Mechanical design indicates differences in mobility among butterfly generations

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ABSTRACT

Question: Do individual generations of temperate butterflies differ in dispersal ability? Evidence from two species, and multiple indirect indices, suggest that this is the case.

Data and method: We measured traits with potential significance for flight of spring and summer generations of eight European butterflies belonging to three families. We assumed that large thoraxes and narrow, pointed wings imply energetically demanding flight, a high wing loading implies limited dispersal, and large wings and low wing loading imply improved dispersal ability. We used multivariate (discriminant function) analyses to separate generations according to the traits.

Results and conclusions: We found significant differences between generations in all species analysed. They mainly concerned wing loading, allocation of mass to thorax and abdomen, and pointedness of wings. The butterflies formed two loose groups. One group contained species well suited for increased dispersal in summer (*Pieris brassicae*, *P. rapae*, *P. napi*, *Leptidea reali*, *Lycaena phlaeas*, *Boloria selene*), resembling previously studied *Araschnia levana*. The traits of the remaining butterflies (*Coenonympha pamphilus*, *Polyommatus icarus*) implied a better dispersal in spring, as in previously studied *Pararge aegeria*.

Keywords: biomechanics, developmental plasticity, dispersal polymorphism, Lepidoptera, voltinism.

INTRODUCTION

Many insects produce multiple generations per year. This allows them faster multiplication than univoltine species, but it may incur costs, especially in regions with seasonally changing conditions, as it exposes different generations to different environmental pressures (e.g. Kingsolver, 1995; Roy and Thomas, 2003; Wiklund and Tullberg, 2004). Developmental plasticity provides a universal answer to this variation (Nylin and Gotthard, 1998; Sultan and Spencer, 2002). It may prevent fine-tuned adaptations to local or temporal conditions (Relyea, 2002), but offers an

evolutionary opportunity, allowing individual generations to exploit seasonally varying resources.

An example of such plasticity is seasonal polymorphism in dispersal. It is best known in aphids (Dixon and Kindlmann, 1999), but exists in Homoptera: Auchenorhyncha, Heteroptera, and Orthoptera as well. Species with dispersal polymorphism alternate between sedentary generations, which have shortened or absent wings and specialize on fast exploitation of resources, and dispersive generations, which specialize on locating novel resources (Harrison, 1980; Roff and Fairbarn, 1991; Rankin and Burchsted, 1992; Halkett *et al.*, 2004). The inter-generation variation in dispersal is not necessarily coupled with such dramatic changes as the absence of wings. For instance, some leafhoppers exhibit 'hidden' allary polymorphism mediated by shifts in bodily proportions (Waloff, 1980).

Several observations from multivoltine butterflies imply that inter-generational variation in mobility might be quite common in this group. Classical migrants may alternate between dispersive and sedentary broods (e.g. Urquhart, 1960; Stefanescu, 2001). Subsequent generations of temperate butterflies often vary in abundance and regional distribution (Ohsaki, 1980; Pollard *et al.*, 1997; Rothery and Roy, 2001) and there have been observations of different mobility among generations (Roer, 1962; Scott, 1975). Finally, Van Dyck and Wiklund (2002) and Fric and Konvicka (2002) reported that successive generations of *Pararge aegeria* (Linnaeus, 1758) (Nymphalidae: Satyrinae) and *Araschnia levana* (Linnaeus, 1758) (Nymphalidae: Nymphalinae) differ in biomechanical design. In both species, one generation possesses a design appropriate for fast but energetically demanding flight, whereas the other generation is designed for long-endurance flight. Fric and Konvicka (2002) hypothesized that members of the former generation tend to remain at the site of their emergence, whereas those of the latter generation tend to disperse. In *A. levana*, demography modelling revealed a higher mobility of the latter (summer) generation (Fric and Konvicka, 2000).

