

Generations of the polyphenic butterfly *Araschnia levana* differ in body design

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ABSTRACT

The European Map Butterfly, *Araschnia levana* (Nymphalidae), represents a classical example of seasonal polymorphism, in which summer and spring generations differ strikingly in wing colour pattern. Although the physiological background to the formation of the two forms is well known, few studies have explored its possible ecological significance. Based on previous findings that both population number and body size tend to be larger in summer, we examined the hypothesis that the summer generation is more capable of long-distance dispersal. We re-analysed mark–recapture data on the butterfly using the multi-strata modelling design in the program MARK, compared biomechanical design traits of the spring- and summer-generation butterflies using canonical variate analysis, and tested for inter-generation differences in allometry relations of design traits to body size. The summer butterflies had shorter residence times and a higher probability of movements among capture sites than the spring butterflies, indicating greater mobility. Regarding the design traits, adults of summer-generation butterflies had a heavier thorax, lower abdomen to body mass ratio, larger wing area, less pointed wings and lower wing loading. The differences in design traits remained significant even after filtering out the effect of sex. Multiple regressions of the biomechanical traits against dry body mass confirmed the above differences between generations and showed that the differences were not only due to different body size. The results support the notion of greater mobility of summer-generation *A. levana* and suggest the possibility that differential dispersal is much more widespread in butterflies with multiple generations. This is well known in insect groups that demonstrate obvious wing size polymorphism, and might manifest itself in butterflies through more subtle shifts in body design proportions.

Keywords: allometry, biomechanical design, canonical variate analysis, dispersal, Lepidoptera, seasonal polyphenism.

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INTRODUCTION

The European Map Butterfly, *Araschnia levana* (Linnaeus, 1758), is a striking example of seasonal polyphenism, the existence of seasonally different generations. Spring and summer generations differ in wing colour patterns to such an extent that they were originally listed as different species (cf. Nijhout, 1991; Windig and Lammar, 1999). The formation of colour pattern is controlled by the timing of ecdysteroid release after pupation (Koch, 1992, 1996). The timing itself is controlled by length of day (short days induce diapause). Although the physiological background to the formation of the two forms is well known, there is uncertainty regarding the ecological significance of the phenomenon. Windig (1999) and Windig and Lammar (1999) studied the evolutionary genetics of the species and found that various wing pattern traits reacted differently to environmental variation and did not overlap across environments. They found neither individual differences in heritability of wing-pattern traits, nor genetic correlations among the traits across different environments. Thus, the two forms were completely discrete, and the authors speculated on such polyphenism as a step towards speciation. However, the possible adaptive value of having two generations that differ entirely in colour pattern is not understood in this species.

One of the hypotheses explaining seasonal polyphenism assumes that phenotypic differences in different generations allow a species to cope with seasonally changing environments (Shapiro, 1976; Brakefield, 1996). If true, then different selective forces should act on different forms of *A. levana*. In a previous paper (Fric and Konvička, 2000), we compared the population structure and behaviour of the two generations and found that diurnal periodicity and mate-locating behaviour were essentially identical in spring and summer butterflies. However, the summer generation was more numerous, and we obtained indirect evidence of its greater mobility, since the summer generation showed a distinctly open (*sensu* Warren, 1992) population structure. The summer-generation butterflies are also larger (Windig, 1999); larger size is likely to be beneficial to dispersing butterflies, since it increases their chances for successful colonization of new habitats (Kuussaari *et al.*, 1996; Thomas *et al.*, 1998). Thus, we hypothesized that the butterflies of the spring generation might preferentially remain on sites where they developed from hibernating pupae, whereas the summer butterflies might tend to leave their sites of emergence.

Here, we explore further the hypothesis that summer-generation *A. levana* are more mobile. We first re-analyse the mark–recapture data of Fric and Konvička (2000) and compare directly mobility-related parameters of the two generations. We use a modelling method that estimates the parameters of residence time (see below), capture probability and rate of migration between capture sites.

Second, we analyse differences in ‘biomechanical design’ between spring and summer butterflies (Wickman, 1992): in allocation of body mass and in shape and area of wings. Differences among species that are exposed to differing selective forces were investigated in the context of palatability and predation (Chai and Srygley, 1990; Marden and Chai, 1991; Srygley and Kingsolver, 1998; Kingsolver and Srygley, 2000) and in studies of mating tactics (Wickman, 1992). More relevantly, biomechanical traits may differ within individuals of the same species originating from isolated and expanding populations (Thomas *et al.*, 1998; Hill *et al.*, 1999a,b; Van Dyck and Matthysen, 1999). Adult butterflies from recently expanding populations, as well as individuals from fragmented landscapes, tend to be larger, which enhances their ability to colonize new habitats. They also tend to have relatively heavier thoraxes, due to a higher investment in flight muscles, and larger wing areas (Thomas *et al.*,

1998; Hill *et al.*, 1999a); that translates into a lower wing loading (Roff and Fairbairn, 1991). Windig (1999) and Windig and Lammar (1999) reported summer-generation *A. levana* to be larger (measured as adult wing area) but, to our knowledge, biomechanical traits have never been analysed across different generations in butterflies.

