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Geographical variation in host plant utilization in the comma butterfly: the roles of time constraints and plant phenology

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Abstract What is the role of time-constraints in determining geographical variation in the resource use of organisms? One hypothesis concerning phytophagous insects predicts a local narrowing of host plant range at localities where a short development time is important (because an additional generation per season is only just possible), with increased specialization on host plants permitting fast development. To test this hypothesis, populations of the polyphagous comma butterfly (Nymphalidae: *Polygonia c-album*) from five European areas (localities in Norway, Sweden, England, Belgium and Spain) were sampled and the preferences of laboratory-reared female butterflies were investigated, by a choice test between *Salix caprea* and the fastest host *Urtica dioica*. The results suggest that females of both of two northern univoltine populations (time-stressed from Norway and time-relaxed from Sweden) accept the slow host *S. caprea* to a higher degree than females of more southern populations with partial additional generations (time-stressed). We thus found partial support for the tested hypothesis, but also conflicting results that cast doubt on its broad generality. Moreover, a split-brood investigation on Swedish stock demonstrated that larval performance is similar on *S. caprea* and *U. dioica* early in the summer, but that later in the season *S. caprea* is a much inferior host. This is reflected by a seasonal trend towards specialization on *U. dioica* and also provides a simpler explanation than the time-constraints theory for avoidance of *S. caprea* (and other woody hosts) in areas with two or more generations of insects per year, illustrating the importance of plant phenology as a constraint on resource use in phytophagous insects. Absolute and relative larval performance on the two hosts varied little among populations across Europe, but lower survival on *S. caprea* in the populations most specialized on *U. dioica* and related plants may be indicative of performance trade-offs.

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Introduction

An increased understanding of the factors that determine niche width has always been a
central aim in evolutionary ecology (Futuyma and Moreno 1988), and in recent years there
has been a resurgence of interest in this topic because of the new emphasis on ecological
speciation (Schluter 2001). The very diverse guild of phytophagous insects are often used
to illustrate and test these ideas (e.g. Ferrari et al. 2006; Nygren et al. 2006; Nosil 2007)
and there is thus a need to determine how niche width evolves in such taxa. For instance,
polyphagous populations have been implicated in facilitating speciation processes in
phytophagous insects (Janz et al. 2001, 2006; Weingartner et al. 2006) as well as in being
particularly prone to climate-driven shifts in distribution (Parmesan 2006; Braschler and
Hill 2007).

Seasonality impacts strongly on insect life histories (Tauber et al. 1986) and is therefore
also likely to be of importance for ecological traits such as niche width. In temperate areas,
the life cycles of insects are restricted by winter and hibernation diapause typically is
possible in only one species-specific developmental stage; i.e. all individuals have to reach
this particular stage before the unfavorable season sets in. By these limitations, a local
insect population with time available for "one and a half" generations, but not for two, will
have surplus time available and is expected to use this time to e.g. achieve larger size (Roff
1980, 1983). The theoretically expected result is then longer development time and higher
adult mass towards lower latitudes and lower elevations within a one-generation (uni-
voltine) area, as season length increases; this is the "converse Bergmann's rule" that
commonly applies to insects and other ectotherms (Mousseau 1997). However, as soon as
an additional generation of insects becomes possible, the favorable season is effectively cut
in two for individuals following this bivoltine pathway (at the same time as other indi-
viduals may still follow a univoltine pathway; i.e. there is partial bivoltinism). The
expectation from optimality reasoning is then shorter development time and smaller adult
size in the former individuals (in the following referred to as being "time-stressed")—
creating a predicted saw-tooth pattern as the process is repeated when a third generation
can be added (Roff 1980, 1983; Nylin and Svärd 1991).

A theoretically closely related hypothesis is a geographical shift in host plant range in
phytophagous insects, since host plants often differ in their effects on larval development
time (Nylin 1988; Scriber and Lederhouse 1992). A general framework for insect-plant
studies is provided by the expectation that females should often prefer to oviposit on those
plants on which their offspring have highest fitness (preference-performance theory;
Thompson 1988; Singer 2002). This expectation is not always met, for various reasons, but
even when it is there can be variation in the degree to which females accept non-preferred
plants. Thus, in areas where there is relaxed selection for fast larval growth the females can
include also "slow" host plants in the host range, reaping the benefits of generalization
such as increased realized fecundity and spreading of risks. In areas with just barely
enough time for an additional generation per year selection for fast larval growth is instead
intense and females should select only "fast" host species. The information previously
available from Swedish and English populations of the comma butterfly *Polygonia c-album*
supports this notion (Nylin 1988), and some North American *Papilio* butterfly species also
show geographical variation in host range consistent with the hypothesis (Scriber and

Lederhouse 1992; Scriber 2002). It is however still unclear to what extent the hypothesis is generally applicable, or even if it applies over the entire geographical distribution of the species from which support has previously been found.

One aim of the present study was thus to extend investigations on *P. c-album*, the species originally used to test the hypothesis, to a wider geographical area. We gave laboratory-reared females originating from different populations a choice between the "fast" herb *Urtica dioica* and the "slow" tree *Salix caprea* and reared their offspring on both species. At least in the previously studied populations *U. dioica* allows *P. c-album* butterflies to develop faster in the laboratory. On the other hand, females raised on *S. caprea* have been found to be larger on average and perhaps more fecund (Janz et al. 1994), a fact which makes this pair of hosts particularly interesting for choice tests. In addition, genetic variation is known to exist for female preference using this set-up, within (Nylin et al. 2005) and between populations (Janz 1998; Nygren et al. 2006).

The present study provided an opportunity to investigate if populations of *P. c-album* across Europe are similar in their life history responses to *S. caprea* and *U. dioica* as larval hosts. Variation among populations could complicate or prevent application of the "time constraints" hypothesis over larger areas. Furthermore, plants are well known to change in quality as hosts over the season. In particular this seems to apply to plants with "quantitative defences" such as woody plants (Feeny 1970; Scheirs et al. 2002; Cizek et al. 2006). A superior host plant may thus in fact be superior only within a specific phenological time frame (Scriber 2002). In particular, different generations of insect herbivores succeeding each other in the same year will have to handle host plants in very different phenological stages (Cizek et al. 2006). For this reason, another aim of the present study was to investigate if the relative differences between *S. caprea* and *U. dioica* in terms of larval performance and fitness consequences remain constant through the summer, or if seasonal trends can cast further light on the variation in host plant preferences seen in *P. c-album*.