To date, few researchers have attempted to detect inter-generational variation in butterfly dispersal. Direct methods, such as mark–recapture, are poorly suited for detecting long-range dispersers (but see Roer, 1962; Knight *et al.*, 1999). Studying successive generations requires more time than studying monovoltine species. Last but not least, most recent mark–recapture studies have focused on endangered species, which tend to be monovoltine (Shreeve *et al.*, 2001).

Comparing biomechanical traits among generations offers a promising alternative to direct methods. We follow this approach here, building on previous studies of *A. levana* and *P. aegeria* and comparing biomechanical traits of different generations of a further eight temperate species belonging to three families. Our principal hypothesis is that generations should differ in dispersal-related traits and that the differences should reflect species ecology.

METHODS

Biomechanical design and dispersal

Biomechanical design in butterflies refers to the allocation of body mass, and shape and area of wings, which affect individual flight performance (e.g. Dudley, 1990). A relationship between biomechanical design and flight performance has been supported experimentally (Kingsolver, 1999; Kingsolver and Srygley, 2000; Berwaerts *et al.*, 2002) and in the field (Berwaerts *et al.*, 1998; Thomas *et al.*, 1998).

One should not expect a universal set of traits suitable for dispersal, because body design also reflects other aspects (Srygley and Chai, 1990; Wickman, 1992). In particular, the sexes may be exposed to different selective forces (Fischer and Fiedler, 2001). For instance, investment in egg production decreases investment in flight muscles in females (cf. Dixon and Kindlmann, 1999). Increased investment in thorax muscles coupled with narrow and pointed wings implies speedy but energetically demanding flight, whereas large, rounded wings imply long-endurance gliding flight. Here, we assume that narrow and pointed wings, plus a high wing loading (due to either thorax or abdomen weight), imply limited dispersal, whereas increased thorax mass coupled with larger and less pointed wings, and a lower wing loading, indicate improved dispersal ability.

Materials

We analysed eight species from three families (Table 1). In three species (*P. brassicae*, *L. phlaeas*, and *B. selene*), we worked only with males, as sufficient numbers of females were not available. We used wild-caught butterflies, collected in 2001 and 2002 in the southwest of the Czech Republic. All analysed species are widely distributed in the country (Benes *et al.*, 2002), inhabiting crop-fields (*P. brassicae*, *P. rapae*) and wet (*P. napi*, *L. reali*, *B. selene*) or dry (*C. pamphilus*, *P. icarus*, *L. phlaeas*) grasslands. In all but two species (*C. pamphilus* and *B. selene*), the summer generation is more abundant than the spring generation.

We processed the material as in Wickman (1992) and Fric and Konvicka (2002). We weighed fresh butterflies, dried them at 60°C, and weighed them to obtain total dry mass, thorax mass, and abdomen mass. Then we placed their wings in a flight-like position, digitized their images, and measured them using Scion Image Beta 4.0.2 (© Scion Corp., 2000). We thus recorded the following measures: forewing length, wing area, and the orthogonal projection of the centroid of the wing area on wing diameter (hereafter 'centroid projection'). We used these 'raw' variables to compute the following 'derived' variables: (i) flight muscle ratio

Table 1. Butterfly species and sample sizes used for analysis of generational differences in biomechanical design

	Spring (M/F)	Summer (M/F)	Autumn (M/F)	Total N
Pieridae				
Pieris napi (Linnaeus, 1758)	25/27	44/16		146
P. rapae (Linnaeus, 1758)	32/13	57/45	17/1	165
P. brassicae (Linnaeus, 1758)	7/0	11/9		27
Leptidea reali (Reissinger, 1989)	37/9	36/20		102
Lycaenidae				
Lycaena phlaeas (Linnaeus, 1761)	13/0	10/10		33
Polyommatus icarus (Rottemburg, 1775)	21/13	34/27		95
Nymphalidae				
Coenonympha pamphilus (Linnaeus, 1758)	63/17	9/5		94
Boloria selene (Dennis & Schiffermüller, 1775)	8/5	7/3		23

(i.e. thorax to total mass), (ii) abdomen ratio (i.e. abdomen to total mass), (iii) wing loading (i.e. fresh body mass divided by wing area), (iv) aspect ratio (i.e. wing span squared divided by wing area), and (v) radius of the first moment of wing area [i.e. the span-wise position of the normalized chord through the centroid of the wing area as a fraction of the wing length (hereafter 'radius')]. The latter two variables describe the pointedness of wings.