Third, we analyse patterns of allometric differences between adults of the two generations to exclude the possibility that differences in design are only due to the different sizes of the butterflies.

METHODS

Modelling mobility parameters

The mark–recapture data were obtained during 1997 using a standard protocol. We marked butterflies within the study site, which was a system of wet meadows and deciduous woodlots, for entire flight periods of the spring (5–26 May) and summer (16 July–13 August) generations, and recorded sex and position of each capture in four separate sections of the study site (details in Fric and Konvička, 2000).

We used the program MARK (White and Burnham, 1999; Cooch and White, 2001) to analyse the data. The program considers pre-defined individual covariates of marked animals (such as sex, site, time of capture, etc.) and allows the construction of multiple models that vary in their numbers of parameters. The parameters are computed by a generalized linear modelling procedure (Lebreton *et al.*, 1992); their parsimony is compared using the quasi-likelihood AIC values (QAICc) (Franklin, 1999).

Since we had four possible locations of capture of each individual, we applied the multi-strata design with the logit link function (Brownie *et al.*, 1993). The capture sites of individual butterflies were entered into the data matrix with sex as the individual covariate. The design allowed us to consider movements of the butterflies among the capture sites and to estimate the following parameters: relative residence (i.e. the probability that a butterfly remains alive at the site of its original capture, S); capture probability (p); and the probability of movement between capture points (Ψ) (Cooch and White, 2001).

The program selects the model that provides the most parsimonious explanation of the mark–recapture data. However, if the ‘best’ models for different data sets differ in their constituent parameters, their parameter values cannot be compared directly. Since this was the case, we looked for a pair of simpler models that contained mutually matching parameters and that still explained a sufficiently high proportion of the original deviance (Lebreton *et al.*, 1992). We used these ‘simplified’ models to compare their parameters.

Biomechanical design

In 1997 and 2001, we collected males and females of both generations of *A. levana* (spring generation: 22♂♂, 13♀♀; summer generation: 36♂♂, 20♀♀) in the field. The material originated from four sites, all of them within 25 km², in the close vicinity of České Budějovice, South Bohemia, Czech Republic (49°02'N, 14°30'E). We processed the butterflies as in Wickman (1992); that is, we weighed their fresh mass, dried them at 60°C to constant weight, and weighed their total dry mass, thorax mass and abdomen mass. We placed the wings in a natural position as in flight and digitized their images. We measured the length of the forewings, wing area and the orthogonal projection of the centroid of the wing area on wing

diameter (hereafter ‘centroid projection’), using the program Scion Image Beta 3b (©Scion Corp., 1998). We used the above values, hereafter referred to as ‘raw’ variables, to compute the following ‘derived’ variables: (1) flight muscle ratio, i.e. thorax mass to total body mass; (2) abdomen ratio, i.e. abdomen mass to total body mass; (3) wing loading, i.e. fresh body mass divided by wing area; (4) aspect ratio, i.e. wing span squared divided by wing area; and (5) radius of the first moment of wing area, i.e. the spanwise position of the normalized chord through the centroid of the wing area as a fraction of the wing length (hereafter ‘radius’). The latter variable is a measure of the pointedness of wings (Wickman, 1992).

We thus had four classes of butterflies – spring males, spring females, summer males and summer females – and our main concern was whether the classes differed in their biomechanical traits. In other words, we examined the hypothesis that the biomechanical traits, being the explanatory variables, can be used to separate the classes. We used canonical variate analysis (CVA), a variant of classical linear discrimination analysis, and performed three separate analyses. The first analysis included all possible explanatory variables; the second included only the raw variables and the third only the derived variables. We used CANOCO for Windows, version 4.0 (Ter Braak and Šmilauer, 1998), which computes CVA as a variant of canonical correspondence analysis with Hill’s scaling and handles the discriminated classes as dummy ‘species data’. We first tested individual effects of all potential explanatory variables, and then forward-selected for the best sets of explanatory variables, using CANOCO’s variance inflation factors for eliminating redundant variables. We assessed the statistical significance of the results using non-parametric Monte Carlo permutation tests (MCPT; 999 runs per analysis).

To eliminate the possibility that significant differentiation among the classes was due to differences between males and females, we subsequently repeated the analyses with two classes only, spring and summer butterflies. Sex was included as a categorical covariable whose influence on the statistical significance of the results was filtered out (Ter Braak and Šmilauer, 1998).

