
5

Seasonal Plasticity, Host Plants, and the Origin of Butterfly Biodiversity

SÖREN NYLIN, KARL GOTTHARD AND GEORG H. NYGREN

Department of Zoology, Stockholm University, Stockholm, Sweden

1. Introduction

Phenotypic plasticity is the term used when different phenotypes can be produced by the same genotype, in different environments. For many years after the Modern Synthesis, research into this area was largely neglected in favour of strictly genetically determined variation, presumably because of the 'Lamarckian' overtones when dealing with environmentally induced traits (Shapiro, 1976; Sultan, 2003). More recently, however, there has been a renewed interest in plasticity (see e.g. Gotthard and Nylin, 1995; Pigliucci, 2001, West-Eberhard, 2003), following an increasing realization that the dichotomy between genetic and plastic variation is false. Instead genotypes have 'norms of reaction', a repertoire of potential phenotypes that are expressed according to environment, and as a result every phenotype is the result of a genotype-by-environment interaction – phenotypic plasticity (Stearns, 1989; Sultan, 2003). Conversely, plasticity is as much a product of evolutionary processes as genetic differentiation, and adaptive plasticity is shaped by natural selection among genotypes with different reaction norms (Gotthard and Nylin, 1995).

The phenomenon of 'counter-gradient variation' is a good illustration of how genes and the environment interact (Conover and Schultz, 1995). Evidence of local adaptation typically consists of phenotypic differences between environments, but, because genotypes are plastic in their phenotypic expression, natural selection can also result in phenotypic similarity. Selection can favour genotypes with reaction norms that oppose maladaptive effects of the environment. For instance, populations from cold and warm environments may have similar developmental rates in the field, but when they are reared in a common environment (a 'common garden' experiment) individuals of the cold-environment population may grow and develop faster, revealing that the populations are in fact locally adapted. Genetics and the environment thus

interact in the truest sense of the word, and for this reason we cannot understand the genetics of evolution without understanding plasticity (Nylin and Gotthard, 1998).

Phenotypic plasticity can be of several different kinds, corresponding to the degree of flexibility in the display of phenotypes. Although the boundaries are not sharp, three types can be recognized.

1. 'Developmental' plasticity is when a certain developmental pathway is taken according to the environment experienced. These environments are known as 'environmental cues' in the case of plasticity which is thought to represent adaptation to variation in the environment. Because of the cascading effects in ontogeny of going down one such pathway and not the other this is often an irreversible process, of particular importance in short-lived organisms such as insects.
2. Vertebrates and other organisms with lives that span more than a year often more conspicuously show reversible plasticity (e.g. birds and mammals that go white in the winter), also known as 'phenotypic flexibility' (Piersma and Lindstrom, 1997).
3. Finally, when the flexibility of the phenotype increases even more, this grades into 'behaviour'. Although behaviour is usually seen as a field of study separate from plasticity, there is no absolute distinction. Moreover, plasticity is often mediated by behaviour. For instance, plastic growth rates may be due to variations in feeding behaviour and plastic clutch size to variation in oviposition behaviour. Not surprisingly, the methods of behavioural ecology, i.e. predictions from optimality followed by experiments or comparative studies, can be fruitfully applied also to plasticity (Nylin, 1994).

For most insects, not the least in temperate areas where the winter season is wholly unfavourable for growth and development, 'seasonal plasticity' is very important. As noted above, in insects this often takes the form of developmental plasticity, where different phenotypes are seen according to the progress of the season and the whole life cycle is regulated by seasonal cues. There are, however, also more flexible elements of seasonal plasticity and there are many where the actual degree of flexibility is uncertain, such as growth rate regulation by external cues, or seasonal plasticity in host-plant choice.

In the following, we primarily use the comma butterfly (Nymphalidae: *Polygonia c-album* L.) and its close relatives to illustrate the ubiquitous presence of seasonal plasticity, and the optimality approach to understanding its role in the evolution of insect life-cycle regulation and host-plant preference. *P. c-album* is strongly polyphagous, with larvae feeding on at least seven different host-plant families, several of which are not closely related (Nylin, 1988; Janz *et al.*, 2001), namely Urticaceae, Ulmaceae, Cannabidaceae, Salicaceae, Grossulariaceae, Betulaceae and Corylaceae. Related species generally feed on a subset of these plant families, resulting in an interesting model group for studying the plastic effects of larval host plants in combination with other environmental factors.

We will touch upon several important features of the insect life cycle where plasticity is important: seasonal polyphenism, life-cycle regulation by photoperiod,

fine-tuning of growth and development, life-history traits, larval host plants and female host-plant preference. For the first two subjects we have included some previously unpublished data, whereas the remaining text reviews other findings from the research project, with a focus on results from the comma butterfly and other *Polygonia*. The final section links phenotypic plasticity to ecological speciation theory.

2. Materials and methods

The term 'polyphenism' is most appropriately used for discrete phenotypes resulting from plasticity, in an analogy with genetic polymorphism (Shapiro, 1976). Seasonal polyphenism, the occurrence of different phenotypic forms in different seasons, is common in insects and an example of plasticity in its most visual form. There is often a correlation with physiological plasticity in terms of developmental pathway taken: diapause or direct development. Butterflies often show striking seasonal polyphenism (Shapiro, 1976; Brakefield and Larsen, 1984; Koch, 1992) but in most cases the adaptive significance is not clear (Gotthard and Nylin, 1995; Kemp and Jones, 2001). One exception is the seasonal variation in the amount of dark pigment in pierid butterflies, which has been convincingly linked to temperature regulation (e.g. Kingsolver, 1995).

For the previously unreported results on seasonal polyphenism and life-cycle regulation in *Polygonia satyrus*, *P. gracilis zephyrus* and *P. faunus*, methods were as follows. Stock originated from females caught in Washington State, USA. As larvae hatched they were randomly divided among different photoperiod treatments (designed to elucidate the role of photoperiod in the adult stage) and raised in environmental cabinets at 20°C. There were four possible treatments (see Tables 5.1 and 5.2):

1. a short larval daylength of 15 h with adults moved to 22 h at eclosion
2. a long larval daylength of 22 h with adults moved to 15 h at eclosion
3. a constant long daylength of 22 h
4. a shift from 15 h to 22 h after 2 weeks (in the fourth larval instar) with adults retained in 22 h.

Adults were frozen 1 week after the eclosion of the last adult and, in the case of females, were later dissected and checked for mature eggs. For *P. g. zephyrus* and *P. faunus* there were only a few hatchlings available, and these were divided among treatments 1 and 4 only.

Wings were removed and taped onto OH transparencies, which were scanned using an HP ScanJet 5100C flatbed scanner. The resulting JPEG images were converted to 16-bit greyscale and the greyness (% white) measured using Corel PhotoPaint software. This was done in two places on the forewing undersides (Fig. 5.1), one of the darkest areas (the most apical section of the central symmetry system; see Nylin *et al.*, 2001) and one of the lightest (in the area between the basal and central symmetry system, immediately apical of the bend in the former).

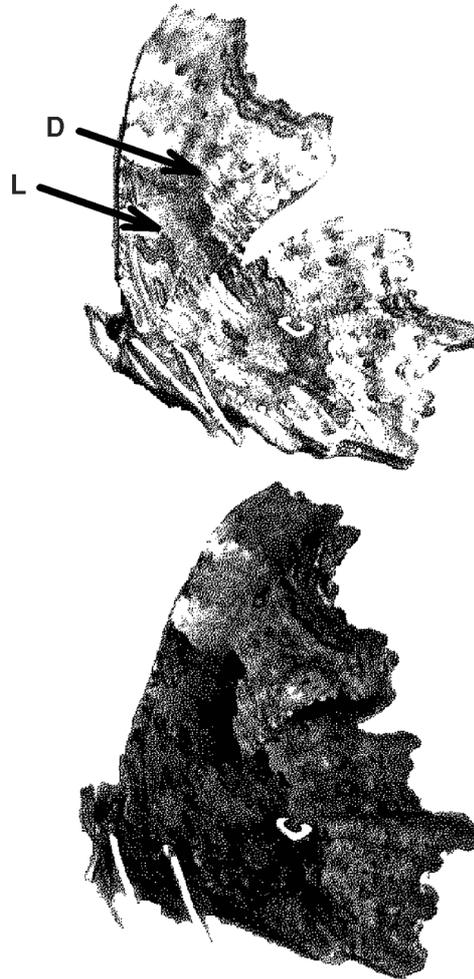


Fig. 5.1. The two seasonal forms of *Polygonia c-album*, as illustrated by two female individuals. *Top*: directly developing form; *Bottom*: hibernating form. The butterflies have been scanned and the images converted to black and white, using the same threshold for both individuals. (*D*) Dark spot measured, (*L*) light spot measured (see text).