Statistical analysis

Each species was analysed separately using multivariate ordination, which does not suffer complications with collinearity among predictors and inflated significance values. We used canonical variate analysis (CVA), a variant of discrimination analysis (Fisher, 1936), which ordinates classes according to traits of individuals. We used CANOCO, version 4.5 (Ter Braak and Smilauer, 1998) for the computing. The program computes CVA as a canonical correspondence analysis with Hill's scaling and tests for statistical significance of the ordinations via the Monte Carlo permutation test (MCPT). It handles dummy variables describing classes as 'species data' and individual traits as 'environmental data'.

   In all analyses, we used individual generations as classes. Sex was entered as a covariate to filter out variation due to sex-specific traits. We then ran three analyses. The first was based on all potential predictors, the second was restricted to raw variables, and the third was restricted to derived variables. Following individual testing of each potential predictor, we identified best subsets of predictors, using CANOCO's forward selection procedure. Following the forward selections, we discarded from each model variables with a high inflation factor, which indicates their redundancy due to strong collinearity with other variables in the respective model.

RESULTS

We found significant differences between generations in all butterflies examined (Tables 2 and 3). Two loose groups of species could be distinguished: one with a smaller spring generation and one with a larger spring generation.

Smaller spring generation

Pieris napi. This species has larger and more rounded wings in summer, and a higher wing loading in spring. Raw analysis pointed to larger wings and a heavier thorax in summer; derived analysis revealed a higher wing loading and radius in spring and a higher aspect ratio in summer (Fig. 1A).

Pieris rapae. In this species, the summer generation is larger, with a heavier thorax and larger wing area. Derived analysis confirmed a higher wing loading (more so in females) and aspect ratio (more so in males) in spring (Fig. 1B). Autumn butterflies grouped with summer butterflies, but had a higher thorax ratio in the derived analysis.

Pieris brassicae. This species has larger and longer wings in summer, more pointed wings in spring (Fig. 1C).

Table 2. Single-term comparisons, and results of forward-selected final models, comparing biomechanical design traits in subsequent generations of multivoltine temperate butterflies

	P. napi	P. rapae	P. brassicae	L. reali	L. phlaeas	P. icarus	C. pamphilus	B. selene
Raw variables								
Total fresh mass	2.58	0.61	0.05	0.46	0.06	**9.83****	1.80	11.71**
Total dry mass	0.03	**4.28***	0.03	**5.35***	0.09	4.25*	3.38	33.48***
Abdomen mass	1.89	1.70	1.23	2.17	3.10	1.95	1.54	**57.36*****
Thorax mass	0.15	5.90**	1.33	2.98**	2.24	2.79	6.06*	9.81*
Wing area	**60.99*****	**27.83*****	9.42**	**22.13*****	5.61*	0.41	0.02	17.05***
Wing length	60.68***	16.64***	3.62	16.01***	**6.41***	3.06	2.96	18.63***
Centroid projection	3.08	15.71***	9.26**	8.78**	1.62	0.78	1.41	2.15
Derived variables								
Flight muscle ratio	**0.05**	2.73	4.81	1.92*	1.92	**3.54***	0.12	4.28*
Abdomen ratio	5.56*	2.31	3.16	**0.72**	**4.82***	0.18	2.09	12.58**
Wing loading	**38.37*****	7.32**	1.41	0.04	0.69	9.56**	**2.15**	3.61
Aspect ratio	0.21	5.71**	**17.51*****	3.19	0.05	4.58*	**9.86****	0.94
Radius	**18.86*****	1.16	11.24**	1.36	0.06	2.17	6.24*	**1.86**
Final model								
First axis								
Eigenvalue	0.54	0.34	—	0.47	0.36	0.22	0.14	0.88
F, P	162.66***	33.73***	—	92.06***	11.07*	24.86***	14.64**	89.28***
All axes								
Trace	0.54	0.37	0.52	0.47	0.36	0.22	0.14	0.88
F, P	40.66***	12.48***	17.51***	30.69***	5.53*	6.21***	7.32**	44.64***