We also controlled for the effects of year and site, again using CVA, with the classes as dummy species data and year/site as categorical explanatory variables.

Allometry

Differences in biomechanical design between generations and between the sexes could be caused solely by different allometric growth patterns in individuals of different size (Klingenberg, 1996). To exclude this possibility, we constructed multiple-regression models in which the effects tested were the design variables from the previous analyses and the explanatory variables were the dry mass of individuals, sex, generation (the two as categorical predictors) and all possible interactions. Dry mass was included in the models as a surrogate of body mass to force the size-related variation to the relationships, while the inclusion of the two categorical predictors tested for differences due to generation or sex independent of body size. The significant relationships explained by the categorical predictors indicate different heights of the respective regression lines; the relationships explained by interactions indicate differences in regression slopes.

Possible part-whole bias (in the models with the ‘derived variables’, such as the flight muscle ratio) was eliminated because total dry mass was included in the multiple regressions. Hence the variation due to the categorical predictors and interactions was assessed after accounting for the background variation.

All the regressions were constructed as linear models, using the program S-plus (S-Plus 2000, 1999). To avoid the false rejection of the null hypotheses in running multiple models, we used the Bonferroni correction, setting α to 0.005 (ten multiple-regression models).

RESULTS

Modelling mobility parameters

In total, we caught 445 spring-generation butterflies (320 ♂♂, 125 ♀♀) and 960 summer-generation butterflies (759 ♂♂, 201 ♀♀). The two ‘best’ models (for spring and summer generations) selected by the MARK program differed substantially in their number of constituent parameters (Table 1). In the spring generation, the residence of the butterflies was independent of sex and position of capture, and males had a higher capture probability

Table 1. The estimated parameter values for the best models that fitted the mark–release–recapture data for the spring and summer generations of *Araschnia levana*

Parameter	Estimated value	Standard error
Spring generation		
$S(\cdot)$	0.647	0.1859
$p(\text{♂♂})$	0.148	0.0771
$p(\text{♀♀})$	0.039	0.0423
$\Psi(\text{from A, B})$	1.000	≈0
$\Psi(\text{from C})$	0.063	0.0433
$\Psi(\text{from D})$	0.175	0.1167
Summer generation		
$S(\text{A ♂♂})$	0.364	0.1124
$S(\text{A ♀♀})$	0.416	0.2552
$S(\text{B ♂♂})$	0.518	0.0755
$S(\text{B ♀♀})$	0.730	0.2482
$S(\text{C ♂♂})$	0.447	0.0635
$S(\text{C ♀♀})$	0.244	0.2198
$S(\text{D ♂♂})$	0.345	0.0904
$S(\text{D ♀♀})$	0.293	0.2519
$p(\text{A ♂♂})$	0.027	0.0160
$p(\text{A ♀♀})$	0.121	0.0720
$p(\text{B ♂♂})$	0.384	0.0720
$p(\text{B ♀♀})$	0.135	0.0731
$p(\text{C ♂♂})$	0.548	0.0961
$p(\text{C ♀♀})$	0.050	0.0406
$p(\text{D ♂♂})$	0.145	0.1221
$p(\text{D ♀♀})$	0.025	0.0267
$\Psi(\cdot)$	0.219	0.0165

Note: S = residence, p = capture probability, Ψ = probability of movement.

Table 2. Evaluation of the best models fitting the mark–release–recapture data of the spring and summer generations of *Araschnia levana* ('Best'), and comparison with models that contained identical parameters as the best model for the other generation ('Equivalent'), with the full model that contained all possible parameters, and with the most reduced models ('Simplified')

Model	AICc	AICc weight	Number of parameters	Model deviance
Spring generation				
Best: $S(\cdot)p(\text{sex})\Psi(A = B, \text{rest: every-to-all})$	129.9	0.355	6	54.4
Equivalent: $S(\text{site}*\text{sex})p(\text{site}*\text{sex})\Psi(\cdot)$	159.3	0.000	17	56.0
Full model: all possible parameters	294.4	0.000	24	185.5
Simplified model: $S(\cdot)p(\cdot)\Psi(\cdot)$	149.9	0.001	3	73.0
Summer generation				
Best: $S(\text{site}*\text{sex})p(\text{site}*\text{sex})\Psi(\cdot)$	1411.8	0.999	17	502.1
Equivalent: $S(\cdot)p(\text{sex})\Psi(A = B, \text{rest: every-to-all})$	5485.0	0.000	6	4597.8
Full model: all possible parameters	1451.2	0.000	40	492.5
Simplified model: $S(\cdot)p(\cdot)\Psi(\cdot)$	1473.7	0.000	3	592.6

Note: S = residence, p = capture probability, Ψ = probability of movement.

than females. The probability of movement between capture sites was high for two sites referred to as A and B in Fric and Konvička (2000) and very low for the other sites (Table 1).