3. Seasonal polyphenism

P. c-album is facultatively multivoltine, with two distinct seasonal forms (Fig. 5.1). Summer-form individuals have light-coloured, yellowish, wing undersides and develop directly to sexual maturation, whereas the darker autumn/spring form spends the winter in reproductive diapause before mating in the spring. Individuals of the spring form may give rise to either form in the next generation, or mixed broods of both forms, depending on the larval environment (Nylin *et al.*, 1989; Nylin, 1992). Temperature regulation seems

an unlikely explanation for this polyphenism, as the species warm up at low temperatures by using 'dorsal basking' (wings held horizontal, exposing the upper sides to the sun) and there is little variation in the dorsal wing surface. Interestingly, the North American *P. interrogationis* and *P. comma* do display dorsal polyphenism, but in this case it is the summer form that is darker (Scott, 1986), further indicating that wing pigmentation is not a major factor in *Polygonia* temperature regulation. More probably, the dark form of *P. c-album* is adapted for being cryptic during hibernation (Nylin, 1991). Evidently, the species hibernates fully exposed on trunks and roots of trees (Thomas and Lewington, 1991), where the dark colour would serve it well, coupled with its extremely cryptic appearance in many other respects (mottled colouration with relief effects, jagged outline etc.). The dark pigment melanin may also have structural importance, making wings more resistant to wear and tear.

Karlsson and Wickman (1989) investigated resource allocation patterns in the two forms of *P. c-album*, which differ dramatically in life history. Whereas the summer form has an expected adult lifespan of at most a few weeks, the hibernating form may live as an adult for up to 10 months. In accordance with predictions from optimality, Karlsson and Wickman (1989) found that the summer form allocates more nitrogen to the abdomen (and hence to reproduction) and the hibernating form more to the thorax and wings (and hence to somatic survival). It has therefore been suggested (Nylin, 1991), that the coloration of the summer form may not be adaptive *per se*, but rather in the sense of saving unnecessary costs of melanization (melanin is rich in nitrogen). In accordance with this idea, Wiklund and Tullberg (2004) have found in experiments with bird predators that the dark form is more efficient at being cryptic than the light form when presented against a 'winter' background consisting of a tree trunk, but that the light form is not superior in this respect against a 'summer' background consisting of stinging nettles.

A very similar polyphenism to that in *P. c-album* is found in the two other Palaearctic species of *Polygonia* for which information is available, namely *P. c-aureum* (Masaki *et al.*, 1989; Koch, 1992) and *P. egea* (Dal, 1981). This demonstrates that a plastic phenotype can be retained over several speciation events (Fig. 5.2). Studies on *P. c-aureum* revealed that the polyphenism is controlled by a hormone which was named 'summer-morph-producing hormone' (Masaki *et al.*, 1989), i.e. independently of hormonal control of adult reproductive diapause, despite a strong correlation. Interestingly, however, the polyphenism is lacking or not very apparent in the Nearctic species (see Scott, 1986), although neither the three Palaearctic species, nor the Nearctic species, form monophyletic groups (Nylin *et al.*, 2001; Wahlberg and Nylin, 2003). The simplest explanation would seem to be that this type of polyphenism is ancestral to the genus but was lost twice after representatives of the genus colonized the New World (Fig. 5.2). It could be speculated that this happened because colonization occurred via a northern route, so that all populations of the incipient species were univoltine for many generations and the reaction norm for expressing both seasonal phenotypes was lost.

In accordance with the phylogenetic interpretation depicted in Fig. 5.2 (polyphenism ancestral) it can be noted that both *P. gracilis zephyrus* and

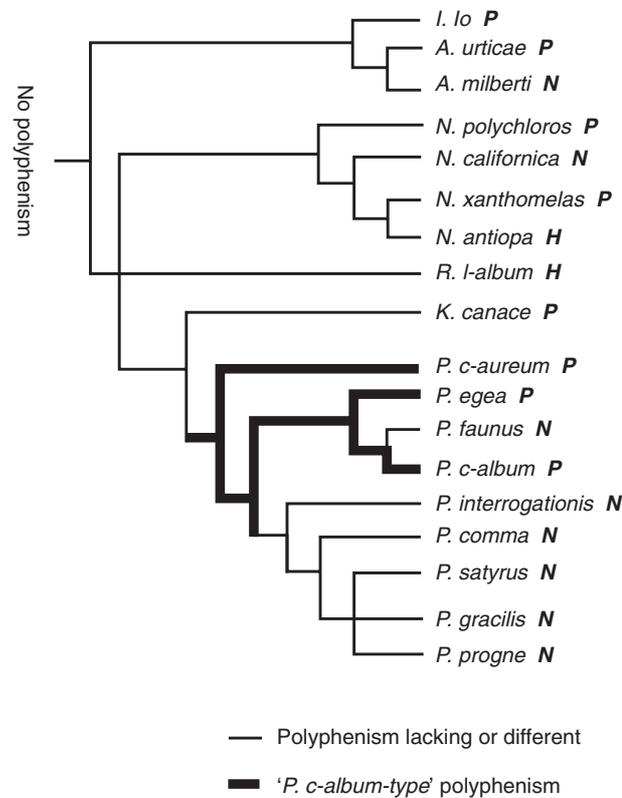


Fig. 5.2. Phylogeny of *Polygonia* and relatives, based on Nylin *et al.* (2001) and Wahlberg and Nylin (2003). Uncertain relationships depicted by *unresolved branching patterns*. *Capital letters* after species names refer to biogeographical region: P = Palaearctic, N = Nearctic, H = Holarctic. Lineages with the type of strong ventral polyphenism found in *Polygonia c-album* and two other Palaearctic species shown with *thick lines*. This trait is reconstructed as being ancestral to *Polygonia* if gains are evolutionarily less likely than losses. Traces of similar polyphenism are found also in *P. satyrus* and *P. gracilis* (see text).

(especially) *P. satyrus* show traces of the Palaearctic form of polyphenism (Table 5.1). Note that the designations of colour forms given in Table 5.1 are rather arbitrary, as the dark and light morphs produced were not as distinct as in, for instance, *P. c-album*. The clearest result was when comparing adults of *P. satyrus* resulting from larvae raised in short or increasing daylengths, respectively, with adults in long daylengths (treatments 1 and 4 in the Materials and methods section). The first treatment can be expected to produce adults initially destined for hibernation, although the reproductive diapause may subsequently be broken by the long adult daylengths. The second treatment is the only one effective in producing a majority of (light-coloured) directly developing adults in the normally univoltine Swedish population of *P. c-album* (Nylin, 1989, 1992) (see next section).

Table 5.1. Proportion of dark-form adults of *Polygonia* species in different photoperiod treatments. Sequence of photoperiod given for young larvae, older larvae/pupae, adults (see text). Sexes pooled. Number of individuals in parentheses.

Species	Photoperiod treatment			
	15–15–22h	22–22–15h	22–22–22h	15–22–22h
<i>P. satyrus</i>	100% (14)	59% (22)	86% (29)	11% (19)
<i>P. gracilis</i>	100% (6)	–	–	21% (14)
<i>P. faunus</i>	100% (8)	–	–	100% (8)

Most individuals in treatment 1 looked darker than most individuals in treatment 4, and this difference could also be quantified. The measured 'dark' area of treatment-1 individuals was on average darker than the corresponding spot in treatment-4 individuals (males 25.2% white vs. 27.1% white, $n = 8 + 8$, t -test: $P = 0.09$; females 25.0% vs. 29.7%, $n = 7 + 4$, t -test: $P < 0.05$) and the 'light' area was also darker (males 33.5% vs. 37.8%, $n = 8 + 8$, t -test: $P < 0.01$; females 31.0% vs. 38.0%, $n = 7 + 4$, t -test: $P < 0.001$). In most cases, however, the ranges in grey values overlapped slightly. This was even more the case in *P. g. zephyrus* and, because of this and the small sample size, no attempt at quantification was made. In *P. faunus*, the sister species to *P. calbum*, no difference in colour between treatments could be detected ($n = 4$ females and $n = 4$ males per treatment).