To these ends, we studied butterflies originating from several additional populations (Norway, Belgium and Spain) along with the previously studied Swedish and English populations. Both *U. doica* and *S. caprea* occur at all of the collection sites, except for the low altitude sites in Spain. There is little information available on actual host use in the field, but from what is known the total range of potential hosts is the same all over Europe, and *U. dioica* and related "fast" hosts are preferred hosts everywhere (e.g. Anonymous 1987; Nylin 1988; Ebert 1993; Tolman and Lewington 1997; Asher et al. 2001). Variation is instead more in the degree of specialization on these preferred plants, making the system ideal for a test of the time-constraint hypothesis.

Materials and methods

Study species and populations

The comma butterfly *Polygonia c-album* (L.) (Lepidoptera: Nymphalidae) is not an indiscriminate generalist, but is polyphagous in the sense that it utilizes larval host plants from several taxa: the "urticalean rosids" *Urtica*, *Humulus* and *Ulmus* and the distantly related *Salix* (Salicaceae), *Ribes* (Grossulariaceae), *Betula* and *Corylus* (Betulaceae) (Nylin 1988; Tolman and Lewington 1997). The typical number of eggs laid at one oviposition event is a single egg, but a few eggs can be laid at the same time on preferred hosts (Bergström et al. 2006). Winter hibernation is in the adult stage. The species is seasonally polyphenic, with the overwintering generation having dark wing undersides

("dark morph") and summer generations of adults (which mature sexually and reproduce the same season) having yellowish wing undersides ("light morph"; Nylin 1989).

For the investigations presented here we collected *P. c-album* females from five different countries (in Spain also from four different altitudes). This geographical transect covers most of the total latitudinal range of the species in Europe. The species is not trivial to collect and the number of wild-caught females available to produce laboratory stock was for this reason small for most populations. This said, the species has an open population structure with no evidence for geographically structured genetic differentiation in host plant use within populations (Nylin et al. 2005), reducing the risk that the founding females are unrepresentative of the genetic variation present in the population. Perhaps for this reason, we have found in the past that results from a particular population are typically highly repeatable despite small initial sample sizes. In the analyses we take steps to further reduce this problem by combining populations. Nevertheless, it must be stressed that results from a particular population are not yet conclusive.

Laboratory stock was initiated from field-mated females collected in:

Norway 63.5°N (Djupdalen, near Trondheim); this is a northern univoltine population assumed to be time-stressed in that there is barely enough time for a single generation. Adults eclose from late July in warm summers, but not until late August and well into September in cooler years (R. Krogen, pers. comm., observations from both the field and from outdoor rearings), i.e. with little time to feed and prepare for hibernation before winter frosts may set in. For the purposes of the present study the population was sampled in the year of 2000 (two wild-caught females, the offspring of which was reared in the laboratory in Stockholm).

Sweden 59°N (vicinity of Stockholm); also a univoltine population but assumed to be time-relaxed, i.e. with surplus time for its single generation (Nylin 1988; Janz et al. 1994). Adults eclose from early July in summers with average temperatures (Nylin 1989). This population served as a control (see below) and hence it was sampled for the geographical study in 2000 (offspring of five females), 2002 (eight females), 2003 (five females) and 2007 (three females). In addition, rearings performed in several other years were included in the phenological study (see below).

England 52°N (vicinity of Oxford); a partially bivoltine population assumed to be under time-stress to complete a second generation (Nylin 1988). It was sampled in 2002 (offspring of one female), included here only to replicate and complement the earlier studies.

Belgium 51°N (vicinity of Antwerp); also a partially bivoltine population under similar time-stress (Maes and Van Dyck 1999). It was sampled in 2003 (offspring of three females).

Spain 42°N (area of Catalonia); all populations have partial second or third generations (C. Stefanescu, pers. observ.) and can thus be expected to be under time-stress, but perhaps to varying degrees. Females were collected in 2002, from four different altitudes: Sea level (El Cortalet; offspring of three females, partially trivoltine population); 200–320 m a.s.l. (Can Liro; five females, partially trivoltine population); 350 m a.s.l. (Sallent; one female, partially trivoltine population) and 1,000 m a.s.l. (El Puig; five females, partially bivoltine population). We also report some complementary results from a family of offspring originating from a female collected in March 2007 in Vallforners, at 700 m a.s.l., which enabled us to perform preference and performance studies earlier in the summer than in the previous study.

Rearings and experiments

Female host plant preferences were studied in choice trials between stinging nettle (*Urtica dioica*) and sallow (*Salix caprea*). Larval performance on the two host plants was measured via: (a) proportion of larvae surviving until pupation, (b) number of days in larval stage,

(c) pupal mass and (d) growth rate ((ln of pupal mass)/days). In the analysis of the rearings (see "Statistics") we specifically investigated the presence of any significant host species * population interactions, since they could indicate genetic differences in relative performance on the two hosts which could be either a cause or an effect of differences in host utilization. The time-constraints hypothesis under study (Nylin 1988) implicitly assumes that such interactions are weak or absent, at least for development time and growth rate.

Two succeeding laboratory generations were reared: in May–July (first larval generation in the following) and July–September (second larval generation). Preference data were recorded with adults of both generations; performance data were recorded in the second generation.

For all experiments the plants used were brought in from field sites adjacent to Stockholm University, and they were thus subject to natural phenological variation.

Wild females of all the different populations could not be collected in the same year, and for this reason the Swedish population was used to control for variation between and within years caused by differences in e.g. exact rearing temperatures and in host plant quality. Thus offspring of females from the other investigated populations was raised in "common-garden" experiments together with Swedish individuals, and the resulting females were then used side-by-side for preference studies.

Larvae of the second generation were split between rearings on *U. dioica* or *S. caprea*. Newly hatched larvae were individually reared in plastic jars with fresh supplies of plants that had access to water through a lower jar through a hole in the bottom. Larvae on *U. dioica* were reared at 17 C at 12 h light/12 h dark for the first 14 days and then moved to 22 C, 22 h light/2 h dark to induce the directly developing light morph (Nylin 1992). Larvae on the "slower" host *S. caprea* were instead moved after 18 days, when they had reached a similar larval development stage (early 4th instar).

In addition, about half of the larvae of the second laboratory generations of Belgian and Swedish stock in 2003 were kept under the initial low temperature/short day conditions for the whole life cycle, to induce hibernating ("dark morph") adults for oviposition trials the next year, after hibernation. These females were also compared to freshly caught Swedish females from the wild, in the spring of 2004.

After eclosion from the pupae, the adults were individually marked and moved to cages of about 1 m³, where mating pairs could be collected. Mated females were placed in cages (0.5 m³) with a sugar solution on a sponge in the middle, and two equally sized and randomly chosen host plants of *U. dioica* and *S. caprea* were presented in bottles put at randomised sides of the cage. Flight cages were illuminated by a 75 W light bulb, hanging at a distance of about 3 dm above the transparent upper part of the cage. Mated female butterflies were arbitrarily placed in the cages; hence Swedish and non-Swedish females were present in the laboratory side by side. A pool of plant specimens were rotated among all cages and replaced only if they had deteriorated visibly; assignment of plant individuals to cages were made by rolling of a dice and flipping of a coin thereafter decided the orientation of the plant pair inside the cages. At the end of each day (8 h of light) the two host plants were taken out and the eggs removed and counted. Average preferences from 2 to 3 days of egglaying by one particular female were used as data points, and days when less than 10 eggs were laid were omitted from this analysis. Females can lay up to around 50 eggs per day initially, but this rate drops after the first 1–2 days.