The best model for the summer generation contained 17 parameters (Table 2). Both residence values and the probability of capture differed between the sexes and among sites, whereas the probability of moving between capture sites did not differ for individual sites. The residence values were lower than in the spring generation for both sexes at most of the sections. An exception was the residence of females at site B, where we regularly observed them to lay eggs at a large patch of *Urtica dioica*, the host plant of the butterfly.

It was not possible to compare the parameters of the two best models directly. The only simplified models that contained identical parameters for both generations, and at the same time achieved sufficiently low deviance values, were the most reduced models. They assumed that residence time, capture probability and probability of moving between sites were identical for both sexes and all capture sections (Table 2). The parameter values of these simplified models suggested, first, that the spring butterflies had longer residence times ($S = 0.654$, standard error = 0.187) than the summer butterflies ($S = 0.405$, standard error = 0.028); second, capture probability was higher in summer (spring: $p = 0.121$, standard error = 0.062; summer: $p = 0.285$, standard error = 0.035); and third, the probability of moving between capture sites was higher in the summer generation (spring: $\Psi = 0.092$, standard error = 0.039; summer: $\Psi = 0.150$, standard error = 0.015).

Hence, the shorter residence times of summer butterflies supported our previous suggestion of higher dispersal capability in the summer generation.

Biomechanical design

Neither year nor site of origin of the material influenced the biomechanical design of the butterflies. (CVA, year: $F = 1.18$, $P = 0.30$; site: 1st axis $F = 0.33$, $P = 0.99$; all axes $F = 0.12$,

$P = 0.99$). This allowed us to pool the samples from different years and sites in further analyses.

The CVA analysis with all variables convincingly and highly significantly separated the four classes (Fig. 1, Tables 3 and 4). Butterflies of the summer generation were significantly larger and had heavier thoraxes. In contrast, the butterflies of the spring generation had a larger wing loading (especially the females) and aspect ratio (especially the males). This indicates the capability of highly energetic, but costly flight. The discrimination using the raw variables was again highly significant (Fig. 2). Thus, the heavier thoraxes, heavier abdomens and larger wings of the summer generation hold even in absolute terms. Finally, the significant model that was based solely on derived variables showed that the effect of abdomen ratio (higher in females) differentiated the sexes, whereas wing loading (higher in spring) separated the generations.

Filtering out the effect of sex on variation in the biomechanical data resulted in a decrease in the variation explained by the individual models (Table 4). Still, the general pattern remained preserved (Table 3). Summer-generation butterflies had significantly larger and longer wings, heavier thoraxes and higher thorax muscle ratios. Spring generation-butterflies had a heavier wing loading (Fig. 3).

In summary, the results suggest two trends. The first was a difference in design between males and females: males were smaller than females in both generations, and had lighter abdomens and heavier thoraxes in both absolute and relative terms. They also had a higher aspect ratio, which is commonly associated with better flight manoeuvrability (Danforth, 1989; Chai and Srygley, 1990). The second trend concerned the differences between generations. The summer butterflies were larger in absolute terms and displayed a higher thorax muscle ratio (or a lower abdomen ratio, as the two measurements were negatively

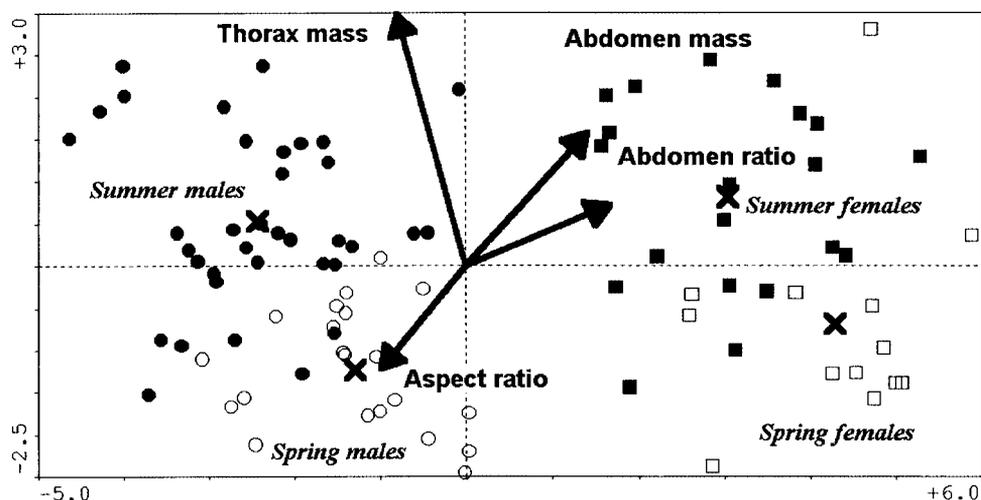


Fig. 1. Canonical variate analysis of biomechanical traits of *Araschnia levana*. Raw and derived explanatory variables combined, sexes within generations treated separately. Only the variables selected by forward selection and included in the final model are shown. The scaling of darts for explanatory variables was multiplied by a factor of 5 for clarity. ○, spring males; □, spring females; ●, summer males; ■, summer females. × = centroids for individual classes.

