

# Host plant defences and voltinism in European butterflies

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**Abstract.** 1. With respect to seasonal availability for herbivores, plants defended by synthesising qualitative compounds differ from those protected by accumulation of quantitative macromolecules, leaf toughness, and low water and/or nutrient content. While the palatability of the former plants remains relatively constant during the season, the palatability of the latter group decreases with leaf age.

2. It was hypothesised that in seasonal temperate environments, quantitative plant defences should restrict the annual numbers of insect generations. To test this hypothesis, European butterflies were used as a model, both non-corrected regressions and tests controlling for phylogeny were carried out, and potentially confounding factors such as body size or occurrence in short-season environments were treated as covariables.

3. Non-phylogenetically controlled regressions corroborated that butterflies feeding on quantitatively protected hosts (woody plants + grasses) form fewer generations than species feeding on qualitatively protected forbs. Plant defences fitted voltinism better than butterfly size, and remained significant even after controlling for short seasons. Using independent contrasts, feeding on woody plants plus grasses, and feeding on woody plants only, predicted fewer generations. These patterns, however, applied exclusively for foliage-feeding species.

4. The association between plant defences and voltinism represents a hitherto overlooked pattern in the ecology of temperate herbivores. It may explain why large insects tend to form fewer generations and feed on structurally complex hosts, and why some species remain monovoltine although they are not restricted by short season.

**Key words.** Butterfly phylogeny, comparative method, herbivory, insect–plant interactions, insect seasonality, Lepidoptera, phenology, plant defences.

## Introduction

Secondary compounds, unpalatable macromolecules that increase leaf toughness, and low water and protein content all serve as plant antiherbivore defences. Qualitative secondary compounds are toxic in low concentrations, and typically protect leaves from bud-burst until senescence. In contrast, quantitative macromolecules accumulate gradually, and both a decreased nutrient content and leaf toughening occur in expanded leaves (Feeny, 1976; Rhoades & Cates, 1976; Scriber & Slansky, 1981; Coley *et al.*,

1985). As the effect of such defences increases with leaf age, they only weakly protect young leaves (Feeny, 1970; Choong, 1996). For insect herbivores, this opens the ‘phenological window of opportunity’ (Martel & Kause, 2002), which closes with the ageing of leaves (Alonso & Herrera, 2000). In contrast, once an insect feeding on a qualitatively protected plant circumvents the plant’s defences, the foliage quality remains relatively stable, leaving the window of opportunity open for a longer time.

The first larval instar is the most food quality-sensitive stage in insect ontogeny (Reavey, 1993). Increasing leaf toughness and decreasing nutrient content lowers the success rate of establishing neonate larvae on host plants and decrease the larval growth rate (Zalucki *et al.*, 2002). In highly seasonal temperate environments, the decrease of food quality is synchronised in most resources available for insects feeding on plants protected by quantitative

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macromolecules, low nutrient content, and leaf toughening (Scriber & Slansky, 1981). Therefore, herbivores feeding on such plants should synchronise hatching with bud-burst (Stamp, 1993; Tikkanen & Julkunen-Tiitto, 2003; Klemola *et al.*, 2003). This may prevent further generations from establishing on maturing foliage, restricting the annual number of generations. In contrast, insects consuming qualitatively protected plants are not subject to such restrictions. It follows that the nature of plant defences should be reflected in the phenology of insect herbivores.

Plants rarely depend on a single type of defence. Nearly any possible combination can be found, depending on evolutionary, ecological, and individual history, as well as on the current situation of a plant. This complicates formulation of a comprehensive plant defence theory, creating 'the quagmire of plant defence hypotheses' (Stamp, 2003). The plant apparency hypothesis (Feeny, 1976; Rhoades & Cates, 1976) allows the formulation of testable predictions relating plant defences to plant growth forms and life histories (Silvertown & Dodd, 1996). It predicts that qualitative compounds should prevail in relatively small-sized and short-lived (= unapparent) plants that do not attract many herbivores in evolutionary time, whereas quantitative compounds, leaf toughness, and low nutrient content should prevail in large and long-lived (= apparent) plants, which are likely to attract more herbivores.