A slightly different set-up was used for the trials involving the second Spanish laboratory generation in 2002, where we positioned the *U. dioica* about one dm below the *S. caprea*. This was done to provoke a higher frequency of *S. caprea* encounters in the cages, because of the late date in the season. The butterflies prefer *U. dioica* to a higher
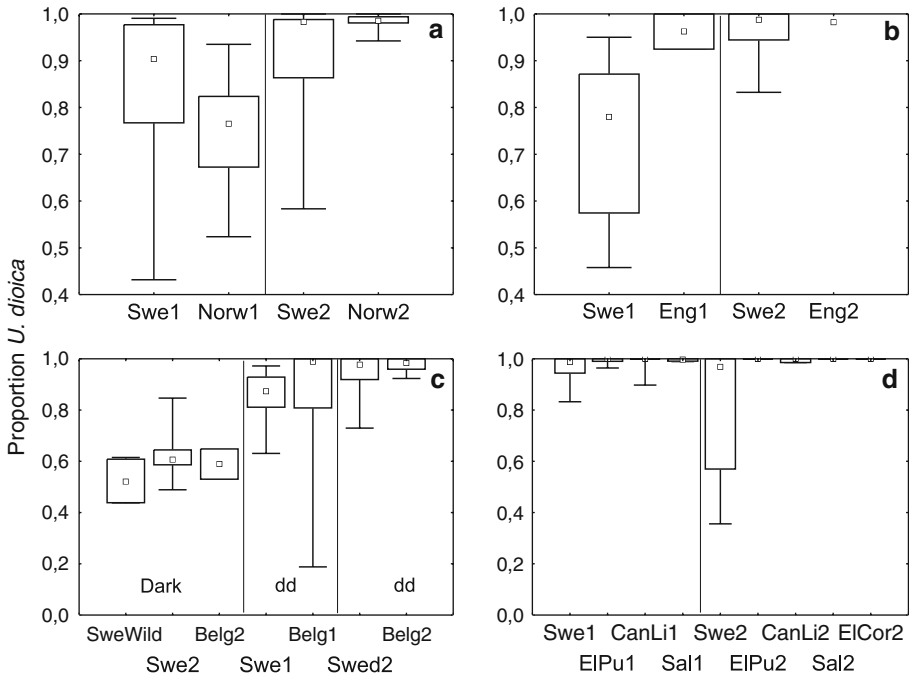
Fig. 1 Comparisons between stock from different populations regarding the proportion of eggs laid on *U. dioica* by females of *P. c-album*, in a choice test between *U. dioica* and *S. caprea*. (**a**) Sweden versus Norway; (**b**) Sweden versus England; (**c**) Sweden versus Belgium and (**d**) Sweden versus the sites in Spain (El Puig, Can Liro, Sallent and El Cortalet). Figures 1 and 2 behind site designations refer to two comparisons in subsequent generations and vertical lines separate comparisons not performed simultaneously. In panel **c**, Dark = Dark (overwintering) females and dd = directly developing females. Figures show medians, 25–75% quartiles (boxes) and total range (whiskers)

degree as the summer proceeds, so that even the Swedish population is relatively specialized late in the season (cf. Fig. 1c) and judging from results in earlier preference studies (Nylin et al. 2005) the procedure was thought necessary in order to be able to observe any differences between populations. In these experiments we thus, strictly speaking, measured "acceptance" of *S. caprea* upon encounter rather than "preference" for one or the other host (to measure preference the hosts should be presented in equal abundance and in the same way (Singer 2002)), although the latter term is used throughout this paper for convenience.

Phenology of host plant quality

To test the implicit assumption of earlier theory (Nylin 1988) that the relative performance on different hosts stays basically constant over the course of the summer, we investigated performance data on Swedish individuals collected at various times over the season in 1996 and in 1999–2003. Each data point in Fig. 4 represents a split-brood experiment where larvae of one family (offspring of one female) were split between *S. caprea* and *U. dioica* and treated as described above to induce direct development. The concentration of data points at three periods (Fig. 4) corresponds to the normal dates for initiation of the first, second and third laboratory generations, respectively. Additional rearings were

performed in 2004 for the specific purpose of complementing earlier data sets, filling out the gaps in the series of dates.

Statistics

All statistical analyses were made with the STATISTICA software. Since the preference data did not conform to the assumptions for parametric tests (unequal variances among categories also after transformations) we used non-parametric tests (Mann–Whitney U-test) and show box-plots with median values rather than averages. For the 2007 preference data it was possible to also use a parametric GLM after ArcSin(Square Root)-transformation had improved homogeneity of variances. For survival and life history traits it was possible to use parametric tests throughout (GLM ANOVAs and regressions).

Survival was analyzed with percentage survival in a family as data points and the sexes pooled. For other performance traits individuals were used as data points and Family was instead included as a factor nested in Population, along with the factors Sex and Host plant. Interactions between Population and Host plant were also included in the model, as they were the particular focus of the study. The three populations reared simultaneously in 2002 were analyzed together, so three separate ANOVAs were performed, one for each year.

Seasonal trends in performance traits (plant phenology study) were studied by regression. We took advantage of the split-brood design and analyzed average family values for performance on *S. caprea* divided by performance values on *U. dioica* for the same family (see Fig. 4). This procedure controls for much of the genetic variation and also reduces the amount of experimental "noise" caused by variation among experiments in e.g. temperature. All families with surviving larva on both host plants were included (individuals per family ranging from 6 to 50, typically 15–20). The sexes were first analyzed separately, but later pooled (see "Results"). A General Linear Model (GLM) ANOVA with Family, Sex and Host plant incorporated as categorical factors and starting Date of the rearing as continuous factor (covariate) was also performed to investigate the interaction Host plant * Date in a statistically more rigorous manner, but in Fig. 4 the regressions are presented, being more illustrative of the phenological trends.