Table 3. Canonical variate analyses of biomechanical traits in males and females of spring- and summer-generation *Araschnia levana*: separate effects of single terms

Explanatory variable	Four-class analyses (generations and sexes)			Two-class analyses (sex as covariable)		
	All	Raw	Derived	All	Raw	Derived
Total fresh mass	#4.12*	0.65	—	0.43	0.06	—
Total dry mass	0.85	1.60	—	0.01	0.03	—
Thorax mass	4.12*	21.67***	—	4.98*	3.84*	—
Abdomen mass	3.88*	21.80***	—	0.00	6.06*	—
Wing area	#23.98***	29.10***	—	#4.44*	3.56*	—
Wing length	#39.80***	1.53	—	#173.36***	173.36***	—
Centroid projection	1.39	1.28	—	0.03	0.24	—
Flight muscle ratio	0.90	—	1.66	7.24*	—	1.72
Abdomen ratio	34.12***	—	34.12***	0.01	—	0.22
Wing loading	#2.98*	—	2.87*	0.73	—	6.39*
Aspect ratio	4.09*	—	0.89	5.70*	—	0.00
Radius	1.41	—	1.41	0.05	—	2.93

Note: Significance of effects of the terms was tested by Monte-Carlo permutation tests (999 permutations). The terms that achieved significance at $P < 0.05$ and had a variance inflation factor < 30 were entered into the construction of the final models.

Denotes the variables with a variance inflation factor > 30 , which were thus excluded from the final models. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Table 4. Canonical variate analyses of biomechanical traits in males and females of spring- and summer-generation *Araschnia levana*: tests of final models

Type of explanatory variables	Four-class analyses (generations and sexes)			Two-class analyses (sex as covariable)		
	All	Raw	Derived	All	Raw	Derived
Class – discriminant var. correlations	0.95	0.92	0.92	0.86	0.84	0.26
Cumulative % variance, 1st axis	43.5	55.1	93.0	73.8	77.1	6.8
Cumulative % variance, 2nd axis	79.5	99.9	100.0	100.0	100.0	100.0
Eigenvalue: 1st axis	0.90	0.85	0.84	0.74	0.71	0.07
MCPT (F): 1st axis	35.2***	34.6***	34.0***	237.2***	209.1***	6.4***
Trace: all axes	2.07	1.55	0.90	0.74	0.71	—
MCPT (F): all axes	22.9***	31.0***	18.9***	47.4***	52.3***	—

Note: Significance of effects of the terms was tested by Monte-Carlo permutation tests (999 permutations). See Table 3 for the tests of model terms.

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

correlated; $R^2 = -0.94$ and $P < 0.0001$) and a lower wing loading. Since the CVA discriminations according to these traits remained highly significant when the variability attributable to sexual differences was filtered out, it follows that there were genuine inter-generation differences in biomechanical design.