Given the sensitivity of first instar larvae, the seasonality of temperate environments, and the decreasing foliage quality of apparent plants in late seasons, it was hypothesised that temperate insects feeding on apparent plants should form fewer generations per year than their relatives feeding on unapparent plants. The hypothesis was tested using a phylogenetically controlled analysis of European butterflies (Lepidoptera: Hesperioidea and Papilionoidea). Butterflies are a particularly suitable model group for such analyses, as their life history and phylogeny are considerably better known than in most insect taxa (Boggs *et al.*, 2003).

## Methods

Lists of European butterflies from Tolman and Lewington (1997) and Kudrna (2002) were combined, excluding species restricted to Russia and Eastern Ukraine (i.e. Crimea), and a few species with uncertain taxonomic status [e.g. *Pieris bryoniae* (Hübner, 1791), *Polyommatus andronicus* (Coutsis & Chavalas, 1995)]. Also, as the hypothesis focused on temperate species, tropical species that reach southernmost Europe but have otherwise tropical distribution were excluded [e.g. *Pelopidas thrax* (Hübner, 1821), *Danaus chrysippus* (Linnaeus, 1758), *Zegris eupheme* (Esper, 1804)] (cf. Larsen, 1986). The total data set contained 369 species, the nomenclature of analysed species (Appendix 1) follows Karsholt and Razowski (1996).

### Butterfly phylogeny

Although there is still no phylogenetic hypothesis for all European butterflies, reconstruction of butterfly phylogeny has flourished during the last few years. A combined tree was constructed, based on published phylogenies of individual groups and, for groups with no phylogeny available, formal classification into genera and sub-

genera (Appendix 2). The following sources were used: Hesperidae – Dennis *et al.* (2000); Papilionidae – Aubert *et al.* (1999), Caterino *et al.* (2001); Pieridae – Hesselbarth *et al.* (1995), Pollock *et al.* (1998), Brunton (1998), M.F. Braby (unpublished data); Lycaenidae – Eliot (1973), Dennis *et al.* (2000), Kandul *et al.* (2004), Pech *et al.* (2004); and Nymphalidae – Aubert *et al.* (1996), Martin *et al.* (2000, 2002), Nylin *et al.* (2001), Wahlberg and Nylin (2003), Wahlberg *et al.* (2003). The formal classification was used mainly within Lycaenidae and Hesperidae, and for species-rich genera within Nymphalidae: Satyrini, such as *Pseudochazara* or *Erebia*. The higher taxa relationship follow de Jong *et al.* (1996).

### Butterfly voltinism

The number of generations was the dependent variable. As voltinism varies in many species, e.g. with latitude and altitude (e.g. Tolman & Lewington, 1997; Gotthard *et al.*, 1999; Fischer & Fiedler, 2002), simplified ordinal coding was used: (i) Exclusively monovoltine species, including semivoltine species developing for more than one year, scored 1; (ii) multivoltine species, i.e. those forming more than one generation per year even if only in part of their range, but not being polyvoltine, scored 2; (iii) and polyvoltine species, occurring for whole season in warmer parts of their range, scored 3.

### Host plant defences

Any categorisation of plants according to their antiherbivore defences is inherently complicated (cf. Stamp, 2003). The plant apparency theory (Feeny, 1976; Rhoades & Cates, 1976) relates plant defences to plant growth forms and life histories, and satisfactorily predicts the nature of plant defences (Yamamura & Tsuji, 1995; Silvertown & Dodd, 1996). Still, plant apparency is necessarily context-dependent: although a dominant tree is clearly more apparent than a forest herb, a perennial forb is more apparent than a spring ephemeral. The situation is further complicated by such factors as local variation in availability of individual plant species, or within-species variation in nutritional quality and/or quantity of defences (e.g. Pullin, 1987; Moyes *et al.*, 2000; Simon & Hilker, 2003; Rogers & Siemann, 2004). A simple, unambiguous, statistically tractable and replicable categorisation of butterfly host plants according to their defences was necessary for comparative analysis. Hence, two binary divisions of host plants were adopted, although it is understood that the issue is more complex in the real world.

**1 Narrow sense apparency.** Plants with hard lignified tissues or woody stems (trees, shrubs, hemi-shrubs) considered apparent, herbaceous plants considered unapparent.

**2 Broader sense apparency.** All woody plants as above, plus grasses (Poaceae, Cyperaceae), considered apparent, forbs considered unapparent. Grasses use quantitative protection (siliceous tissues), and in many cases dominate their biotopes.