Note: Canonical variate analysis (CVA), raw and derived predictors combined. Variables that were selected for the final models are in **bold**.
Monte-Carlo permutation tests (999 runs): * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Table 3. Predictors selected for raw and derived models comparing biomechanical design traits in generations of multivoltine temperate butterflies, and characteristics of final models

	P. napi	P. rapae	P. brassicae	L. reali	L. phlaeas	P. icarus	C. pamphilus	B. selene
Raw analysis								
Total fresh mass	×	×						
Total dry mass		×				×		
Abdomen mass	×			×				×
Thorax mass	×			×			×	
Wing area	×	×	×	×				
Wing length			×		×		×	
Centroid projection	×				×		×	
CVA – first axis								
Eigenvalue	0.55	0.34	0.61	0.53	—	—	0.14	—
F, P	167.43***	33.73***	23.6***	117.87***	—	—	15.23**	—
CVA – all axes								
Trace	0.55	0.37	0.61	0.53	0.23	0.10	0.14	0.82
F, P	33.49***	12.48***	11.76***	39.29***	6.41*	9.83**	7.61**	57.36***
Derived analysis								
Flight muscle ratio		×				×		
Abdomen ratio					×	×		
Wing loading	×	×				×		×
Aspect ratio	×	×	×				×	×
Radius	×			×			×	
CVA – first axis								
Eigenvalue	0.32	0.16	—	—	—	0.22	0.14	0.69
F, P	66.89***	14.59***	—	—	—	26.07***	14.64**	26.48**
CVA – all axes								
Trace	0.32	0.20	0.52	0.03	0.19	0.22	0.14	0.69
F, P	22.30***	4.59***	17.51***	3.19	4.82*	8.69***	7.32**	13.24**

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

Leptidea reali. This species has a larger wing area in summer, and a higher abdomen ratio and total mass in spring; derived analysis pointed to a higher aspect ratio in spring (Fig. 1D).

Lycaena phlaeas. This species has a higher abdomen ratio in spring and longer wings in summer (Fig. 1E).

Boloria selene. This species has a heavier abdomen in spring, and a higher abdomen ratio and wing loading in summer (Fig. 1H).

Larger spring generation

Polyommatus icarus. In this species, individuals are larger in spring, with a heavier abdomen and higher aspect ratios. Summer butterflies are smaller, with a heavier thorax (Fig. 1F).

Coenonympha pamphilus. This species has a higher wing loading in spring and more pointed wings in summer. The aspect ratio was the strongest predictor distinguishing the generations (Fig. 1G). Raw analysis showed that spring butterflies have a heavier thorax and summer butterflies have longer wings.

DISCUSSION

We found that in eight species of multivoltine temperate butterflies, the generations differ in biomechanical design traits that are likely to be responsible for differential dispersal. The main differences between the generations, in addition to body size, were wing loading, allocation of mass to thorax and abdomen (the two being collinear), and pointedness of wings. Together with *Araschnia levana* and *Pararge aegeria* (Fric and Konvicka, 2002; Van Dyck and Wiklund, 2002), such inter-generational differences are now known for ten butterflies from three families.

The butterflies studied form two loose groups. One resembles *A. levana* in possessing traits that imply better dispersion of the summer (non-diapausing) generation. The other resembles *P. aegeria* in possessing traits for better dispersion of the spring (diapausing) generation.