For comparison, we measured grey values in four typical adults of *P. c-album* (both forms and sexes, the females are shown in Fig. 5.1), and in these individuals the 'dark' areas differed in amount of white between forms by 5% in the males and 7% in the females, and the light areas by 24% in the males and 28% in the females. In treatments corresponding to those applied here, there were no intermediate individuals (Nylin, 1989, 1992). It is thus questionable whether the weaker polyphenism in the two studied Nearctic species is adaptive, but the fact that it can be detected demonstrates that traces of the ancestral reaction norm are still present.

4. Photoperiodic control of the life cycle

As noted in the Introduction, insects in seasonal areas, especially in the temperate zone, must regulate their life cycles in accordance with the progression of the seasons. Notably, they enter a hormonally controlled diapause in preparation for the winter, well in advance of the onset of unfavourable conditions. This is done using information from the environment, known as seasonal 'cues', of which photoperiod is the most important (Danilevskii, 1965; Tauber *et al.*, 1986). The most common response is that individuals enter diapause (in a species-specific developmental stage) after a having experienced short daylengths, signalling the approaching winter, in a preceding stage. The 'critical daylength' is the threshold value below which a majority of the individuals enter diapause.

It is often difficult to determine whether phenotypic plasticity is an adaptation to environmental variation, and not an unavoidable 'direct' effect of the environment, but plasticity in response to token cues such as photoperiod is one of the most convincing cases (Gotthard and Nylin, 1995). This is both because of the existence of physiological 'machinery' specialized for photoperiod detection and response, and because it can be demonstrated that this machinery is locally adapted. Since the amplitude of daylength variation over the year increases with increasing latitude, it can be predicted from optimality that the critical daylength should vary accordingly over the geographical range of a species (Danilevskii, 1965), and this has also repeatedly been found to be the case. In fact, one of the most convincing examples of local adaptation available in any organism is the geographical variation in photoperiodic thresholds in the pitcher-plant mosquito (Bradshaw, 1976). Such examples also provide good illustrations of how plasticity – the response to photoperiod – can be determined by adaptive genetic variation in reaction norms. Variation among families in the propensity for diapause is commonly found, for instance in *P. c-album* (Nylin, 1992), and this is the raw material for natural selection.

The comma butterfly follows the latitudinal pattern, with individuals of the English population developing directly at a larval daylength of 18 h or 20 h, whereas insects of the more northern Swedish population enter hibernation diapause (Nylin, 1989). However, a further complication is that there is also a response to the direction of change in daylength. In the Swedish population, diapause is averted by a change in the larval stage from 16 h to 18 h (Nylin, 1992) or 18 h to 20 h (Nylin, 1989), presumably signalling a date before summer solstice and time for a second generation. English insects enter diapause after a decrease from 20 h to 18 h (Nylin, 1989), i.e. well above the critical constant daylength but indicating a date after summer solstice.

Such true sensitivity to changing daylengths in diapause induction has rarely been demonstrated, and in butterflies it may be associated with the unusual habit of adult hibernation diapause, which results in eggs and larvae early in the summer. Since the same daylengths occur before and after summer solstice, they may provide conflicting information regarding the date in the season if larvae occur early enough to experience both spans of daylength (Nylin, 1989). Misinterpreting a daylength in a late larval stage means several weeks of juvenile development and adult maturation and mating, before the second generation can be initiated, and a risk of failing to complete this generation before winter. This problem can be solved by also using information on the direction of change, or in some cases by postponing the 'decision' on whether to enter diapause or not until the adult stage.

We expected polyphenic species to be relatively insensitive to adult daylengths as a cue for entering diapause or not, since they should have already 'decided' before eclosion, the decision signalled by the irreversible adult morph (Nylin, 1989). This is a potential cost of polyphenism, as it would result in some loss of developmental flexibility. Non-polyphenic species could be predicted to instead make use of the information from adult daylengths. Such a response (where adult daylengths override any cues during the larval stage) is seen in the related non-polyphenic butterfly *Aglais urticae* (Voigt, 1985).

P. gracilis showed a higher proportion of females with eggs after an increase in daylength during the larval stage, as predicted for a polyphenic species. Also as predicted, the non-polyphenic *P. faunus* did not seem to be sensitive to an increase in daylength during the larval stage (Table 5.2). However, for both of these species, sample sizes were small and missing treatments prevented a real test of the hypotheses outlined above. Concerning *P. satyrus*, short adult daylengths did not prevent egg maturation. This is as expected for a polyphenic species (Table 5.2), and as found in *P. c-album* (Nylin, 1989). However, egg maturation was not well correlated with coloration (cf. Tables 5.1 and 5.2), a further demonstration that the observed polyphenism is not necessarily adaptive in this species. Note especially that the constant long-day treatment was the most effective in inducing direct development in females (Table 5.2), but resulted in a high proportion of individuals of the dark morph.

In summary, few clear conclusions could be drawn from this small-scale experiment, but we believe that it may still serve to demonstrate that the research field of comparative studies on insect life-cycle regulation lies open for exploitation.

5. Life-cycle fine-tuning and adaptive growth rate plasticity

Once a certain main developmental pathway has been taken, other responses than diapause (or not) and morphological polyphenism may follow. In particular, the optimal development time for a directly developing insect can be expected to be shorter than that for an insect destined to enter diapause, at least in near-critical conditions (e.g. near the critical daylength). This is because the former is aiming to fit a whole extra generation of offspring into the same season, whereas the latter should accordingly have surplus time left to develop up to the stage where it can itself enter diapause. In mixed broods of *P. c-album*, for instance, development time is shorter for individuals that later eclose as the light form than for the dark form, and this is a result of both shorter larval and pupal developmental times (Nylin, 1992). Such responses may be viewed as a physiological polyphenism, and perhaps also a behavioural polyphenism if shorter larval times are a result of more pronounced larval feeding. However, it is not always clear whether differences in development

Table 5.2. Proportion of females found to contain eggs in different photoperiod treatments. Sequence of photoperiod given for young larvae, older larvae/pupae, adults (see text). Number of individuals in parentheses.

Species	Photoperiod treatment			
	15–15–22h	22–22–15h	22–22–22h	15–22–22h
<i>P. satyrus</i>	25% (4)	60% (5)	88% (8)	71% (7)
<i>P. gracilis</i>	0% (2)	–	–	88% (8)
<i>P. faunus</i>	50% (4)	–	–	50% (4)

time are purely effects of the developmental pathway taken, or whether they may to some extent be the cause, as poor environments may act as a cue for diapause induction (see Section 9).

In addition to such qualitative differences between pathways, photoperiod and other seasonal cues can also be used to fine-tune growth and development within pathways (Nylin and Gotthard, 1998). In our work, evidence of the adaptive nature of such reaction norms comes primarily from satyrine butterflies. Here, development time is progressively shorter in shorter daylengths when they can be expected to signal progressively later dates in the summer after the summer solstice, in *Pararge aegeria* (Nylin *et al.*, 1989) and *Lasiommata petropolitana* (Gotthard, 1998; Nylin *et al.*, 1996a). In related species hibernating as larvae, such as *L. maera*, the late larval stages instead occur before the summer solstice when daylengths are still on the increase, and here development time is instead shorter in longer daylengths (Gotthard *et al.*, 1999b; Nylin *et al.*, 1996a). Moreover, in the same individuals development time is, as predicted, shorter in shorter daylengths in the early larval stages, before hibernation (Gotthard *et al.*, 1999b). Finally, experiments on *L. maera* show that even the plastic growth rate response to rearing temperature is influenced by photoperiod; individuals experiencing daylengths indicating a higher degree of time-stress increase their growth rates more at higher temperatures compared with individuals under less time-stress (Gotthard *et al.*, 2000a).

In *P. c-album*, studies on the quantitative effects of photoperiod are complicated by the sensitivity to changes in daylength, but there is a clear tendency towards a gradual response, with progressively shorter development times in shorter daylengths (Nylin, 1992).

6. Life-history patterns and plasticity

Life-history traits can be defined as those that quantitatively describe the life cycles of organisms, notably juvenile development time, size at sexual maturity, egg weight, clutch size and lifespan (Nylin, 2001).