As the hypothesis tested here predicts that feeding on apparent plants constrains the number of generations, all butterflies feeding on both apparent and unapparent plants were classified as feeding on unapparent plants. This strengthened the null hypothesis of no difference in voltinism between species feeding on

differently protected hosts. It also avoided potential biases in species that form different numbers of generations on differently protected hosts (Wedell *et al.*, 1997).

#### Butterfly covariables

Several life history traits that affect butterfly voltinism are likely to be independent of host plant defences. To control for their effects, the following traits were considered as covariables.

**Body size.** Larger insect species require more time for development (Stamp, 1993), and thus form fewer generations per year than smaller species. Wingspan information from Higgins and Riley (1970) was used. For species not covered by Higgins and Riley (1970), illustrations in Tolman and Lewington (1997) were measured.

**Short season environments.** Species confined to arctic and/or alpine regions are constrained in number of generations by adverse climate (Strathdee & Bale, 1998). Such species were distinguished using maps in Kudrna (2002) and information on altitudinal distribution in Tolman and Lewington (1997). Scored binarily.

**Myrmecophily.** The mutualistic association with ants enhances juvenile survivorship and hastens development (Pierce *et al.*, 2002). Scored binarily, without distinguishing among types of myrmecophily (cf. Fiedler, 1991).

**Flower feeding.** Feeding on nutrient-rich flowers and developing fruits may accelerate development, allowing increase in number of generations. On the other hand, exclusive flower feeders might be confined to one generation simply because the appropriate plant parts are available only for a short time. Scored binarily: exclusive foliar feeders, 0; species exploiting flowers in any part of larval development, 1.

**Gregariousness.** Gregarious caterpillars feed more efficiently and develop faster than solitary ones (e.g. Clark & Faeth, 1997). Scored binarily.

#### Analyses

Both analysis – not controlled and controlled for phylogeny – were performed.

**1** For the analysis not controlled for phylogeny, generalised linear models with an assumed Poisson distribution of the dependent variable were applied. After testing the independent effects of all predictors, multiple regressions were constructed using the stepwise forward selection procedure in S-plus (S-plus 1999, 2000). Model fits were assessed using the Akaike information criterion (AIC).

**2** For the phylogenetically controlled analysis, the method of independent contrasts (Felsenstein, 1985) was applied. It is based on the weighted differences between the character values of sister nodes of phylogenetic trees. Essential information is the length of individual tree branches: a longer evolutionary time will likely produce larger differences between sister branches that would attain greater weights in statistical analyses. Hence, contrasts have to be standardised to account for branch lengths (Garland *et al.*, 1992), and appropriate standardisation of branch lengths may vary from trait to trait (Garland *et al.*, 2002).

Naturally, information on ‘true’ branch lengths was not available. Methods for setting unknown branch lengths include Grafen’s, Nee’s, Pagel’s, and ‘constant’ (Garland *et al.*, 1992, 2002). Appropriate standardisation of branch length requires that absolute values of standardised independent contrasts for each trait are independent of their errors. On the other hand, a significant linear or non-linear relationship between contrast values and their errors indicates inadequate standardisation.

The contrasts were computed using PDAP ver. 6.0 (Garland *et al.*, 2002). The program allows analyses with categorical variables and computes contrasts from partially unresolved trees. To select appropriate standardisation, each variable was checked (separately for the above four methods of setting branch lengths) for the relationships between contrasts and their standard deviations, and the standardisation with the weakest and/or least significant relationship was selected for further analysis. Finally, linear least-square regressions of standardised contrasts of the number of generations against the standardised contrasts of individual predictors were forced through the origin (Garland & Diaz-Uriarte, 1999; Garland *et al.*, 1999; Garland & Ives, 2000).

Following analyses with the complete data set, analyses that excluded species from short-season environments, myrmecophilous species and species feeding on flowers were performed. Analysing this ‘reduced data set’ (193 species) avoided biases caused by climate-caused monovoltinism, and by retention of species not entirely depending on leaves during larval development.

## Results

The analysis not controlled for phylogeny (Table 1) revealed that broader-sense apparency of host plants decreased the numbers of generations, whereas narrow-sense apparency had no effect. This applied to both the complete and the reduced data sets. In addition, fewer generations were associated with large bodies (both data sets) and short-season environments (complete data set). There was also a marginal increase ( $P = 0.06$ ) in the number of generations due to myrmecophily (complete data set). The multiple regression for the complete data set (Fig. 1) pointed to additive effects of short-season and broader-sense apparency of host plants in decreasing numbers of generations. Therefore, even after considering the climate-induced monovoltinism in short-season environments, butterflies developing on forbs form more generations than butterflies developing on either woody or gramineous plants. In the reduced data set, the only predictor selected by the multiple regression was broader-sense apparency.