Results

Preference

Contrary to expectations from the time-constraints hypothesis, females of the time-stressed Norwegian population did not oviposit to a higher degree on the "fast" host *U. dioica* when given a choice with *S. caprea*. The preferences of the Norwegian females did not differ significantly from the Swedish population in either the first or the second laboratory generation of directly developing adults (Mann–Whitney U-test on average of female preference $n_{(Gen1)} = 21$, $Z = 1.59$, $P = 0.11$; $n_{(Gen2)} = 18$, $Z = 0.69$, $P = 0.49$). In fact, in the first laboratory generation the trend was rather towards more eggs laid on *S. caprea* by Norwegian females (Fig. 1a), and this trend is significant if the comparison is extended to include also Swedish females of the same generation in the other years of the study ($n_{(Gen1\ 2000–2003)} = 37$, $Z = 2.07$, $P < 0.05$). Thus, if a consistent difference between the two populations does exist, it is likely to be in the direction of higher rather than lower acceptance of *S. caprea* in Norway.

The preferences of English females were consistent with the previously shown higher levels of specialization on *U. dioica* when compared to Swedish females (as predicted by the time-constraints hypothesis; Fig. 1b). The difference between populations was not statistically significant in the present study, but there was a strong tendency in the first laboratory generation (Mann–Whitney U-test on average of female preference, $n_{(females)} = 10$, $Z = 1.83$, $P = 0.068$). In the second generation there was only a single mated English female, so no statistics were attempted.
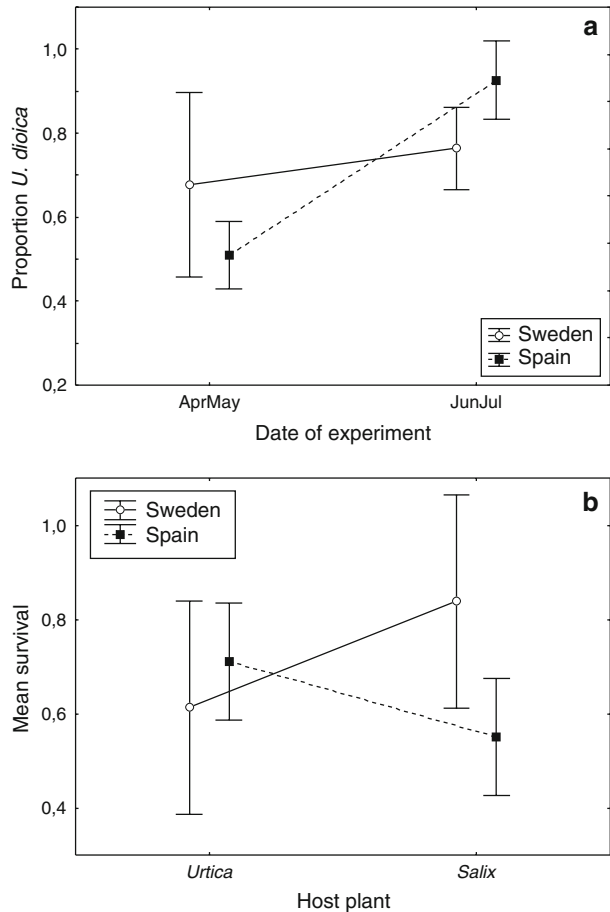
Belgian females tended to be only slightly more specialized on *U. dioica* than Swedish females (the hypothesis under test would predict a clear difference). There were no significant differences in single experiments, but a tendency in this direction at least in the first laboratory generation of directly developing females (Fig. 1c; Mann–Whitney U-test on average of female preference; first generation: $n_{(females)} = 29$, $Z = 1.84$, $P = 0.066$; second generation: $n_{(females)} = 19$, $Z = 0.41$, $P = 0.68$). The difference between populations is significant if both directly developing generations are pooled ($n_{(females)} = 48$, $Z = 2.15$, $P < 0.05$). Statistical tests comparing categories of dark morph females after hibernation were not undertaken due to a low mating frequency among hibernated adults resulting in low sample sizes, but these results are shown in Fig. 1c (leftmost) to demonstrate that the much lower level of specialization on *U. dioica* in the spring is seen in females of both populations. The difference between hibernating and directly developing generations is significant in both cases (Sweden $n_{(females)} = 15$, $Z = 2.94$, $P < 0.01$; Belgium $n_{(females)} = 11$, $Z = 2.12$, $P < 0.05$).

In the Sweden versus Spain comparisons there was a very high degree of preference for *U. dioica* for all investigated females, due to the late date in the season for these experiments. Still, the few eggs laid on the "slow" host plant *S. caprea* were almost exclusively laid by females of the Swedish population (Fig. 1d). No significant differences in host plant choice could be detected between the different Spanish samples from different altitudes (Kruskal–Wallis ANOVA $n_{(Gen1)} = 37$, $H = 1.54$, $P = 0.46$; $n_{(Gen2)} = 14$, $H = 3.67$, $P = 0.30$). Thus the Spanish females were instead pooled, and they were then found to be significantly more specialized on *U. dioica* than the Swedish females in both laboratory generations (Fig. 1d; Mann–Whitney U-test; generation 1: $n_{(females)} = 50$, $Z = 2.73$, $P < 0.01$; generation 2: $n_{(females)} = 21$, $Z = 2.46$, $P < 0.05$).

Since all Spanish populations should be time-stressed, this result is in line with the hypothesis under study. We initially chose to study stock from several Spanish populations in order to include varying levels of time stress over altitudinal as well as latitudinal gradients. However, upon closer investigation of the phenology of the four Spanish populations from which stock could be collected, they were all found to show a variable number of generations (C. Stefanescu, unpublished data from the Catalan Butterfly Monitoring Scheme) and thus they all had to be classified as time-stressed in the sense of the hypothesis. We believe this may in fact well be a very common situation in insects with good dispersal capacity, limiting the applicability of the time constraints hypothesis in its strongest sense (the predicted "saw-tooth" pattern) to more sedentary species. Climatic variability, coupled with gene-flow between local populations, should mean that for mobile species there are no discrete time-relaxed populations with a fixed number of complete generations in all years—except for the univoltine areas.

Finally, disregarding the fact that the separate experiments were not performed simultaneously, results from all directly developing females of normally univoltine populations (Sweden and Norway; $n = 93$) can be contrasted with all those of potentially bi- or multivoltine populations (Belgium, England and Spain; $n = 76$). This comparison (which violates the time-constraints hypothesis by pooling time-stressed Norwegian with

Fig. 2 (**a**) Comparison between
stock from Sweden and Spain
regarding the proportion of eggs
laid on *U. dioica* by females of *P.
c-album* in spring and in summer
2007, in a choice test with *S.
caprea*. Figure based on
untransformed data for clarity.
(**b**) Mean survival of families of
larvae of *P. c-album* from
Sweden and Spain (offspring of
females in panel **a**) when reared
on *U. dioica* or *S. caprea*. Bars
show standard errors

time-relaxed Swedish populations) illustrates that even though preferences for *U. dioica* were very high overall in these summer generation females, only the latter category of females totally rejected *S. caprea* (median of 100% eggs on *U. dioica*, compared to 93% in univoltine populations; Mann–Whitney *U*-test, $Z = 6.90$, $P < 0.001$). The difference could be due to an evolved stronger avoidance of late-season *S. caprea* in populations where females are normally exposed to this situation, if this host deteriorates relatively more in quality over the season than *U. dioica* (as suggested by the plant phenology study; see below).