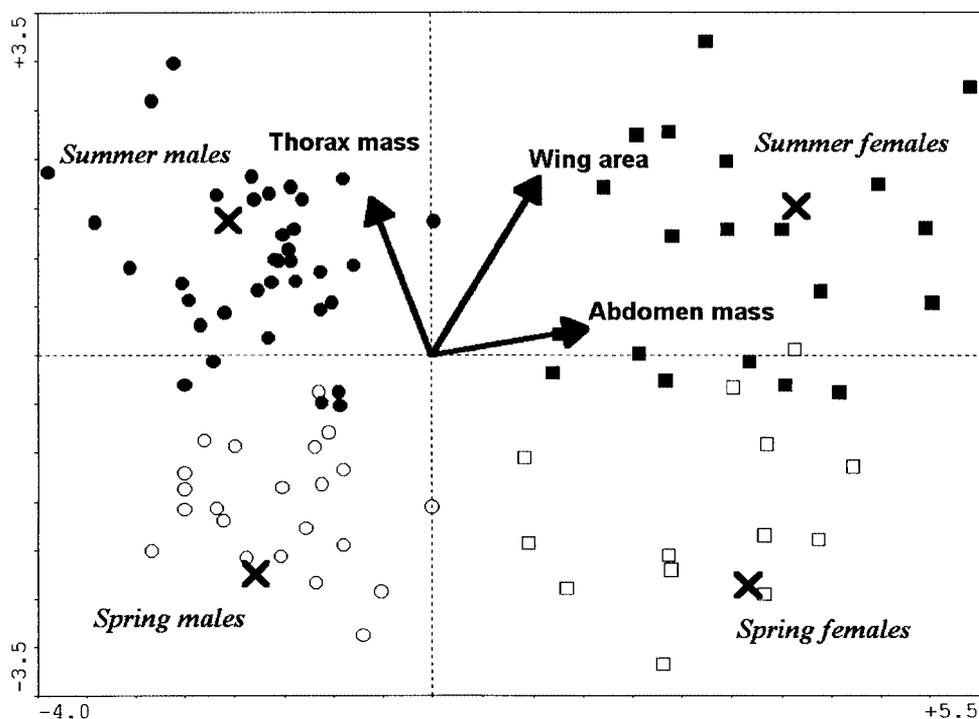


Fig. 2. Canonical variate analysis of biomechanical traits of *Araschnia levana*. Raw variables, sexes within generations treated separately. Only the variables selected by forward selection and included in the final model are shown. The scaling of darts for explanatory variables was multiplied by a factor of 5 for clarity. ○, spring males; □, spring females; ●, summer males; ■, summer females. × = centroids for individual classes.

Allometry

The multiple regression models of the biomechanics variables against dry body mass, sex and generation and their interactions (Table 5) confirmed the larger investment by the spring generation in abdomen mass (also manifested as a higher wing loading) and the larger investment by the summer generation in wing size and flight muscles.

Tests for the effects of generation were significant, after subtracting the trivial (and highly significant) variation due to overall body size, for all the variables tested except the centroid projection and the aspect ratio. That the factor 'generation' produced significant differences in the slope of the regression lines for eight of the ten models suggests that the between-generation patterns were not due to differences in body size alone.

DISCUSSION

Both the comparisons of the residence and mobility parameters of the mark–recapture models, and the analyses of biomechanical traits, provided strong support for our initial hypothesis of greater mobility of summer-generation *Araschnia levana* butterflies. The summer-generation butterflies had shorter residence times (which, in fact, combines

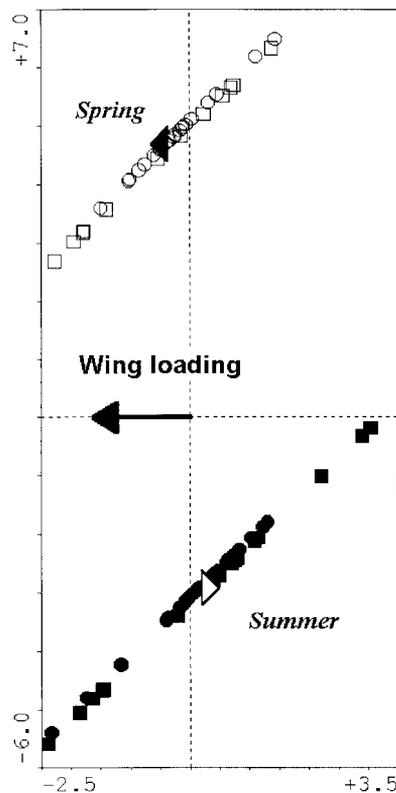


Fig. 3. Canonical variate analysis of biomechanical traits of *Araschnia levana*. Derived variables, sexes within generations treated as covariables. Only the variables selected by forward selection are shown. The scaling of the dart for the explanatory variables was multiplied by a factor of 2 for clarity. ○, spring males; □, spring females; ●, summer males; ■, summer females; ▲, centroid for the spring generation; △, centroid for the summer generation.

survival and site-tenacity) and higher ‘emigration probability’ than the spring butterflies. However, the modelling approach provides only indirect insight into mobility. Nevertheless, it is one of the few approaches available for inter-generation comparison of the mobility of this species. Alternatives, such as mark–recapture across an array of multiple habitat patches (e.g. Hanski *et al.*, 2000; Roland *et al.*, 2000), would be technically prohibitive in *A. levana* because of its distinctly open population structure in summer.

The second line of evidence is the inter-generation differences in biomechanical design. The summer generation conformed to what was expected for butterflies designed for crossing relatively large distances. Not only were they larger, but they also had higher thorax muscle ratios and larger (in terms of both area and length) and less pointed wings. As a result, their wing loading was lower than that of spring butterflies.