Notably, butterflies feeding on apparent plants were larger. This applied to both the complete data set (narrow sense,  $22.8 \pm 7.32$  vs.  $19.7 \pm 4.48$  mm,  $t_{367} = 3.11$ ,  $P < 0.01$ ; broader sense,  $20.2 \pm 4.92$  vs.  $18.5 \pm 4.22$  mm,  $t_{367} = 3.40$ ,  $P < 0.001$ ) and the reduced data set (narrow sense,  $29.4 \pm 4.17$  vs.  $21.5 \pm 4.80$  mm,  $t_{191} = 8.88$ ,  $P < 0.0001$ ; broader sense,  $24.0 \pm 4.92$  vs.  $20.7 \pm 4.27$  mm,  $t_{191} = 3.75$ ,  $P < 0.001$ ). While adding broader-sense apparency to the models containing body size considerably improved the fit (comparing two-term and one-term models, complete data set:  $F_{1,366} = 19.9$ ,  $P < 0.0001$ ; reduced data set:  $F_{1,190} = 25.1$ ,  $P < 0.0001$ ), adding body size to models containing apparency did

**Table 1.** Regressions not controlled for phylogeny showing the effects of host plant apparency, and butterfly life history traits, on the number of generations in European butterflies. Generalised linear models with the number of generations (coded on an ordinal scale) as a dependent variable and link-function log. The +/- signs indicate the directions of the relationships, *F*- and *P*-values refer to comparison with null models.

	d.f.	Residual dev.†	Model dev.‡	AIC	<i>F</i>	<i>P</i>
Complete data set						
Null	368	91.9		92.4		
Narrow sense apparency	1,367	91.8	0.1	93.0	0.14	N. S.
Broader sense apparency [1]	-1,367	84.6	7.3	85.7	27.38	****
Arctic/alpine [2]	-1,367	79.2	12.6	80.2	53.31	****
Myrmecophily	+1,367	90.8	1.0	91.9	3.60	+
Flower feeding	1,367	91.8	0.1	92.9	0.29	NS
Gregariousness	1,367	91.8	0.1	93.0	0.09	NS
Body size	-1,367	88.9	3.0	90.0	10.63	**
Final model ([1] + [2])	2,366	74.4	17.4	75.8	38.84	****
Reduced data set§						
Null	192	53.8		54.4		
Narrow sense apparency	1,191	53.5	0.3	54.7	1.09	NS
Broader sense apparency	-1,191	45.9	8.0	46.9	30.12	****
Body size	1,191	52.0	1.8	53.2	5.98	*
Gregariousness	1,191	53.8	0.1	55.1	0.01	NS

+*P* < 0.1; \**P* < 0.05; \*\**P* < 0.01; \*\*\**P* < 0.001; \*\*\*\**P* < 0.0001;

†Residual deviation, not accounted for by the model.

‡Model deviation, accounted for by a fitted model.

§Final model for reduced data set contained broader sense apparency only.

not cause any improvement (complete data set:  $F_{1,366} = 3.40$ ,  $P = 0.09$ ; reduced data set:  $F_{1,190} = 1.47$ ,  $P = 0.23$ ). It follows that feeding on quantitatively protected plants decreased the number of generations even after controlling for body size.

When controlled for phylogeny (Table 2) both narrow- and broader-sense apparency lowered the number of generations in the reduced data set (Fig. 2). None of these predictors, however, had an effect in the complete data set. Here, the factors significantly reducing the number of generations included short-season environments, large bodies, and larval gregariousness. Controlling for phylogeny thus revealed that if the analysis was restricted to exclusively foliage-feeding butterflies not limited in voltinism by short seasons, species feeding on forbs formed more generations per year than species feeding on woody plants and/or grasses.