A common feature in the studies reported in the previous section is that much of the variation in larval development time is achieved by plasticity in larval growth rates, rather than by variation in final pupal or adult weight, the trade-off often assumed in life-history theory (Abrams *et al.*, 1996; Nylin and Gotthard, 1998). Evidently growth rates are typically not maximized in butterflies. This may be because there is often surplus time due to the constraint of species-specific diapause stages and because high growth rates carry costs such as higher risk of starvation and predation when feeding activity is increased (Gotthard *et al.*, 1994; Gotthard, 2000).

In an experiment designed to investigate the effects of different growth rate levels on the trade-offs between development time and final weight, *P. c-album* was used as a contrast to the presumed growth rate maximizer *Epirrita autumnata* (Tammaru *et al.*, 2004). This geometrid moth feeds in the spring on the foliage of trees, which rapidly declines in quality as food for larvae, and consequently larval growth rates are very high. The Swedish population of *P.*

c-album studied, on the other hand, has plenty of surplus time for its single generation and is known not to maximize its growth rate (Janz *et al.*, 1994). Last-instar larvae of both species were deprived of food for short periods of time. As predicted, pupal weight was little affected by this treatment in the comma butterfly, because larval development time was extended to compensate for the loss of food. In addition, compensatory growth occurred, with faster growth on the day after the starvation treatment. Surprisingly, however, very similar results were obtained from *E. autumnata*, indicating that not even this seasonally constrained species maximizes growth rates. Such universal plasticity in growth rates has profound consequences for life-history theory (Abrams *et al.*, 1996; Nylin and Gotthard, 1998).

One example is the theory on latitudinal and altitudinal patterns of size variation. It has been proposed that insects should often be expected to follow a 'saw-tooth' pattern in development time and (consequently) size (Roff, 1980, 1983). At high latitudes or altitudes the favourable season is short and the optimal development time short. Going south or to lower elevations the optimal time for growth and development becomes longer and hence adult size should increase. Such patterns of increasing size to the south have been documented in crickets (Mousseau and Roff, 1989) and other ectotherms (Mousseau, 1997), including butterflies (Nylin and Svård, 1990). However, in facultatively multivoltine species an additional generation in the same season can be added when the season becomes long enough to allow it. This effectively cuts the season into two parts and can be predicted to lead to a decrease in size, before it starts increasing again. The best-documented saw-tooth patterns in size are those found in crickets (Mousseau and Roff, 1989), whereas in butterflies they are less apparent but may well be present (Nylin and Svård, 1990).

Phenotypic plasticity has several roles in creating such geographical patterns, or in preventing them. First, the shift in generation number involves plasticity, since environmental cues are typically used to determine whether an additional generation is possible (see above). However, if the insect is sedentary enough, there may also be a genetic component, a propensity to enter diapause that is locally adapted, i.e. a greater propensity to the north or higher up a mountain than in the long-season areas. In species with strong gene flow any geographical pattern observed must instead be wholly due to plasticity. Second, plasticity determines whether trends in development time will in fact produce trends in size. Roff's theory really predicts variation in development time, and then assumes a direct relationship with adult size. One way that this could happen is by plastically adding extra larval instars when time allows, as suggested by Roff (1983). However, as we have seen, plasticity could also work to prevent the expected size patterns. In *P. c-album*, larvae of the partially bivoltine English population have shorter development times than those of the more northern and univoltine Swedish population – as predicted by saw-tooth theory – but this is achieved by higher growth rates and does not result in decreased adult size (Nylin, 1992).

Another 'classic' life-history trait is lifespan (Stearns, 1992). This trait obviously has a strong plastic component, presumably in most cases representing

non-adaptive effects of poor environments. In the speckled wood butterfly *Pararge aegeria*, for instance, levels of drought and food shortage have a strong impact on adult longevity (Gotthard *et al.*, 2000b) (see also next section for an example from *P. c-album* involving host-plant quality). Perhaps more surprisingly, a strong genetic component to variation in ageing and lifespan can also often be demonstrated, a fact that has led to considerable discussion (Stearns, 1992). One approach to explaining such variation is in terms of future reproductive success. From an optimality perspective, it does not pay to invest in a very durable soma if there is little chance of reproduction in late life. In a previous section we saw a result of this equation, with the light form of *P. c-album* investing more in current reproduction and less in a durable thorax and wings than the long-lived dark form; a demonstration that plastic variation in lifespan can sometimes be adaptive.

Extending this reasoning to different populations, with different expectations of future reproduction, we compared *P. aegeria* from Sweden and Madeira. In Sweden there is strong seasonality and populations are synchronized by winter diapause. Females mate only once and there is a strong fitness premium on males being already present when they emerge, but a low premium on long male life. On Madeira there is a relatively non-seasonal climate and female emergence is unpredictable for males over the year and *vice versa*. As predicted, there is strong protandry in Sweden (i.e. males enter the population earlier), which is lacking in Madeira (Nylin *et al.*, 1993), and virgin butterflies from Madeira are more prone to mate as soon as they meet compared with Swedish ones (Gotthard *et al.*, 1999a). Moreover, male lifespan is shorter in the Swedish population, where there is little chance of a mating once the peak of female emergence has passed (Gotthard *et al.*, 2000b). This reasoning can also be extended to species differences. *P. c-album* is a highly polyandrous species, with females mating 2–3 times on average, whereas females of the peacock butterfly *I. io* mate only once. As predicted, males of *P. c-album*, which can expect matings over a longer fraction of the summer than peacock males, live longer relative to females (Wiklund *et al.*, 2003).

7. Responses to host-plant quality

For phytophagous insects, the larval host plant constitutes a very important environmental variable, but plastic responses by larvae to host-plant quality are typically not reported as part of the literature on plasticity but rather in the context of insect–plant associations. However, one influential genetic model of the evolution of phenotypic plasticity used examples framed in terms of an insect utilizing one or several host plants (Via and Lande, 1985), which shows the close connection.

Since plants vary in their properties, we can also expect them to vary in quality as host plants. Most often we are concerned with variation among plant species, but sometimes the variation among plant individuals may be greater than among species (Singer and Lee, 2000). What, exactly, is host-plant ‘quality’? This is a complicated subject, but it should at least be noted that there

are two different aspects of quality that cannot always be separated. One is the 'objective' quality, i.e. the amount of resources contained in the plant, the water content and the amount of material that is hard or impossible to digest, or even toxic. For instance, high nitrogen levels, high water content and low leaf toughness in a plant equals high quality for many insects, unless specific chemistry prevents use of the plant. The other aspect is the 'subjective' quality for the particular insect, taking into account its specific adaptations (or fortunate chance properties) for utilizing the particular resources in the plant, metabolizing toxins and perhaps even sequestering them for its own use. Since one insect's toxin may be another insect's resource, host-plant quality can more unequivocally be defined in terms of a specific insect's 'performance' on that plant.

Performance, in turn, is measured as the fitness of offspring reared on the plant (relative to other potential hosts) summed over the whole life cycle. That is, ideally not only survival, larval development time and final mass (and hence average growth rate) should be measured, but also male and female reproductive success (Nylin *et al.*, 1996b). In the case of the polyphagous comma butterfly, the preferred host plants (the stinging nettle *Urtica dioica* and the hop *Humulus lupulus*) rank highly in terms of most of these fitness correlates. Survival is higher, development time shorter, and growth rate higher than on alternative hosts, which are bushes and trees (Nylin, 1988; Janz *et al.*, 1994; Nylin *et al.*, 1996b). This can probably to some extent be explained in terms of 'objective' quality, as the herb *U. dioica* has higher water and nitrogen contents than the alternative hosts (N. Wedell, N. Janz and S. Nylin, unpublished data; *H. lupulus* not measured). However, a more 'subjective' aspect of quality is also likely to be involved, since larvae also perform well on other (preferred) hosts in the order Urticales with lower levels of nitrogen and water, including the tree *Ulmus glabra*. It seems probable that such phylogenetically constrained patterns of responses to host plants have a historical component, and indeed the tribe Nymphalini has a long history of association with this plant order (Janz *et al.*, 2001).

When it comes to adult fitness components, the high nitrogen content of stinging nettles adds another bonus for utilizing this plant as host. Males reared on nettles can produce spermatophores that are richer in nitrogen than males reared on, for instance, willow *Salix caprea* (Wedell, 1996; N. Wedell, N. Janz and S. Nylin, unpublished data). This nitrogen is transferred to the females, and it has been shown by radioactive labelling that it is incorporated into eggs (Wedell, 1996). Furthermore, females mated to nettle-males can increase their own reproductive effort but still live longer than females mated to willow-males, and would probably have higher lifetime fecundity if allowed to remate at will (Wedell, 1996). Most aspects of performance that have been measured are poorer on other host plants than on nettle, but larvae reared on willow pupate at higher weights (after a longer time) and eclose as larger adults, and are also more fecund in the case of females (Janz *et al.*, 1994; Nylin *et al.*, 1996b).