This interpretation was supported by a complementary comparison between Swedish and Spanish females performed in 2007 when we had access to Spanish females earlier in the season, at the time when Swedish females normally oviposit. As can be seen from Fig. 2a preferences were actually similar for females of the two populations when they were given a choice between spring plants, with a large proportion of eggs laid on *S. caprea* (Mann–Whitney *U*-test, $n_{(females)} = 25$, $Z = 1.67$, $P = 0.11$), but already in the next generation of offspring Spanish females displayed a higher preference for *U. dioica* (Mann–Whitney *U*-test, $n_{(females)} = 32$, $Z = 2.51$, $P < 0.05$). The seasonal trend was thus much steeper for Spanish females, and in a parametric GLM ANOVA-model, analyzing both generations together, this was reflected by a significant interaction between source

population and generation of experiment ($n_{(females)}$ = 57, $P_{(country)}$ = 0.54, $P_{(gen)} < 0.001$, $P_{(country*gen)} < 0.05$).

Performance

Table 1 shows a summary of traits recorded (averages of family averages) in each pair-wise comparison of populations where two or more populations were reared side-by-side. Few of the differences are significant in *t*-tests (not shown). This is despite the fact that there are many possible separate comparisons and high risk of spurious results. To avoid this problem, in the following we instead report results of GLM ANOVAs on each separate experiment, where different factors can be analyzed simultaneously. Of particular interest for the present study is the existence of any significant Population * Host plant interactions, indicating that the populations responded differently to the hosts.

Sweden versus Norway experiment in 2000

Survival did not differ between Norwegian and Swedish stock, on any of the host plants. There was no significant effect of country of origin or interaction between country and host plant, although the effect of host plant was significant—with lower survival on *S. caprea* for both populations (GLM ANOVA $n_{(families*host plants)}$ = 32, $P_{(country)}$ = 0.17, $P_{(host plant)} < 0.001$, $P_{(c*h)}$ = 0.38).

The results for other performance traits were similar, in that there were clear effects only of Host plant. Development time: GLM ANOVA $n_{(families)}$ = 18, $P_{(family)} < 0.05$, $P_{(sex)}$ = 0.10, $P_{(country)}$ = 0.38, $P_{(host plant)} < 0.001$, $P_{(c*h)}$ = 0.48. Pupal mass: $P_{(family)} < 0.001$, $P_{(sex)}$ = 0.59, $P_{(country)}$ = 0.20, $P_{(host plant)} < 0.01$, $P_{(c*h)} < 0.05$. Growth rate: $P_{(family)} < 0.01$, $P_{(sex)}$ = 0.15, $P_{(country)}$ = 0.06, $P_{(host plant)} < 0.001$, $P_{(c*h)} < 0.41$. The significant interaction for Pupal mass was due to Norwegian individuals growing larger on *U. dioica* (cf. Table 1).

Sweden versus England and Spain in 2002

In this experiment relative survival on the two host plants differed between stock, as a significant interaction was found: GLM ANOVA $n_{(families*host plants)}$ = 16, $P_{(country)}$ = 0.06, $P_{(host plant)} < 0.05$, $P_{(c*h)} < 0.05$. A Tukey HSD post hoc test shows that this was mainly due to lower survival on *S. caprea* in English stock compared to Swedish, but the difference between Spanish and Swedish stock on this plant was similar (cf. Table 1). Furthermore, the latter result was repeated in the 2007 replicate experiment performed earlier in the summer (Fig. 2b; GLM ANOVA; $n_{(families*host plants)}$ = 26, $P_{(country)}$ = 0.29, $P_{(host plant)}$ = 0.72, $P_{(country*host plant)} < 0.05$).

It can be noted from Table 1 that the butterflies from England and Spain also tended to show poorer performance in other respects on *S. caprea* than Swedish butterflies, with longer development time, slower growth and (for English stock) lower mass. Such differences were not evident when larvae were reared on the preferred host for both populations, *U. dioica* (Table 1). However, the GLM ANOVA for all three populations together (or in pairwise comparisons; not shown) do not show evidence of the country * host plant interactions that these patterns would indicate, with the possible exception of pupal mass ($n_{(families)}$ = 9). Development time: $P_{(family)}$ = 0.06, $P_{(sex)}$ = 0.20, $P_{(country)}$ = 0.01, $P_{(host plant)} < 0.001$, $P_{(c*h)}$ = 0.52. Pupal mass: $P_{(family)} < 0.001$, $P_{(sex)}$ = 0.70,

Table 1 Larval performance on *U. dioica* (U) or *S. caprea* (S) in stock from different populations of *P. c-album* reared in common-garden experiments

Trait	Host	Population	N	Mean	±SE	Population	N	Mean	±SE
Time	U	Norway	6	19.7	0.25	Sweden	14	20.5	0.38
	S		4	28.4	1.6		9	27.8	0.83
Mass	U		6	299	7.3		14	277	5.6
	S		4	269	6.3		9	274	8.4
Rate	U		6	0.29	0.004		14	0.28	0.006
	S		4	0.20	0.013		9	0.20	0.006
Survival	U		6	0.77	0.08		10	0.59	0.06
	S		6	0.27	0.1		10	0.23	0.07
Time	U	England	2	22.6	0.17	Sweden	5	21.3	0.46
	S		2	30.3	0.75		3	27.3	0.38
Mass	U		2	339	16		5	320	9.0
	S		2	288	6.9		3	314	6.3
Rate	U		2	0.26	0.0003		5	0.27	0.006
	S		2	0.19	0.004		3	0.21	0.003
Survival	U		2	0.66	0.27		6	0.64	0.09
	S		2	0.25	0.15		6	0.79	0.05
Time	U	Spain	4	21.8	0.28	Sweden	5	21.3	0.46
	S		3	31.7	3.7		3	27.3	0.38
Mass	U		4	331	7.1		5	320	9.0
	S		3	325	22		3	314	6.3
Rate	U		4	0.27	0.003		5	0.27	0.006
	S		3	0.19	0.02		3	0.21	0.003
Survival	U		4	0.88	0.03		6	0.64	0.09
	S		4	0.57	0.06		6	0.79	0.05
Time	U	Spain	4	21.8	0.28	England	2	22.6	0.17
	S		3	31.7	3.7		2	30.3	0.75
Mass	U		4	331	7.1		2	339	16
	S		3	325	22		2	288	6.9
Rate	U		4	0.27	0.003		2	0.26	0.0003
	S		3	0.19	0.02		2	0.19	0.004
Survival	U		4	0.88	0.03		2	0.66	0.27
	S		4	0.57	0.06		2	0.25	0.15
Time	U	Belgium	4	20.7	0.24	Sweden	3	20.4	0.12
	S		3	27.9	0.59		4	26.4	0.43
Mass	U		4	288	9.5		4	296	8.9
	S		3	270	6.2		4	272	16
Rate	U		4	0.27	0.003		3	0.28	0.003
	S		3	0.20	0.004		4	0.21	0.005
Survival	U		4	0.63	0.12		4	0.77	0. 02
	S		4	0.40	0.14		4	0.67	0.11

