the early spring individuals overwinter as pupae, the late spring butterflies as larvae. The late-spring or larva-overwintering individuals were darker and larger than early spring or pupa-overwintering butterflies. The former live for a very long period as larvae (from autumn to early spring) and continue feeding when the ambient temperature is up to only a few degrees Celsius (6.5°C, LEES, 1962; 3-4°C, WIKLUND *et al.*, 1983). The adults of the second generation all develop and pupate in late spring or early summer conditions which vary less, and we found wing length and wing colour also to be less variable in the summer generation of both years. The temporal trends were similar in both years, but we found lower mean wing colour values in the second generation of 1993 compared to 1994. It is worth remarking that the spring of 1994 (when the summer butterflies were larvae) was wet and cold by Belgian standards. Nevertheless the wing features can be influenced by the same environmental variables (suggested by their conjuction expressed in the PC1). Wing colour and spotting are known to be developmentally independent since different physiological pathways are used (NIJHOUT, 1991). Therefore it is unlikely that the correlation between pigmentation and spotting is causally linked. Moreover, we did find individuals with a dark wing colour and low spotting (or vice versa) as indicated by the weightings of PC 3 of the principal component analysis.

Knowing that (1) the first individuals of the summer generation are the offspring of the first of the spring generation (although there may be some overlap between the two emergences of the first generation), (2) the pattern of spot type of the spring generation was repeated in the summer generation, while this was not the case for wing colour and wing length, we suggest that the genetic control for spotting is considerably larger than for colouration and size. This suggests that there would be seasonal variation in the genetic composition of the population. In other satyrine butterflies, spot pattern characters have been shown to have a considerable heritability (BRAKEFIELD, 1984; BRAKEFIELD & VAN NOORDWIJK, 1985; WINDIG, 1994). In the tropical satyrine *Bicyclus anynana* heritabilities of wing patterns vary with ambient temperature (WINDIG, 1994) and within temperature regimes development time was related to the wing phenotype (BRAKEFIELD & REITSMA, 1991; WINDIG, 1992). In the speckled wood, complex variation in development rates and even developmental pathways have been documented (e.g., NYLIN *et al.*, 1989), but possible associations with phenotype production remain obscure. Experiments are now underway rearing several families under controlled climatic conditons in order to examine some of these interactions.

ACKNOWLEDGEMENTS

HVD was supported by a grant of the Flemish Institute for the Promotion of Scientific and Technological Research (IWT). EM is research associate with the National Fund for Scientific Research (NFWO).

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