Thus, the differences between the spring and summer generations of *A. levana* were congruent with those found in the satyrid butterfly *Pararge aegeria* (Berwaerts *et al.*, 1998) and the lycaenid butterfly *Plebejus argus* (Hill *et al.*, 1999a) from fragmented habitats, as opposed to continuous habitats. The patterns in the summer-generation *A. levana* corre-

Table 5. Results of the multiple regressions of the biomechanical traits against body size (expressed as total dry mass; continuous predictor), sex and generation (categorical predictors) and their interactions in the spring- and summer-generation *Araschnia levana*

Trait	Tdmass		Sex		Gen		Sex*Gen		Sex*Tdmass		Gen*Tdmass		Sex*Gen*Tdmass	
	coeff.	F	coeff.	F	coeff.	F	coeff.	F	coeff.	F	coeff.	F	coeff.	F
Thorax mass	0.2581	70.5***	0.0008	254.9***	0.0006	54.8***	-0.0002	1.7	-0.1137	30.0***	-0.0169	0.1	0.0181	0.6
Abdomen mass	0.5801	3150.6***	0.0004	248.6***	-0.0004	42.1***	0.0007	0.1	0.0636	2.7	0.0011	1.3	-0.0403	1.8
Wing area	1281.119	123.6***	14.5037	16.5**	38.0864	113.9***	13.5403	1.1	179.816	0.0	-583.052	2.2	-473.482	0.4
Wing length	89.3627	101.0***	0.7357	0.0	1.4374	137.2***	0.2345	0.0	-28.2158	1.8	-24.4323	1.6	-4.7019	0.0
Centroid projection	1.7506	6.8	0.0221	6.9	0.0137	7.16	-0.0232	0.1	-1.0956	0.1	-1.4403	0.4	1.4947	2.6
Flight muscle ratio	-5.0670	387.8***	-0.0635	256.9***	0.0346	27.7***	-0.0169	0.4	-0.4334	0.1	-0.9534	0.0	1.1973	0.8
Abdomen ratio	10.1241	477.6***	0.1597	316.6***	-0.0157	22.0**	0.0314	2.1	-4.0825	16.8**	-0.3051	1.3	-1.5078	1.0
Wing loading	0.0071	121.1***	0.0000	7.0	-0.0001	41.1***	-0.0000	4.0	-0.0009	0.2	0.0014	2.9	0.0006	0.2
Aspect ratio	26.7379	11.8**	0.0998	32.5***	-0.0879	2.0	-0.1562	0.0	-25.5260	2.4	-1.8392	0.2	10.0212	0.4
Radius	67.5707	78.5***	0.6634	5.7	0.8303	26.0***	-0.2847	0.0	-28.2074	0.9	-34.3405	1.8	22.8160	1.4

Note: The generations were coded as 0 for spring and 1 for summer; the sexes were coded 0 for males and 1 for females. Hence, positive coefficients for generation indicate an increase in the summer generation, and positive coefficients for sex indicate an increase in females. Gen = generation; Tdmass = total dry mass; coeff. = regression coefficient.

Significance was adjusted by the Bonferroni correction. * $P < 0.005$; ** $P < 0.001$; *** $P < 0.0001$.

sponded similarly with the changes in biomechanical design reported for two butterflies, *Pararge aegeria* and *Hesperia comma*, during expansion of their ranges (Thomas *et al.*, 1998; Hill *et al.*, 1999b). Last but not least, the allometry analysis confirmed that the inter-generation differences in design were independent of differences in body size.

In contrast to our study, the studies cited in the previous paragraph used captive-reared butterflies, which allowed family effects to be assessed – that is, the heritable components of variation in the biomechanical data. We did not study heritability of the design traits, since we were primarily interested in differences between generations. To obtain a captive lineage of *A. levana* spanning multiple generations would require rearing the species for at least 2 years. This is not feasible at the moment, since the butterfly displays complex pre-mating behaviour, including lekking by males (Fric and Konvička, 2000), and such insemination techniques as hand-pairing are not available. Also, working with captive-reared butterflies, the authors that studied *Pararge aegeria*, *Plebejus argus* and *Hesperia comma* worked with individuals of the same imaginal age. This was not the case in our study, which could have introduced some ‘noisy’ variation into our data. For instance, the abdomen mass of females decreases with age in butterflies, as progressively more eggs are laid. Despite this, the highly significant differences between generations found in our canonical variate analyses, and the finding that the inter-generation differences were not caused solely by the effects of sex or total body size, render our main conclusion robust.

It can be argued that some of the design differences may not be connected to dispersal capability. First, an increased flight muscle ratio, which we found in summer butterflies, may be beneficial in reproductive competition. We have shown previously that the summer generation is more numerous (Fric and Konvička, 2000) and reproductive competition may intensify at high population densities. However, the summer-generation males had a lower wing loading than the spring males. An increased wing loading translates into better manoeuvrability, which is of special concern for butterflies with a perching mate-locating tactic (Wickman, 1992), a tactic used by males of both generations of *A. levana* (Fric and Konvička, 2000). This rules out the higher flight muscle ratio in summer males being a trait selected for by reproductive competition. Otherwise, the wing loading should have been higher in summer males. Also, an increase in flight muscle ratio for reproductive reasons does not make sense in females, who do not depend on energetic flight for mate acquisition (cf. Wickman, 1992; Van Dyck *et al.*, 1997).