Studied populations: Trondheim (Norway), Oxford (England), Antwerp (Belgium) and Can Liro (Spain). *N* = number of families (number of individuals >500 for each comparison). Traits compared are development time (days), pupal mass (mg), growth rate (ln(mass)/days) and survival (proportion of larvae surviving until pupation). Trait values are averages of family averages, with standard error

$P_{(country)} = 0.09$, $P_{(host\ plant)} < 0.001$, $P_{(c*h)} < 0.05$. Growth rate: $P_{(family)} = 0.054$, $P_{(sex)} = 0.21$, $P_{(country)} < 0.01$, $P_{(host\ plant)} = 0.05$, $P_{(c*h)} = 0.38$. A Tukey HSD post hoc test suggests that the significant interaction for pupal mass is mostly due to differences between England and Spain.

Sweden versus Belgium in 2003

The general pattern of survival in the comparison between Belgian and Swedish stock was a slightly lower survival for Belgian stock on both hosts (not significant) and for larvae reared on *S. caprea* in both populations (Table 1), but no significant interaction between the two factors: GLM ANOVA: $n_{(families*host\ plant)} = 16$, $P_{(country)} = 0.059$, $P_{(host\ plant)} < 0.001$, $P_{(c*h)} = 0.60$).

Analysis of other performance traits (GLM ANOVA, $n_{(families)} = 6$) indicates that responses to host plants in the populations may however differ in other performance traits: Development time: $P_{(family)} < 0.05$, $P_{(sex)} = 0.85$, $P_{(country)} < 0.01$, $P_{(host\ plant)} < 0.001$, $P_{(c*h)} < 0.01$. Pupal mass: $P_{(family)} < 0.001$, $P_{(sex)} = 0.54$, $P_{(country)} = 0.15$, $P_{(host\ plant)} < 0.01$, $P_{(c*h)} = 0.72$. Growth rate: $P_{(family)} < 0.01$, $P_{(sex)} = 0.62$, $P_{(country)} < 0.01$, $P_{(host\ plant)} = 0.70$, $P_{(c*h)} < 0.05$. Tukey HSD post hoc tests suggest that the reason for the significant interactions is that the Belgian stock has relatively longer development time and relatively lower growth rate on *S. caprea*.
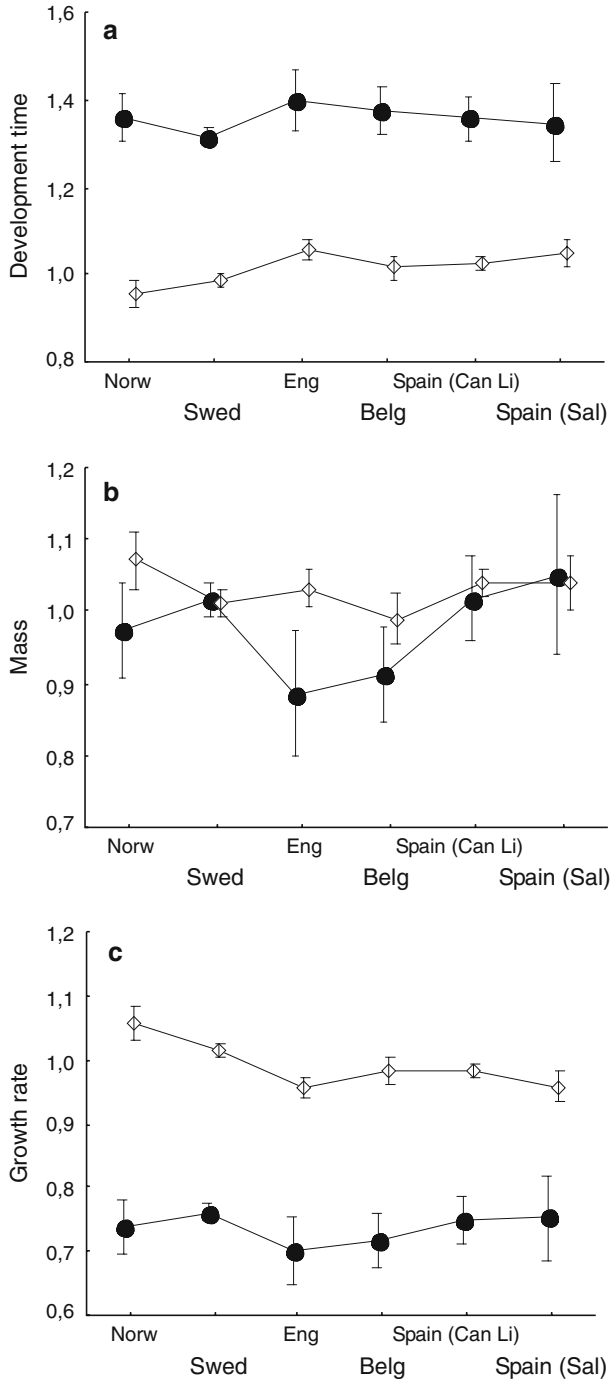
Combined analyses

We also performed analyses where all stock from univoltine areas (Norway, Sweden) were pooled, and all from potentially multivoltine areas (Belgium, England, Spain), with a factor Generations included in GLM ANOVAs instead of Country. This did not add much to the results of the separate analyses, in that for all four performance traits there were weak effects of Sex, highly significant effects of Host plant and Generations, but no significant interactions between the two (not shown). Nevertheless, the fact that in the separate analyses reported above there were consistent trends towards poorer relative performance on *S. caprea* in stock from potentially multivoltine areas indicates that significant interactions of this type might well be found in future studies, in experimental designs with more statistical power.

With the exception of survival, such interactions seem to be rather subtle, however. For the other performance measures, trait values were similar across populations when compared in common-garden experiments (Table 1) and even differed in the same way between host plants in each population (Fig. 3).