8. Performance and the evolution of host-plant preference

The subject of host-plant preference (in insects where the ovipositing females make the choice of host for their offspring) is traditionally even less tied to plasticity theory than that of larval performance, but since preference and performance are closely connected themes (Thompson, 1988), and 'performance' is just another word for plastic responses to host plants, the preference-plasticity connection is equally strong. To the extent that plant preference is determined by performance on different host plants, it can be said to be determined by reaction norms of offspring, in response to host plants selected by the females. Preference and performance are not always well correlated (Thompson, 1988), but a search for such a correlation is a good starting point in attempting to understand preference. If it is not found, this may be because the most relevant fitness correlates have not been measured, because field characteristics of performance (e.g. predation) are of crucial importance for the preference patterns, because realized fecundity is more important than careful selection of hosts in the study species, or because the insect is in fact not behaving optimally (Nylin *et al.*, 1996b). If a good correlation is found, there is reason to assume that females have been selected to prefer the plants of highest 'quality' in terms of offspring performance. In *P. c-album* there is a good general agreement between preference and performance (Nylin, 1988; Janz *et al.*, 1994; Nylin *et al.*, 1996b).

Phytophagous insects are often specialized on one or a few host plant species (or genera, or families) (Fig. 5.3; Thompson, 1994), and the reason (or reasons) for this pattern has generated much discussion (Strong, 1988). The traditional explanation is in terms of the 'jack-of-all-trades is a master of none' idea (Pianka, 1983), a general ecological principle which makes intuitive sense. Since plants vary in their properties, optimal performance should not be possible on more than one type of plant simultaneously, and hence the population should specialize to improve performance. But exactly how does this principle apply (if it does)? In the language of plasticity theory there may be a 'negative genetic covariance' across environments (Via and Lande, 1985). That is, a genotype's performance on one plant is not independent of its performance on another plant, but instead they are negatively correlated, perhaps because of physiological trade-offs when larvae are metabolizing plant chemistry. Such negative covariance can have the effect that adaptation for good performance occurs most rapidly to the most frequently used host plant, whereas adaptation to perform well on more rarely used plants is greatly slowed down (Via and Lande, 1985). Ovipositing females which to a higher degree prefer the already most frequently used host may then in the meantime be selectively favoured, and hence specialization occurs.

Physiological trade-offs have seldom been demonstrated from genetic evidence; in fact genetic covariance in performance among hosts is more often positive rather than negative, reflecting, for example, generally higher growth rates in some families of offspring (Fox and Caldwell, 1994). This has led interest to shift instead to other aspects of the 'jack-of-all-trades' principle, such as faster and more accurate host-plant choice in specialists (Fox and Lalonde,

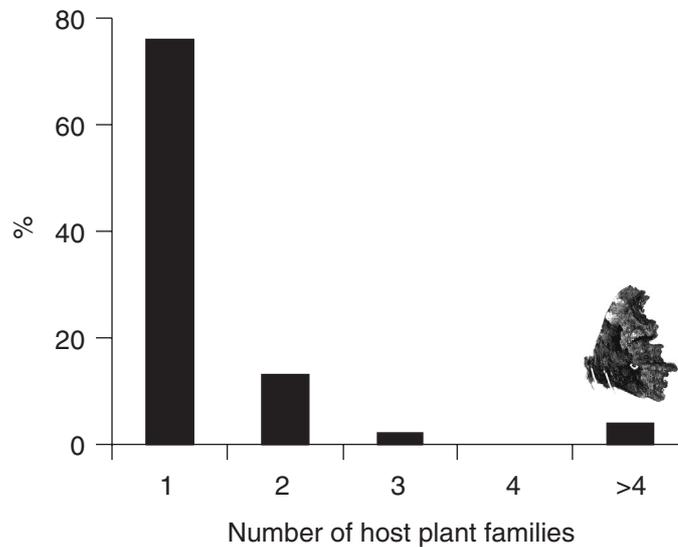


Fig. 5.3. The proportion of Swedish butterflies utilizing one, two, three, four or more than four plant families as larval host plants. *Polygonia c-album* belongs to the last category. Data from sources given in Janz and Nylin (1998).

1993; Bernays and Wcislo, 1994; Janz and Nylin, 1997; Nylin *et al.*, 2000). However, it has been suggested that we can expect to see negative covariance for performance among genotypes only under rather restricted conditions, in populations adapted to use more than one host, close to the genetic equilibrium (Joshi and Thompson, 1995). In other words, negative covariance patterns are evolved traits of generalist populations, as has been experimentally demonstrated using *Drosophila* (Joshi and Thompson, 1997). Consistent with this idea, Ballabeni *et al.* (2003) found positive covariance for performance on two potential hosts in populations of the sedentary beetle *Oreina elongata* that normally use a single host plant, but a lack of covariance among hosts in a two-host population. This population may then be on its way to evolving genotypes that are differentially adapted to showing good performance on one or the other host plant. Clearly, this more restricted view of when to expect to see trade-offs means that the principle can also be less generally applied to the evolution of specialization.

Trade-offs or not, however, the universal existence of performance hierarchies can hardly be denied. Whenever a phytophagous insect has the choice of more than one potential host plant, performance is bound to be better on some of these plants, if all fitness components are summed together. In the case of the comma butterfly, overall performance is good on the hosts in the plant order Urticales (especially *Urtica dioica* and *Humulus lupulus*), intermediate on the hosts in the genera *Salix* and *Ribes*, and poor on hosts in the genera *Betula* and *Corylus* (Nylin, 1988; Janz *et al.*, 1994; Nylin *et al.*, 1996). Other *Polygonia*

species, such as *P. gracilis*, instead do better on *Ribes* than on *Urtica* (Janz *et al.*, 2001). It is hard to see why such patterns should exist, unless it is because it is difficult to perform equally well on several plant species, especially when they are distantly related and consequently chemically different. Perhaps, then, the most universal reason for specialization is simply that it is best to specialize on the plant (or set of similar plants) where overall performance is best (Nylin and Janz, 1999). One could instead fruitfully ask why there are any generalists at all, and look for explanations in terms of higher realized fecundity or spreading of risks in variable environments.

9. Host plants, other environmental factors and life-cycle regulation

The strong effects of host plants in terms of larval growth rate and development time provide parallels to the sections above on insect life-cycle regulation and fine-tuning by photoperiod. Besides photoperiod, temperature and host plant quality also commonly affect the propensity to enter diapause, and in addition there may be sexual differences. One suggested adaptive interpretation of such patterns is that they arise because the optimal development time under direct development is typically shorter than when the insect is destined for diapause (because in the former case it is aiming to fit an additional generation into the season), and hence optimal growth rates are also typically higher. Low growth rates, and any environmental factors constraining growth rates to be low, may then have been selected to act as cues for diapause development (Wedell *et al.*, 1997; Nylin and Gotthard, 1998).

In several studied butterflies, male and female reaction norms differ in that males are more prone to enter diapause than females are (Wiklund *et al.*, 1992; Nylin *et al.*, 1995; Wedell *et al.*, 1997). This may relate to selection for protandry (Wiklund and Fagerström, 1977). At least in species hibernating as pupae or adults, protandry is achieved by different mechanisms in directly developing generations compared with generations that enter diapause. Protandry in the spring after hibernation diapause can probably be achieved without costs, because males and females have been synchronized by diapause and males simply need to emerge earlier. In order to achieve protandry under direct development, however, males need a shorter development time, which can only be achieved at the cost of either lower pupal weight or higher growth rate. Possibly, males 'choose' diapause at near-critical conditions when females are still developing directly, rather than taking on these costs (Wiklund *et al.*, 1992).