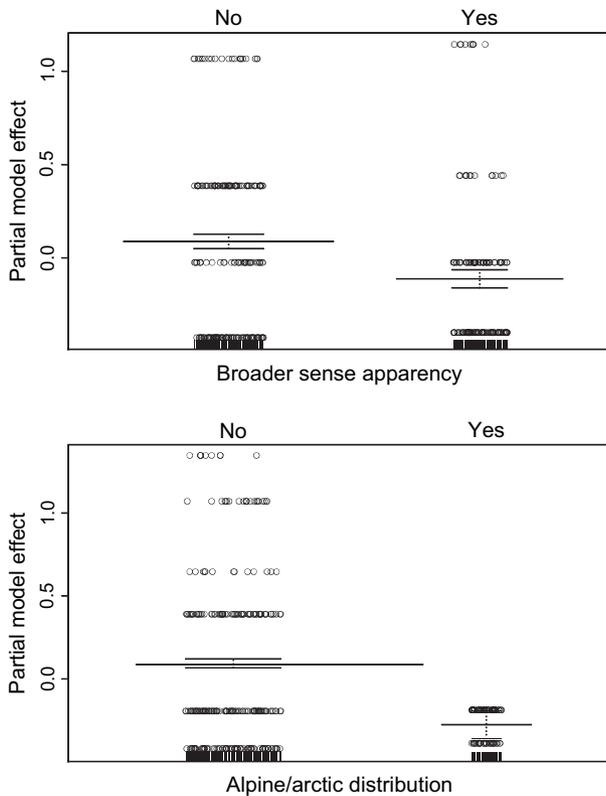
## Discussion

These results support the hypothesis that feeding on quantitatively protected plants restricts the annual number of generations in European foliage-feeding butterflies. Strictly speaking, this applies only to butterflies not restricted in voltinism by other constraints. There was also a difference between analyses controlled and not controlled for phylogeny, as feeding on woody hosts decreased the number of generations only in phylogeny controlled regressions. The difference was influenced by the species-rich and grass-feeding subfamily Satyrinae, which contains seven multivoltine and 108 monovoltine species and thus outweighed the effect of monovoltine species in other taxa. This highlights the importance of controlling for phylogeny in comparative analyses.

It is not claimed here that the nature of plant defences constitutes a single factor affecting butterfly voltinism. Both grass-

feeding and arboreal-feeding species with multiple generations per year, and monovoltine herb-feeding species exist. The effect of host defences was robust, it held despite these deviating cases, and withstood controlling for phylogeny. On the other hand, many of the species that deviated from the general pattern seem to represent the exceptions that prove the rule. For instance, monovoltine species developing on spring ephemeral herbs face a temporal constraint not unlike the constraints faced by species of short-season environments (Valimaki & Itamies, 2005). Furthermore, polyvoltine grass-feeding Satyrinae, such as *Coenonympha pamphilus*, tend to feed on exceptionally weakly protected grasses that re-sprout late in the seasons (cf. Goverde *et al.*, 2002). The binary categorisations of host plants used here obviously could not capture all the variation, but a more detailed categorisation would necessarily introduce subjectivity into the analyses.

The link between butterfly voltinism and host plant defences is further supported by indirect evidence. For the facultatively bivoltine and polyphagous species, *Polygonia c-album*, feeding on woody hosts slows down the growth rate (Janz *et al.*, 1994) and initiates diapause, leading to monovoltine development (Wedell *et al.*, 1997). Multivoltine and even monovoltine butterflies that utilise woody hosts seem to exploit the natural variability among their hosts, preferring individuals that are presumably less well defended. McKay (1991) demonstrated this for the monovoltine species *Gonopteryx rhamni* (McKay, 1991) and anecdotal observations exist for other species, both multivoltine (e.g. *Iphiclides podalirius*) and monovoltine (e.g. *Apatura* spp., *Limenitis populi*, or *Satyrion ilicis*) (cf. Fuldner, 1997; Ebert & Rennwald, 1991; Hermann & Steiner, 2000). Nymphalids feeding on woody hosts and overwintering as larvae grow slowly on mature autumn foliage and rapidly on young spring foliage (Dell *et al.*, 2005).



**Fig. 1.** Multiple regression model of factors influencing the number of generations in European butterflies, complete data set containing 369 species (GLM, link function log, apparent host plants defined as woody plants plus graminoids). Predictors were entered as 1/0 factorial variable (Yes/No in the plots). The plots show fitted partial effects of each of the predictors on log-transformed number of generations (long horizontal lines) plus standard errors of the estimates (shorter horizontal lines). White dots are residuals of individual observations, whereas the short whiskers at *x*-axis show counts of occurrences of respective states in the data.