Effects of plant phenology on performance

The subject of the plant phenology study was the Swedish (Stockholm) population, for which data was available from families that had been split between the two host plants and reared at various times in the spring and summer. A GLM ANOVA ($n = 58$ families) on larval development time showed weak effect of Sex ($P < 0.05$), strong effects of Family ($P < 0.001$), Host plant ($P < 0.001$), Start date ($P < 0.001$) and, importantly, the interaction between Host plant and Start date ($P < 0.001$). Similarly for pupal mass: Sex ($P = 0.69$), Family ($P < 0.001$), Host plant ($P < 0.001$), Start date ($P = 0.67$), Host plant * Start date ($P < 0.001$); and for growth rate: Sex ($P < 0.05$), Family ($P < 0.001$), Host plant

($P < 0.001$), Start date ($P < 0.001$), Host plant * Start date ($P < 0.001$). Thus, the quality of the host plants changed over the season, but in different ways for the two plants. This is best illustrated by the following regression analyses.

In this Swedish population, life history differences between sexes are small and inconsistent regarding direction, if present at all (Nylin 1992). Also in the present study life history differences between sexes were very slight, especially compared to effects of host plant and date in the season (see above). The regression analyses below were first performed separately for each sex, but the results were close to identical between sexes. Hence the sexes were subsequently pooled in the analyses so that also families with only one sex represented could be included, again with very similar results.

Relative time spent in the larval stage on *S. caprea* compared with siblings reared on *U. dioica* increased significantly over the year (Fig. 4a; regression, $n = 61$ split families, $P \ll 0.001$, $r^2 = 66\%$).

The relative mass of butterflies raised on *S. caprea* compared to *U. dioica* decreased significantly over the year (Fig. 4b; $n = 65$ split families, $P \ll 0.001$, $r^2 = 31\%$). Initially larvae reared on *S. caprea* on the average reached higher masses than the *U. dioica* larvae (cf. Janz et al. 1994), but the opposite was true later in the season.

The relative growth rates were similar on the two host plants in the beginning of the summer (Fig. 4c; close to 1.0) but then decreased relatively more on *S. caprea* during the summer, producing a negative slope of the regression ($n = 61$ split families, $P \ll 0.001$, $r^2 = 69\%$).
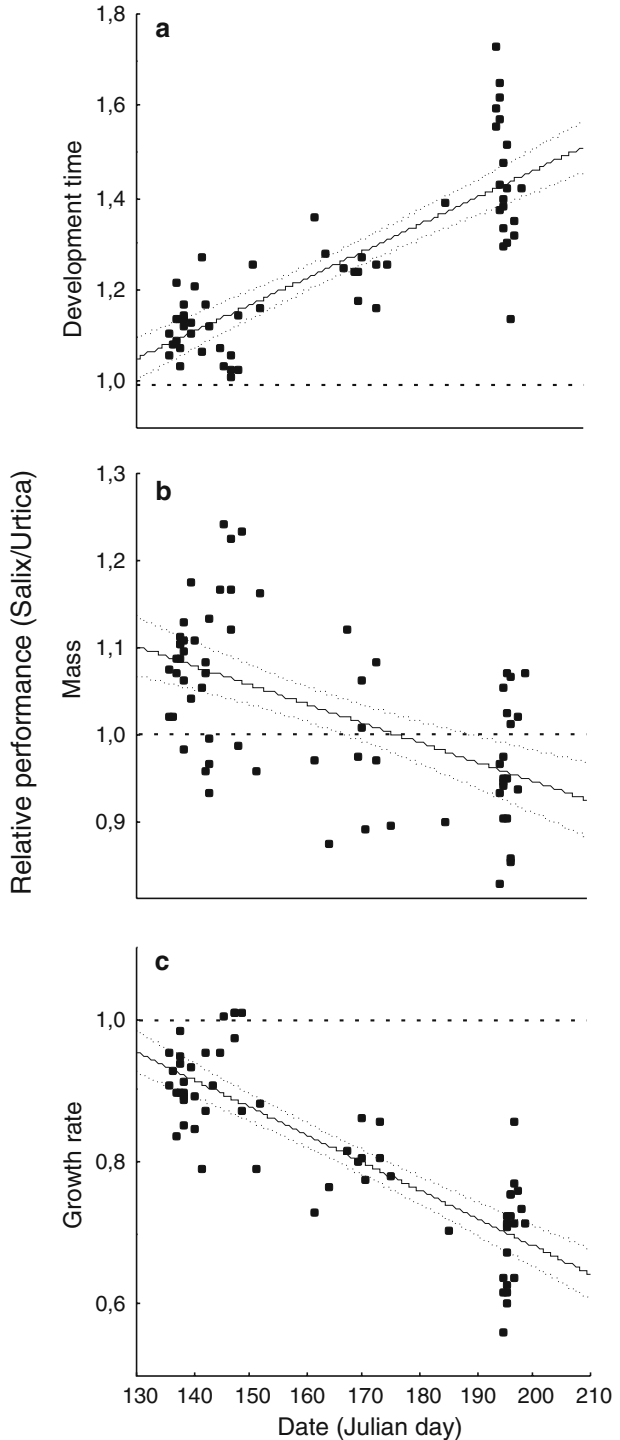
It is of great interest to relate the performance studies reported in the previous section to this information on the effects of plant phenology on larval life history. This can be done by noting the actual start dates for each of the experiments reported in Fig. 3, and use them to plot the relative performance values (*S. caprea*/*U. dioica*) onto the graphs of the effects of plant phenology (Fig. 4). If this is done (not shown), all these points fall on or close to the regression lines, except for pupal mass of English stock (at $x \approx 170$, $y \approx 0.85$ in Fig. 4b). This indicates that stock from all populations responded similarly not only to the two host plants (Fig. 3) but also to the phenological variation in host plant quality.

Discussion

What is the role of time constraints in determining geographical variation in the resource use of organisms? One hypothesis concerning phytophagous insects predicts an increasing degree of specialization on the "fast" host plants in locally time-stressed populations—i.e. those with partial generations or with little time for even a single generation (Nylin 1988; Scriber and Lederhouse 1992). Based on this idea, females of the partially bivoltine English population were initially predicted to be more specialized on *U. dioica* and its herbaceous relatives than females of the univoltine Swedish population (Nylin 1988), and this was also found to be the case (Nylin 1988; Janz 1998). A further prediction was that directly developing light morph females should be more specialized than dark morph females, because the former category is always part of a bi- or multivoltine life cycle and will more often be under time stress (Nylin 1988). This prediction has also been repeatedly corroborated (e.g. Nylin 1988 and present study). Hence, time constraints have been seen as potentially explaining most of the variation in host plant range in *P. c-album* (e.g. Janz et al. 1994).