Why does low temperature often increase diapause propensity (Danilevskii, 1965)? Doubtless, temperature often acts as an additional seasonal cue, with low temperatures signalling a late date. Danilevskii (1965) strongly favoured this view, and dismissed the earlier notion that diapause is induced by conditions unfavourable for growth as 'mistaken'. At the time of publication of Danilevskii's seminal book, it was important to establish that diapause is not a consequence of unfavourable conditions, but instead a state that the insect

enters in advance of poor conditions in order to survive them. However, there may have been a sense in which the early entomologists were correct. Low temperatures are relatively poor seasonal cues, but they do indicate that high growth rates are costly, at least now and perhaps also in the future. This may tip the balance towards diapause development as the optimal pathway in near-critical conditions (Wedell *et al.*, 1997; Nylin and Gotthard, 1998).

The same type of reasoning holds for host plants: they strongly affect performance and (since the plant is unlikely to change, at least in the butterfly case) is also a good cue for predicting future conditions. Specifically, high growth rates (optimal under direct development) are likely to be costly on poor host plants. Perhaps not surprisingly, effects of host-plant quality on the propensity to enter diapause is often found in the rare cases when this is explicitly investigated, as in *Choristoneura rosaceana* (Lepidoptera: Tortricidae) (Hunter and McNeil, 1997). In the case of the comma butterfly, significant effects of sex, temperature, larval growth rate and host plant, besides photoperiod, were found on the propensity to enter diapause (Wedell *et al.*, 1997). The effect of host plant paralleled the performance hierarchy (highest incidence of direct development on stinging nettle, lowest on birch) and remained after statistically controlling for the effect of larval growth rate itself, suggesting that the plant is truly used as a cue for future conditions (Wedell *et al.*, 1997).

Within developmental pathways, the choice of host plant can act to fine-tune development time. From optimality, it may be predicted that plants permitting fast growth and short development time should be used when the optimal development time is short. Hence, these 'faster' hosts should be used later in the season. In the comma butterfly, the directly developing generation of adults, which always occurs late in the summer and in addition is always associated with a bivoltine life cycle, is more specialized on *U. dioica* than the hibernating generation (Fig. 5.4; Nylin, 1988). It is still unclear whether this really results from an innate higher propensity to prefer *U. dioica* in light-form adults or is an effect of a more drastic seasonal decrease in quality for the alternative hosts, which are trees and bushes rather than herbs, or whether both factors contribute. Experiments to disentangle these factors must be done with the two forms ovipositing simultaneously, when the plants are at the same phenological stage, and this has been difficult to achieve. With the discovery that dark-form adults break reproductive diapause in long days (C. Wiklund, Stockholm, 1999, personal communication), we will be able to establish the respective roles of these factors (G.H. Nygren and S. Nylin, unpublished). In other words, do dark-form females also show an increased preference for nettle later in the season, if manipulated to oviposit, but not to the extent that light-form females do? If so, this adds yet another component to the battery of traits involved in the seasonal polyphenism of *P. c-album*.

Expanding this type of reasoning to variation among populations, we can expect populations under time-stress to be more specialized on 'fast' hosts, even if other hosts are superior in other ways (Nylin, 1988; Scriber and Lederhouse, 1992; Janz *et al.*, 1994). In the comma butterfly, the higher degree of specialization on nettles in the partially bivoltine English population

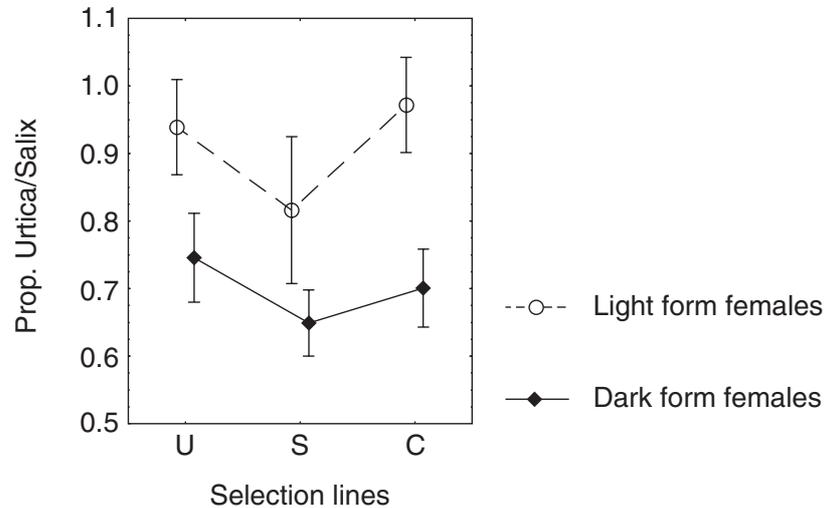


Fig. 5.4. The proportion of eggs laid on *Urtica dioica*, in a choice with *Salix caprea*, by light-form (open symbols) and dark-form (closed symbols) of *Polygonia c-album*. Bars show standard errors from variation among females. Data are from the F_3 generation of a selection experiment: U, S = lines selected for *Urtica* and *Salix* preference, respectively. C = unselected control line (Nylin *et al.*, 2005).

than in the univoltine Swedish population has been interpreted in this manner (Nylin, 1988). This body of theory is tightly linked to 'saw-tooth' theory regarding latitudinal variation in development time and size, described above, since preferentially using 'fast' hosts is another way of decreasing development time in offspring. Such preference is expected when the season is very short, as in the extreme north of the distribution, and in areas where there is a shift in generation number, i.e. barely enough time for the additional generation. Achieving a short larval development time in this manner has its advantages. It should typically not carry a cost in either reduced final weight (but here the comma butterfly is an exception if the alternative is to use *Salix caprea*; Janz *et al.*, 1994) or in the form of costs of high growth rates. This is because presumably the 'fast' hosts allow high growth rate because they are plants of high 'quality', i.e. they present a good environment for larval performance.

Other types of costs may be present, however. If the 'fast' hosts are not abundant enough there will be time lost in searching, perhaps even negating the time gained in the next generation. This may explain why females of an extreme short-season population of *P. c-album* from Norway does not specialize on nettles but rather prefers the more abundant willow (G. Nygren, S. Nylin and R. Krogen, unpublished). In addition, any advantage of a wide host plant range that may originally have promoted its evolution, such as higher realized fecundity or spreading of risks, will be lost by specialization on 'fast' hosts.

10. Speciation and the origin of biodiversity

The prevailing view of speciation has been that the most important process is allopatric speciation, often with a high degree of randomness with respect to the ecology of the incipient species. Populations become geographically isolated (for random, climatic or geological, reasons), and after some time they have diverged enough, through selection, genetic drift and mutation, so that they are reproductively isolated if they meet again. Recent years have seen an increase in the interest in 'ecological speciation', i.e. processes of speciation where ecological factors play an important role (Schluter, 2000). In the extreme case, ecological specialization of different genotypes may lead directly to a degree of reproductive isolation (e.g. 'host-plant races') and eventually sympatric speciation (Bush and Smith, 1997). However, ecology can play an important role also in allopatric speciation, and in intermediate modes of speciation. If two populations of a species become geographically isolated, gene flow between the populations is interrupted, and they can each adapt to their respective environments. Against a background of the type of results reported above, it is easy to see how, for instance, two insect populations isolated along a north-south gradient could adapt and diverge ecologically, with respect to traits such as life history, life-cycle regulation, seasonal polyphenism and host plant utilization. Co-adapted gene complexes may form, since these traits affect each other in various ways. The populations may not diverge enough to become completely reproductively isolated in case of a secondary contact, but the ecological differences may be enough to promote a process of 'reinforcement'. If hybrids are less fit because they are bad compromises between two ecologies, selection in favour of assortative mating may occur, perhaps simply by divergent choice of habitat.

We have already seen that all of the ecologically relevant traits mentioned in the previous paragraph have strong plastic components; in other words plasticity cannot be ignored in ecological speciation. It is high time to break with the view of genetics and plasticity as contrasts, where a plastic phenotype is seen as an alternative to genetic differentiation, hence making genetic divergence and speciation less probable. In fact, all genotypes are plastic with respect to most traits, and we must instead concern ourselves with the shape of reaction norms. It has been suggested that, rather than making genetic divergence more difficult, plasticity may provide the necessary raw material of varying phenotypes for natural selection to act upon (West-Eberhard, 1989). After all, imagine two geographically isolated populations of the same species, both fixed for a highly canalized genotype, one that can only produce phenotype A whatever the environment. If the environment changes in the area inhabited by one of the populations, so that phenotype B is now optimal, it must wait for a fortuitous mutation before it can respond. If, instead, both populations have the plastic genotype AB, capable of producing either phenotype, the populations are likely to diverge phenotypically much faster, in response to the environmental change (Fig. 5.5).