The restriction of the annual numbers of butterfly generations by quantitative defences of their host plants may contribute to explaining several patterns in butterfly biology. They include (i) the association between large bodies and low number of generations; (ii) the association between large bodies and feeding on large (or woody) hosts; and (iii) monovoltinism found in some species that are not restricted by short season.

The observation that large butterflies tend to form fewer generations per year was previously reported, e.g. by Garcia-Barros (2000), who attributed it to longer development of larger species. The author also observed that large butterflies often feed on large or 'structurally complex' plants – which typically employ quantitative defences (Silvertown & Dodd, 1996). As body size became redundant for explaining voltinism after considering plant defences, attributing both patterns to plant defences provides a more parsimonious interpretation than attempts to interpret the patterns separately. Any insect may increase its fitness either by maturing early and forming more generations, or by growing larger and producing more eggs. As the former option is closed

for species developing on quantitatively defended plants, the only remaining option is to increase in size. In addition, larger larvae can digest foliage of lower quality, extending the feeding period opened for larger butterflies (Yang & Joern, 1994; Cizek, 2005).

If host plant defences rather than climate mediate voltinism, it becomes clear why the majority of butterflies developing on woody plants or grasses form one generation per year, even if they are not restricted by the length of the growth season. Indeed, the majority of European butterflies occurring as adults in late summer/early autumn are forb-feeding polyvoltines. Likewise, because monovoltine species tend to consume quantitatively protected plants, they should emerge relatively early in the season. This explains the relative rarity of late-season monovoltines, noted by Shapiro (1975) and Shapiro *et al.* (2003) as an enigmatic pattern. Again, late-season monovoltines may represent exceptions proving a rule. They tend to be large (e.g. *Hipparchia* or *Chazara* satyrids, the hairstreak *Thecla betulae* or the skipper *Hesperia comma*, are the largest European representatives of their clades) and typically feed on quantitatively protected hosts. Their late emergence then might represent a trade-off with large size (cf. Janz *et al.*, 1994).

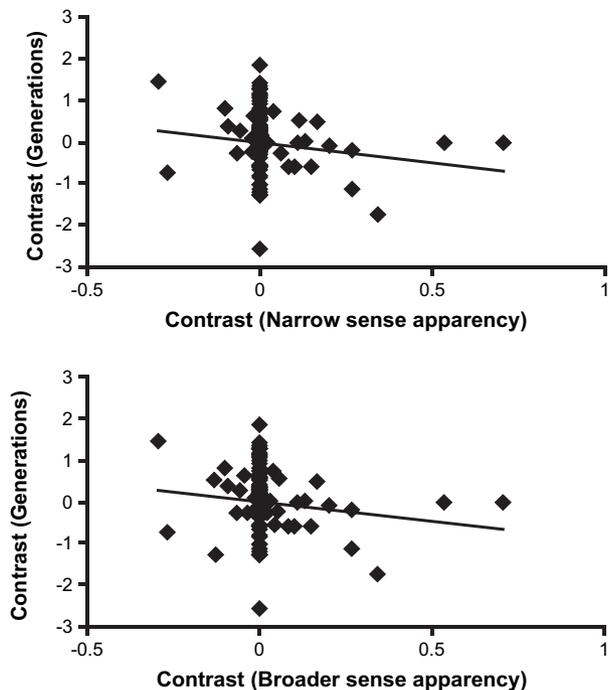
Dennis *et al.* (2004) recently showed that British butterflies feeding on stress-tolerant plants (*sensu* the RCS scale of Grime, 1974) tend to be monovoltine. All else being equal, stress-tolerant plants tend to be long living and hence more apparent than short-living ruderal plants (Coley *et al.*, 1985). The same applies for plants with a competitive strategy, however; both stress-tolerant and competitive plants may include various growth forms (woody plants, forbs, or grasses), and both groups may employ either quantitative or qualitative defences. If plant defences rather than ecological strategies affect voltinism, they should correlate with numbers of generations better than positions of the plants on the RCS continuum. This is exactly what Dennis *et al.* (2004) would have observed, had they considered voltinism of their butterflies within entire distribution ranges, not only in Britain. Some of their species that feed on stress-tolerant herbs are monovoltine in Britain (e.g. *Erynnis tages*, *Boloria selene*) but form second generations elsewhere, whereas all their monovoltine butterflies feeding either on stress-tolerant grasses (e.g. *Hipparchia semele*) or on competitive woody plants (e.g. *Satyrion w-album*) remain monovoltine across their ranges. The nature of plant defences thus broadens and expands the perspective adopted by Dennis *et al.* (2004).