The second trait closely connected to reproduction is relative abdomen mass. Abdomens are filled with developing eggs in butterfly females and we agree with Rutowski (1997) that the larger absolute size of females is reproductively beneficial. However, both the absolute and relative mass of the abdomen was higher in spring even after subtracting the effect of body size (Table 5). This suggests that summer-generation butterflies invest more resources in body structures other than the reproductive ones. This supports the finding of Dixon and Kindlmann (1999), who observed that dispersing (winged) generations of aphids invest relatively less in the gonads than do sedentary (unwinged) generations. Although it is important for dispersing animals (whether aphids or butterflies) to invest heavily in reproduction – otherwise they would not establish their progeny at a new site – dispersal apparatus is costly, and building it diminishes investment in reproductive structures via a trade-off.

In summary, the evidence indicates that the spring generation of *A. levana* is more sedentary and invests heavily in reproduction, whereas the summer generation tends to disperse in search of new inhabitable space. Such a strategy is perfectly reasonable: the

butterfly probably suffers increased mortality during winter diapause (cf. Windig, 1999), and any site in which the overwintering butterflies finish their development is thus by definition both relatively empty and suitable for reproduction. In summer, on the other hand, population densities are much higher and it may pay for the butterflies to leave, exactly as proposed for mobile butterflies by Baker (1984).

This reflects the situation found in other insect groups, in which different generations are polymorphic with respect to dispersal. Examples include aphids, Homoptera, Heteroptera and Orthoptera, in which sedentary generations typically have shortened or absent wings (Harrison, 1980; Waloff, 1980; Roff and Fairbairn, 1991; Rankin and Burchsted, 1992; Dixon and Kindlmann, 1999). Because no such dramatic dispersal-related adaptations are found in butterflies, the possibility that different generations might vary in their mobility was never sought in this group, with the important exception of 'classical' migrants (e.g. Urquhart, 1960; Dingle *et al.*, 1999). However, many butterflies that are not 'classical' migrants still possess considerable dispersal abilities (Vandewoestijne *et al.*, 1999), and different abundances of seasonal broods have been observed in several species (e.g. Ohsaki, 1980; Tabashnik, 1980; Pollard *et al.*, 1997; Rothery and Roy, 2001). Good dispersal capability and variation in abundance among generations are obvious prerequisites for inter-generational dispersal polymorphism. It follows that inter-generational differences in dispersal capabilities might be more common in butterflies than previously thought. As exemplified by 'hidden' alary polymorphism in the Homoptera (Waloff, 1980), such differences may not manifest themselves in different development of the wings, but by relatively subtle shifts in body proportions. Also, and unlike in *A. levana*, inter-generational polymorphism in dispersal capability does not have to be associated with polyphenism in wing pattern and coloration.

We propose that differential dispersal should be beneficial for species that inhabit seasonally changing environments, allowing individual generations to utilize varying resources. If so, the patterns in biomechanical design of individual generations of multivoltine butterflies should be 'ecologically predictable'. Specifically, the generation that utilizes seasonally predictable but spatially unpredictable increases in resources should be designed for better mobility. Examples of such situations should be sought, for example, among temperate-zone Pieridae, which track ample supplies of their host plants at cultivated fields in late summer, or among wetland-inhabiting temperate species, which should be more mobile during more humid months. The same might apply to tropical species with dry-season and wet-season forms (cf. Windig *et al.*, 1994; Dingle *et al.*, 1999).

To test the above hypotheses, two lines of evidence should be explored. First, corresponding differences in biomechanical design as those demonstrated for *A. levana* should be found in a broader array of multivoltine species, including species that are not polyphenic in wing patterns. Indeed, seasonal differences in 'wing shape' have been reported for an African polyphenic *Precis* butterfly (McLeod, 1984) and for the satyrid *Pararge aegeria*, in which the generations differ even in their behaviour (Van Dyck *et al.*, 1997). Second, since biomechanics provides indirect evidence only for mobility, it should be demonstrated that the generations that potentially differ in mobility do in fact utilize different ranges of habitats (or differ seasonally in abundances in particular habitat types). In any case, studies of inter-generational differences in biomechanical traits appear to be a cost-efficient way of revealing ecologically relevant patterns in dispersal and colonization capabilities of butterflies and other insect groups.

ACKNOWLEDGEMENT

We are grateful to Oldřich Čížek for help with the material, to Jiří Beneš, Ferenc Kassai, Pavel Kindlmann, Jan Lepš, Vojtěch Novotný, Petr Šmilauer and Josef Settele for inspiring discussions on various drafts of the manuscript, and to Derek Roff for valuable comments and patient editing. The study was funded by grant FRVŠ 21-1295 to Z. Fric.

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