Admittedly, it will initially not be a genetic difference, but genotypes within the populations are likely to differ in the exact form of their reaction norms

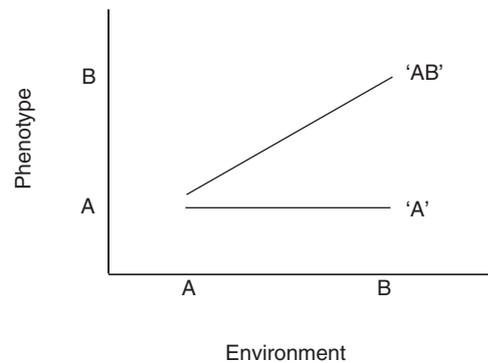


Fig. 5.5. Reaction norms of two genotypes: one ('A') is capable of producing only phenotype A regardless of environment, the other ('AB') is more plastic and produces phenotype A in environment A and phenotype B in environment B. If the plasticity is adaptive, phenotype A leads to higher fitness in environment A than the alternative phenotype, and *vice versa*.

(genotype-by-environment interactions are ubiquitous). If the environments are stable enough, reaction norms producing phenotype A under prevailing conditions are likely to be favoured by selection in one of the populations, and *vice versa* for phenotype B in the other population. If this happens by increasing the range of environmental conditions that produces the optimal phenotype, there will be a process of 'genetic assimilation' (Waddington, 1953; Pigliucci and Murren, 2003), in the extreme case leading to canalized phenotypes in the two populations, what we see as a pure 'genetic difference' between them. Even if the potential to produce both phenotypes is retained, 'common garden' experiments can still reveal that the populations now differ genetically in the shape of reaction norms. In other words, upon secondary contact they will display different phenotypes in the same environment, and some form of ecological speciation may then occur.

It is interesting to apply this sort of scenario to insect–host plant interactions. Phytophagous insects make up a very large proportion of the biodiversity, and this does not seem to be a historical coincidence. Clades of phytophagous insects are consistently more rich in species than the most closely related group of insects feeding on something else (Mitter *et al.*, 1988), suggesting that evolution of the ability to feed on plants somehow opens up a new adaptive zone, promoting diversity. But is it the enormous amount of resources that becomes available by using a lower trophic level that is the key, or is it the diversity of this resource – diversity feeding diversity? This is still unclear, but detailed phylogenetic studies *within* clades of phytophagous insects may provide clues.

Zooming in on the comma butterfly, one of the examples given by Mitter *et al.* (1988) is the comparison between Lepidoptera and its sister-clade Trichoptera, or perhaps between basal Lepidoptera and the 'higher' forms such as butterflies and large moths (the ancestral diet of Lepidoptera is uncertain). In any case, the phytophagous clade is much more diverse. The phylogenetic

relationships among and within 'higher' moth families are still very unclear, but we have some understanding of relationships within the butterflies, an understanding which is rapidly improving through the advent of simple techniques for molecular phylogenetics. Starting with such hypotheses of phylogeny, it is possible to 'map' host-plant utilization onto the phylogenies using the principle of character optimization (Brooks and McLennan, 1991). Simply put, if two closely related species use the same host plant, the simplest explanation is that this host plant was used already by the ancestor of the two species (see Fig. 5.2 for another example of character optimization).

This technique was applied to a butterfly phylogeny, put together from various available sources, by Janz and Nylin (1998). The reconstruction suggested that the ancestral butterfly was a specialist on trees in (or near) Fabaceae. Most modern butterflies are also specialists on one (or a few related) plant families (Ehrlich and Raven, 1964), but obviously not all on the same families. This pattern by itself suggests that butterflies sometimes shift plant clades, but then specialize again. There is evidence that shifts to more closely related plants are more likely than shifts to distantly related plants, perhaps because they are chemically more similar and there is some degree of pre-adaptation for good performance (Janz and Nylin, 1998). At the time of shifts, the butterflies must by necessity pass through a phase when they are capable of using at least two clades (the ancestral and the new) as larval food. However, they must re-specialize rather quickly, considering the rarity of polyphagous butterfly species.

The comma butterfly belongs to the family Nymphalidae, one of the most diverse butterfly families both in terms of species and host-plant utilization. Relationships within the family are still poorly known, and so we are not yet in a position to reconstruct host plant utilization at this phylogenetic level. However, hypotheses of phylogeny are available for the tribe Nymphalini (Nylin *et al.*, 2001; Wahlberg and Nylin, 2003). It seems clear from mapping host plants onto these phylogenies (Janz *et al.*, 2001; Wahlberg and Nylin, 2003) that the ancestor of Nymphalini was a specialist on Urticaceae (and perhaps other hosts in Urticales), like the basal genera *Hypanartia*, *Antanartia*, *Mynes*, *Symbrenthia* and *Araschnia* and many of the more derived species are today. Many of the plant families used in *Polygonia* are shared with the sister-genus *Nymphalis* (Nylin, 1988; Nylin and Janz, 1999). The probable interpretation is that a wider host-plant range evolved in the ancestor of this clade (Janz *et al.*, 2001). In the present context it is interesting to note that the *Polygonia+Nymphalis* clade is more diverse than its sister clade *Aglais+Inachis*, which retains the ancestral specialization on hosts in Urticales (Nylin and Janz, 1999).

Possibly, evolution of a wider host-plant range opens up avenues for higher rates of speciation, analogous to evolution of a plastic phenotype. In fact, as pointed out above, an insect that is capable of feeding on more than one plant clade is more plastic compared with a specialist. But how does this ability promote speciation? In some phytophagous insects, one way may be via the formation of host-plant races and sympatric speciation. This could perhaps happen even in some sedentary butterflies (Pratt, 1994), but does not seem likely in strong fliers such as *P. c-album*, where available evidence suggests a

very open population structure with high levels of gene flow (Nylin *et al.*, 2005). However, a wide host plant range may also allow a species to expand its geographical range to areas where the ancestral host is lacking or not very abundant. This sets the scene for speciation, if the species is then over time secondarily broken up into populations with some degree of geographical isolation. Specialization on the host plant which locally provides best performance (also taking into account field characteristics such as relative plant abundance, phenological fit to butterfly life history, predation etc.) may follow. Such emerging differences in host plant utilization, in turn, have the potential to become an important component in ecological speciation. They may provide some degree of reproductive isolation in time and space, as well as being at the core of co-adapted gene complexes with possibly unfit hybrids between them.

Most of this scenario is at present highly speculative as to how it applies to speciation in *Polygonia* or elsewhere, but there is evidence for one of the necessary components. A dynamic pattern of oscillations in host-plant range is seen in the *Polygonia+Nymphalis* clade, where several species in the clade have re-specialized on a subset of the common plant families or, in a few cases, on novel hosts (Janz *et al.*, 2001). Evolutionary dynamics in host-plant range is now emerging as a general property of insect-plant associations (Thompson, 1998; Janz *et al.*, 2001; Nosil, 2002) and it is possible that such oscillations in host-plant range have played a crucial role in the origin of the Earth's biodiversity.

11. Conclusions

Not so long ago the study of plasticity was considered almost a suspect subject in evolutionary biology, but the situation has changed dramatically over the last two decades (West-Eberhard, 2003). Over the coming decades the study of plasticity *per se* will doubtlessly continue and lead to new insights into the evolution of reaction norms and the mechanistic determination of plasticity. Even more importantly, however, it can be hoped that plasticity in traits will increasingly be given the consideration that it deserves as a natural part of any ecological or evolutionary study.

As we hope to have illustrated here, using primarily *Polygonia* butterflies, seasonal plasticity offers a conceptual framework that connects most aspects of insect evolutionary ecology; life-cycle regulation, life-history theory, optimal design, sexual selection, resource use, species interactions and ecological speciation. Some of these are highly theoretical fields, but using the seasonal life cycle of the insect as a starting point helps to ensure that sight is never lost of the organism in its real ecological situation, complete with its constraints and complexities.

Acknowledgements

This study was supported by grants from the Swedish Research Council to S.N. and K.G.