The *levana*-type design is easier to interpret. Since winter diapause increases mortality, the best strategy in spring is to increase in numbers at suitable sites. As risk of crowding increases in summer, moving away becomes profitable. Three representatives of this group (*Pieris* whites) develop on short-living plants, whose supplies vary at specific sites within any year, creating a further incentive to disperse (Courtney, 1986). In addition, *P. brassicae* and *P. rapae* may exploit rich supplies of their host plants at crop fields in summer. These two species, unlike the remaining species analysed here, also behave as classical migrants in parts of their range (Courtney, 1986), and their migrations tend to occur in summer (Asher *et al.*, 2001). Similarly, *L. phlaeas* is a long-range migrant, whose host plants (*Acetosella* spp.) thrive at early successional sites, whose locations again vary among years (Pickering *et al.*, 2003).

For the second group, the interpretation is more difficult. It is easiest for *P. aegeria*, which inhabits closed-canopy forests, utilizing sunny spots there. Its dispersal might be easier in spring, when canopy foliage has not yet fully developed (cf. Shreeve, 1984). The extent of the
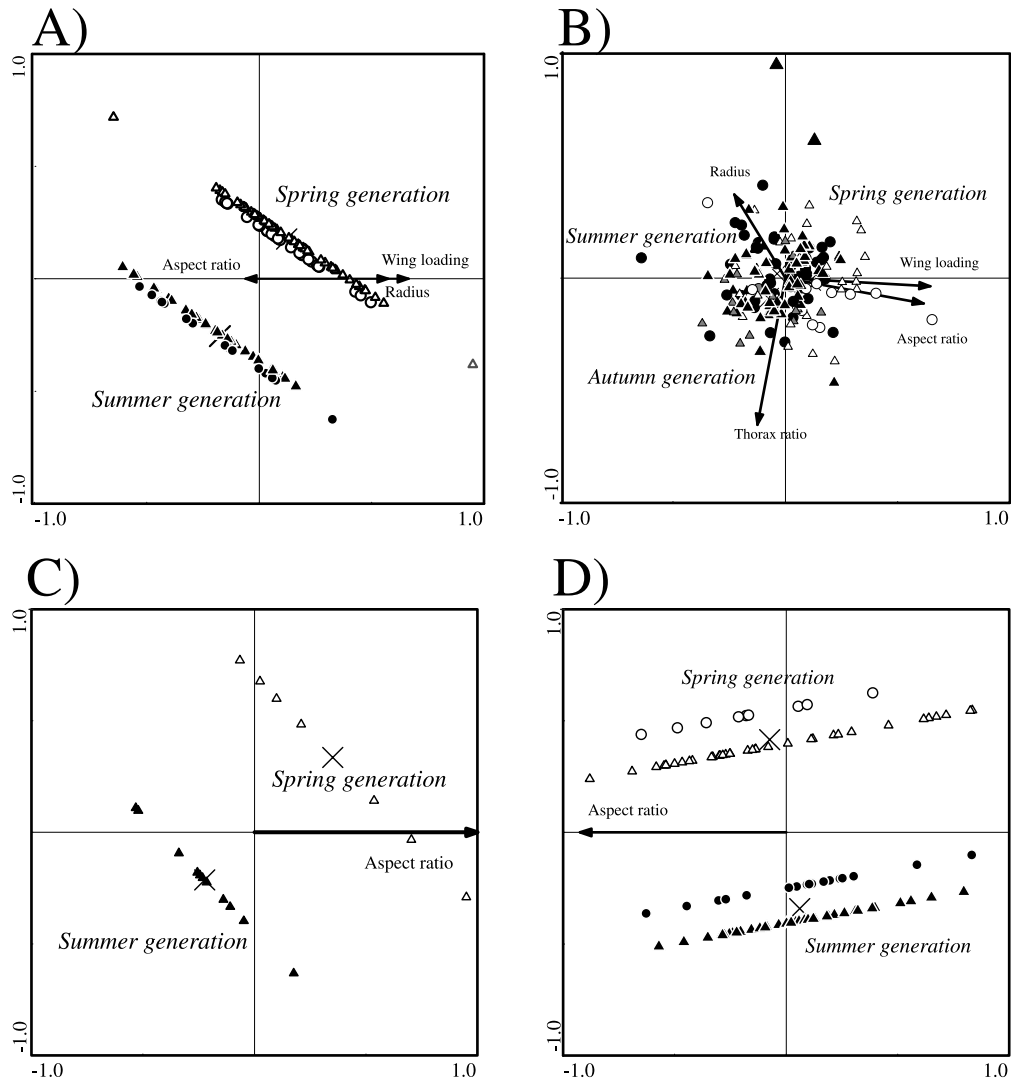
Fig. 1. Ordination diagrams (canonical variate analysis) comparing biomechanical design traits of multivoltine butterflies. Derived variables only. Triangles are for males, circles for females; open symbols are for spring generation, solid symbols for summer generation. For *Pieris rapae* there is an