All the above arguments support the initial hypothesis, at least for temperate butterflies. As the physiological patterns associated with leaf ageing likely affect all insects consuming quantitatively protected plants (Kause *et al.*, 2001; Cizek, 2005), however, the restriction of numbers of generations in such herbivores may represent a more general pattern. Although hitherto overlooked, it likely contributes to structuring herbivore communities. For instance, European grasshoppers feed on quantitatively protected grasses and exhibit prolonged monovoltine development. In herbivore communities on deciduous trees, seasonal peaks of occurrence of externally feeding moth larvae precede peaks of leaf mining and gall-making larvae (Southwood *et al.*, 2004). Because plant phenology is seasonally synchronised in all terrestrial biomes (Sakai, 2001), the restricted numbers of generations associated with feeding on quantitatively protected hosts may apply even to regions beyond

**Table 2.** Comparison of phylogenetically independent contrasts for the effects of host plant apparency, and butterfly life history variables, on the number of generations in European butterflies. The comparisons were done by regressions of contrasts for the number of generations against contrasts for individual predictors forced through the origins. The  $+/-$  signs indicate the slope of the regressions, and the letters under the headings 'type of transformation' stand for the most appropriate transformations of branch lengths for each particular trait which was used in individual analyses: C, constant; G, Grafen's; N, Nee's; and P, Pagel's (see Garland *et al.*, 2002).

Model	Branch length standardisation		$r^2$	d.f.	$F$	$P$
	Voltinism	Predictor				
Complete data set						
Narrow sense apparency	C	G	0.002	1, 367	0.59	NS
Broader sense apparency	C	G	0.001	1, 367	0.38	NS
Arctic/alpine distribution	C	N	-0.118	1, 367	49.07	***
Myrmecophily	C	N	0.001	1, 367	0.36	NS
Flower feeding	C	P	0.002	1, 367	0.66	NS
Gregariousness	C	G	-0.011	1, 367	4.04	*
Body size	C	C	-0.020	1, 367	7.56	**
Reduced data set						
Narrow sense apparency	N	G	-0.021	1, 191	4.08	*
Broader sense apparency	N	G	-0.020	1, 191	3.86	*
Body size	N	P	-0.032	1, 191	6.28	*
Gregariousness	N	N	-0.025	1, 191	4.80	*

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



**Fig. 2.** Plots illustrating the regressions through the origins used in the phylogenetically controlled analyses of factors influencing the number of generations in European butterflies. Standardised contrasts for the number of generations are regressed against standardised contrasts for individual predictors. Reduced data set, i.e. after exclusion of species restricted to short-season environments, myrmecophilous species and species with flower-feeding larvae. Narrow sense apparency (top) includes woody hosts, broader sense apparency (bottom) includes woody plus gramineous hosts.

temperate zones. This hypothesis deserves further testing, as it might, for example, contribute to understanding of patterns of herbivore seasonality in different regions. Besides butterflies, obvious candidates for testing are moths with externally leaf-chewing larvae, which vary in voltinism, whose phylogeny is at least partially known, and for which an association between monovoltinism and feeding on woody plants has occasionally been reported (Yela & Herrera, 1993; Loder *et al.*, 1998).

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## Supplementary material

Supplementary material is available at [www.blackwell-synergy.com](http://www.blackwell-synergy.com) under DOI reference doi:10.1111/1365-2311.2006.00783.x

## Appendix 1

Sheet containing original data used for the analyses. Butterfly nomenclature follows Karsholt and Razowski (1996), the important exceptions being that (i) subgeneric names were used when provided by the authors, and (ii) the following species not

recognised by K and R were included: *Pontia edusa* (Fabricius, 1777), *Pseudophilotes panoptes* (Hübner, 1813), *Agrodiaetus ainsae* (Forster, 1961), and *Polyommatus violetae* (Gomez-Bustillo *et al.*, 1979).

## Appendix 2

Combined tree showing phylogenetic relationships among 369 species of European butterflies, used for the phylogenetically controlled analyses of relationships between voltinism and host plant defences.