However, in its straightforward version the hypothesis is based on the implicit assumption that the relative quality of different hosts is similar across geographical

Fig. 4 (**a**) Larval development time, (**b**) pupal mass and (**c**) larval growth rate on *S. caprea* (relative to *U. dioica*) in families of the Swedish population of *P. c-album* when larvae were reared at different times in the season, and broods split between the two host plants. The average start date (in Julian days) for each family is given on the *x*-axis, and the average life history value for each family on *S. caprea*—divided by the average on *U. dioica*—is given on the *y*-axis. The values obtained on *U. dioica* in each family can thus be found at 1.0 on the *y*-axis (dotted horizontal line). Figures show regression slopes and the 95% confidence intervals

localities as well as over the season. We show here that the first assumption more or less holds for the two investigated hosts of the comma butterfly, but the second does not, because the relative quality of *S. caprea* as host plant compared to *U. dioica* is much poorer later in the summer. Obviously this fact provides a simpler explanation for the seasonal trend towards increasing specialization on *U. dioica* and its relatives later in summer, especially since this trend is seen both between spring and summer generations (e.g. Figs. 1c, 2a), between different summer generations (e.g. Fig. 1a–c) and even within each generation (Nylin et al. 2005). Moreover, the phenological data also provides an alternative explanation for the geographical trends: in southern populations there should be strong selection in the summer for specialization on herbaceous host plants, because they are the only ones on which larval performance is still good at the late time in the season when the second and third generations of *P. c-album* occur. Thus, the major geographical pattern of host use in the comma butterfly seems to be a division between northern univoltine populations and southern potentially multivoltine populations, rather than a saw-tooth pattern.

We still do see a role for time constraints in explaining levels of host plant specialization in *P. c-album*, and they may be of particularly great importance in transition areas between univoltine and bivoltine populations. A broader host plant range will be easier to achieve in time-relaxed populations such as the Swedish univoltine population (Janz et al. 1994) where using slow hosts does not necessarily decrease fitness, and in transition areas there is a potential for the differences in voltinism among genotypes to contribute to disruptive selection in host plant utilization and other traits that affect development time (Burke et al. 2005). This process could even result in speciation (Nygren et al. 2006).

It should be noted that the results presented here must be seen as tentative when it comes to conclusively establishing the characteristics of particular populations of the comma butterfly in the field. The preference results should thus only be seen as parts of the overall test of the generality of the time constraints hypothesis, and the performance results mainly as an exploration of patterns that should be corroborated in future studies. Although relative larval performance on the two hosts was in general strikingly similar across populations, populations may differ in a few cases, and interestingly local host plant preference correlated with larval performance in some respects. The two populations with the lowest preference for *S. caprea* (England and Spain) also had the lowest survival on this host plant, and perhaps also poorer growth performance. A tempting but preliminary interpretation is that these are examples of a trade-off between performance abilities on the two host plants, with English, Spanish and, to some extent, Belgian populations accumulating performance genes fitting their local host plant preference to the exclusion of genes better fitting other alternative hosts (Futuyma and Moreno 1988; Joshi and Thompson 1995; Via and Hawthorne 2005). This will be the subject of future more exhaustive studies.

Returning to preference, available field observations suggest that English (Asher et al. 2001) and Spanish (C. Stefanescu, pers. observ.) populations rarely if ever use hosts in *Salix*, *Ribes* or *Betula*. Our results from the spring of 2007 with similar preference shown by Swedish and Spanish females (Fig. 2a) were thus unexpected, and they could indicate that *S. caprea* is more important as a spring host in Spain than previously thought. There is however also a possibility that the result is a cage artifact, if Spanish females do not often approach *S. caprea* when searching for oviposition sites in the field. In any case a clear difference was seen in the next generation, where Spanish females rejected *S. caprea*, and it now seems probable that the demonstrated genetic preference differences between populations of the comma butterfly (Janz 1998; Nygren et al. 2006) are shaped by the degree to which females are repelled by the characteristics of mature leaves of trees and

bushes. Such findings are of interest with regards to the suggestion that the role of habitat avoidance (in contrast to attractant traits) in shaping the preference hierarchies of phytophagous insects has been underestimated. This possibility may in turn have important consequences for the genetics of differentiation and speciation (Forbes et al. 2005; Feder and Forbes 2007).

In the present investigation the only clearly time-relaxed population was the one from Sweden, with ample time for its single generation. The observed higher degree of specialization on *U. dioica* in the English and Spanish populations is in line with the time-constraints hypothesis, as they could all benefit from only using "fast" hosts in order to achieve additional generations. Results from the Belgian population are equivocal in that this stock originates from a latitude similar to the English one, and apparently shows similar partial voltinism (Maes and Van Dyck 1999), but is less specialized. This may indicate that the Belgian population is more affected by gene flow from northern univoltine areas. Finally we found no evidence that the Norwegian females, originating from an area where females should be severely stressed for time to complete a single generation, show an increased degree of specialization on the fast host plant *U. dioica*.

This last result points to an issue of interest for future studies that aim to further elucidate the role of time constraints for populations of phytophagous insects. Specialization on the "fastest" hosts in populations at the limit of having time for a single generation has indeed been found for populations of the *Papilio glaucus/P. canadensis* species group of butterflies in USA (Scriber 2002). That we did not find this pattern in the Norwegian population of *P. c-album* could be because it is less severely time-constrained than assumed by us, but another strong possibility is that time constraints are indeed present but that they instead affect oviposition directly. Females may have a faster oviposition rate if they have a broader host plant range, in particular if they then include locally abundant plants (Courtney 1982; Janz et al. 2005; Doak et al. 2006). Springtime in the Trondheim area has a high proportion of cloudy and rainy days, limiting the time available for oviposition, and *S. caprea* is a common plant in the region, with a wider distribution than *U. dioica* (R. Krogen, pers. comm.). A more rapid oviposition also means more time left of the summer for the larvae to grow, and this may be enough to counterbalance the loss of time due to development on a "slow" host plant.

In conclusion, time constraints may be important for local host plant range in phytophagous insects, but they can interact with temporal host quality variation in several ways. In insects that (unlike *P. c-album*) have to rely entirely on hosts that decline strongly in quality over the season, or on ephemeral flowers or fruits rather than leaves, such phenological effects may even by themselves cause the most important time constraint so that the length of the favorable season is set by the host plants rather than by the local climate. This is most clearly observed in "flush-feeding" insects, where only spring leaves are suitable for growth (e.g. Ayres and MacLean 1987). Interestingly, in a recent phylogenetic comparative study it was observed that butterflies feeding on the foliage of woody plants generally have fewer generations per year (Cizek et al. 2006), in line with the results of the present study. Phenological variation in absolute and relative host plant quality presents a severe challenge to the development of theory on geographical variation in host plant range, and so do the integration of effects of selection on adult oviposition rates rather than on offspring performance. These challenges have to be met, however, before we can hope to understand patterns in the niche width of phytophagous insects and how they connect to life history evolution, climate-driven shifts in distribution and to speciation in these important model taxa.

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