sunny spots diminishes later in the season, which might favour remaining at suitable sites. *Coenonympha pamphilus*, another grass feeder, prefers low-sward grasslands (Asher *et al.*, 2001; Benes *et al.*, 2002). Because many grasslands grow taller in summer, it may be more risky to leave optimal spots later during the year.

The lycaenid *P. icarus* represents an enigma with regard to the differences between generations. Males of both generations are considerably larger than females. Additionally, spring males are larger than summer males, *and* have a higher wing loading. This suggests differences in mobility between the sexes, with males the more mobile sex. *Polyommatus icarus* is
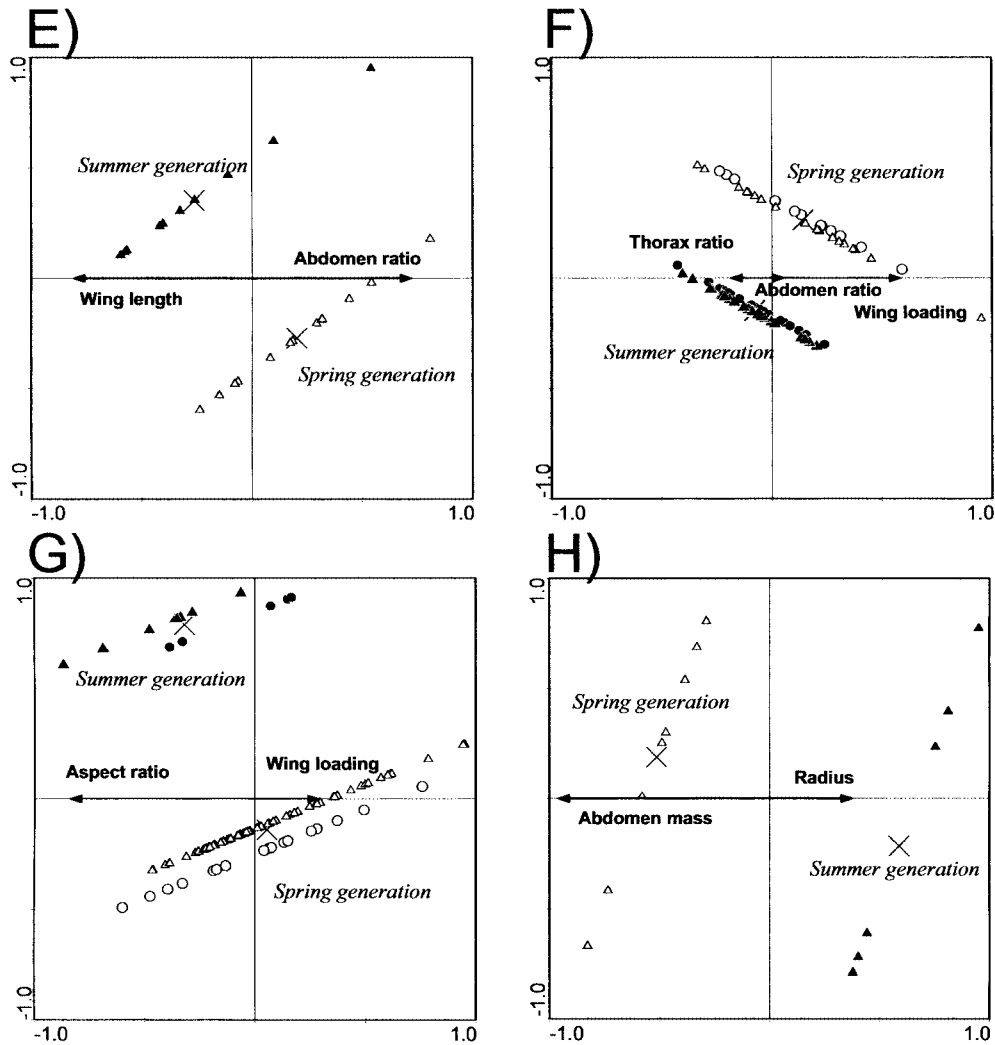
additional intermediate symbol (grey triangles) for the autumn generation. (A) *Pieris napi*, (B) *P. rapae*, (C) *P. brassicae*, (D) *Leptidea reali*, (E) *Lycaena phlaeas*, (F) *Polyommatus icarus*, (G) *Coenonympha pamphilus*, (H) *Boloria selene*.

the most widely distributed lycaenid in Europe (Kudrna, 2002) and male mobility might suffice to maintain genetic cohesion within the species.

Some behavioural traits of the studied species are also consistent with differences in mobility. According to A. Shapiro (personal communication), spring individuals of *P. rapae* avoid wind-exposed locations (which might contribute to lower dispersal), whereas summer individuals utilize ascending thermal currents (consistent with a higher wing area and higher dispersal). Of course, the existence of this pattern does not prove a connection with dispersal, and alternative explanations are possible. In this respect, it might be worthwhile

exploring the biomechanical design of single-brooded populations of some of the studied species. A good candidate would be populations of the *P. napi*-complex inhabiting extreme arctic and alpine conditions (cf. Eitschberger, 1983). Had they resembled spring broods of multi-voltine populations, then the design would primarily evolve out of a need to maintain control over flight in the extreme environments.

Analyses of quantitative distribution data are required to ascertain whether differences in design among generations do indeed influence dispersal. The putatively more mobile generations of individual species should exhibit wider distributions than sedentary generations, even after controlling for differences in abundance. Such analyses have yet to be conducted. However, the world's largest set of butterfly distribution data, the British distribution atlas (Asher *et al.*, 2001), provides some indices. Phenograms for selected species (Asher *et al.*, 2001, pp. 391–398) allow visual inspection of abundance and distribution of separate generations. The *levana*-like pattern of higher abundance *and* distribution in summer applies to *Pieris napi*, *P. rapae*, *P. brassicae*, and *Lycaena phlaeas* (in line with our results), plus *Plebejus argus* (Linnaeus, 1758), *Aglais urticae* (Linnaeus, 1758), and *Polygonia c-album* (Linnaeus, 1758).

Traits such as body size and allocation of mass are plastic and subject to relatively fast selection (Dempster, 1991; Moczek and Nijhout, 2003). The same applies to annual numbers of generations, which is phylogenetically plastic (Marcon *et al.*, 1999; Fric *et al.*, 2004) and often varies within species, including the frequent occurrence of partial generations (e.g. Sibly *et al.*, 1997; Gotthard, 1998). This variety implies that a wide diversity of biomechanical responses to external conditions could have evolved.

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