References

- Abrams, P.A., Leimar, O., Nylin, S. and Wiklund, C. (1996) The effect of flexible growth rates on optimal sizes and development times in a seasonal environment. *American Naturalist* 147, 381–395.
- Ballabeni, P., Gottbard, K., Kayumba, A. and Rahier, M. (2003) Local adaptation and ecological genetics of host-plant specialization in a leaf beetle. *Oikos* 101, 70–78.
- Bernays, E.A. and Wcislo, W.T. (1994) Sensory capabilities, information processing, and resource specialization. *Quarterly Review of Biology* 69, 187–204.
- Bradshaw, W.E. (1976) Geography of photoperiodic response in a diapausing mosquito. *Nature* 262, 384–385.
- Brakefield, P.M. and Larsen, T.B. (1984) The evolutionary significance of dry and wet season forms in some tropical butterflies. *Biological Journal of the Linnean Society* 22, 1–12.
- Brooks, D.R. and McLennan, D.H. (1991) *Phylogeny, Ecology, and Behavior: A Research Program in Comparative Biology*. University of Chicago Press, Chicago.
- Bush, G.L. and Smith, J.J. (1997) The sympatric origin of phytophagous insects. In: Dettner, K., Bauer, G. and Volkl, W. (eds) *Vertical Food Web Interaction*. Springer, Berlin, pp. 3–19.
- Conover, D.O. and Schultz, E.T. (1995) Phenotypic similarity and the evolutionary significance of countergradient variation. *Trends in Ecology and Evolution* 10, 248–252.
- Dal, B. (1981) *Fjärilar i Naturen 2*. Wahlström and Widstrand, Stockholm.
- Danilevskii, A.S. (1965) *Photoperiodism and Seasonal Development of Insects*, English edn. Oliver and Boyd, Edinburgh and London.
- Ehrlich, P.R. and Raven, P.H. (1964) Butterflies and plants: a study in coevolution. *Evolution* 18, 586–608.
- Fox, C.W. and Caldwell, R.L. (1994) Host-associated fitness trade-offs do not limit the evolution of diet breadth in the small milkweed bug *Lygaeus kalmii* (Hemiptera, Lygaeidae). *Oecologia* 97, 382–389.
- Fox, C.W. and Lalonde, R.G. (1993) Host confusion and the evolution of insect diet breadths. *Oikos* 67, 577–581.
- Gotthard, K. (1998) Life history plasticity in the satyrine butterfly *Lasiommata petropolitana*: investigating an adaptive reaction norm. *Journal of Evolutionary Biology* 11, 21–39.
- Gotthard, K. (2000) Increased risk of predation as a cost of high growth rate: an experimental test in a butterfly. *Journal of Animal Ecology* 69, 896–902.
- Gotthard, K. and Nylin, S. (1995) Adaptive plasticity and plasticity as an adaptation: a selective review of plasticity in animal morphology and life history. *Oikos* 74, 3–17.
- Gotthard, K., Nylin, S. and Wiklund, C. (1994) Adaptive variation in growth rate: life history costs and consequences in the speckled wood butterfly, *Pararge aegeria*. *Oecologia* 99, 281–289.
- Gotthard, K., Nylin, S. and Wiklund, C. (1999a) Mating system evolution in response to search costs in the speckled wood butterfly, *Pararge aegeria*. *Behavioral Ecology and Sociobiology* 45, 424–429.
- Gotthard, K., Nylin, S. and Wiklund, C. (1999b) Seasonal plasticity in two satyrine butterflies: state-dependent decision making in relation to daylength. *Oikos* 84, 453–462.
- Gotthard, K., Nylin, S. and Wiklund, C. (2000a) Individual state controls temperature dependence in a butterfly (*Lasiommata maera*). *Proceedings of the Royal Society of London, Series B* 267, 589–593.

- Gotthard, K., Nylin, S. and Wiklund, C. (2000b) Mating opportunity and the evolution of sex-specific mortality rates in a butterfly. *Oecologia* 122, 36–43.
- Hunter, M.D. and McNeil, J.N. (1997) Host-plant quality influences diapause and voltinism in a polyphagous insect herbivore. *Ecology* 78, 977–986.
- Janz, N. and Nylin, S. (1997) The role of female search behaviour in determining host plant range in plant feeding insects: a test of the information processing hypothesis. *Proceedings of the Royal Society, Series B* 264, 701–707.
- Janz, N. and Nylin, S. (1998) Butterflies and plants: a phylogenetic study. *Evolution* 52, 486–502.
- Janz, N., Nylin, S. and Wedell, N. (1994) Host plant utilization in the comma butterfly: sources of variation and evolutionary implications. *Oecologia* 99, 132–140.
- Janz, N., Nyblom, K. and Nylin, S. (2001) Evolutionary dynamics of host-plant specialization: a case study of the tribe Nymphalini. *Evolution* 55, 783–796.
- Joshi, A. and Thompson, J.N. (1995) Trade-offs and the evolution of host specialization. *Evolutionary Ecology* 9, 82–92.
- Joshi, A. and Thompson, J.N. (1997) Adaptation and specialization in a two-resource environment in *Drosophila* species. *Evolution* 51, 846–855.
- Karlsson, B. and Wickman, P.-O. (1989) The cost of prolonged life: an experiment on a nymphalid butterfly. *Functional Ecology* 3, 399–405.
- Kemp, D.J. and Jones, R.E. (2001) Phenotypic plasticity in field populations of the tropical butterfly *Hypolimnas bolina* (L.) (Nymphalidae). *Biological Journal of the Linnean Society* 72, 33–45.
- Kingsolver, J.G. (1995) Fitness consequences of seasonal polyphenism in western white butterflies. *Evolution* 49, 942–954.
- Koch, P.B. (1992) Seasonal polyphenism in butterflies: a hormonally controlled phenomenon of pattern-formation. *Zoologische Jahrbücher, Abteilung für Allgemeine Zoologie und Physiologie der Tiere* 96, 227–240.
- Masaki, T., Endo, K. and Kumagai, K. (1989) Neuroendocrine regulation of the development of seasonal morphs in the Asian comma butterfly, *Polygonia c-aureum* L.: stage-dependent changes in the action of summer-morph producing hormone of the brain-extracts. *Zoological Science* 6, 113–119.
- Mitter, C., Farrel, B. and Wiegmann, B. (1988) The phylogenetic study of adaptive zones: has phytophagy promoted insect diversification? *American Naturalist* 132, 107–128.
- Mousseau, T.A. (1997) Ectotherms follow the converse to Bergmann's rule. *Evolution* 51, 630–632.
- Mousseau, T.A. and Roff, D.A. (1989) Adaptation to seasonality in a cricket: patterns of phenotypic and genotypic variation in body size and diapause expression along a cline in season length. *Evolution* 43, 1483–1496.
- Nosil, P. (2002) Transition rates between specialization and generalization in phytophagous insects. *Evolution* 56, 1701–1706.
- Nylin, S. (1988) Host plant specialization and seasonality in a polyphagous butterfly, *Polygonia c-album* (Nymphalidae). *Oikos* 53, 381–386.
- Nylin, S. (1989) Effects of changing photoperiods in the life cycle regulation of the comma butterfly, *Polygonia c-album* (Nymphalidae). *Ecological Entomology* 14, 209–218.
- Nylin, S. (1991) Butterfly life-history adaptations in seasonal environments. PhD thesis, Stockholm University, Sweden.
- Nylin, S. (1992) Seasonal plasticity in life history traits: growth and development in *Polygonia c-album* (Lepidoptera: Nymphalidae). *Biological Journal of the Linnean Society* 47, 301–323.

- Nylin, S. (1994) Seasonal plasticity and life-cycle adaptations in butterflies. In: Danks, H.V. (ed.) *Insect Life-Cycle Polymorphism*. Kluwer Academic, Dordrecht, The Netherlands, pp. 41–67.
- Nylin, S. (2001) Life history perspectives on pest insects: what's the use? *Austral Ecology* 26, 507–517.
- Nylin, S. and Gotthard, K. (1998) Plasticity in life history traits. *Annual Review of Entomology* 43, 63–83.
- Nylin, S. and Janz, N. (1999) The ecology and evolution of host plant range: butterflies as a model group. In: Drent, R., Brown, V.K. and Olff, H., (eds) *Herbivores, Plants and Predators*. Blackwell, Oxford, pp. 31–54.
- Nylin, S. and Svärd, L. (1990) Latitudinal patterns in the size of European butterflies. *Holarctic Ecology* 14, 192–202.
- Nylin, S., Wickman, P.-O. and Wiklund, C. (1989) Seasonal plasticity in growth and development of the speckled wood butterfly, *Pararge aegeria* (Satyrinae). *Biological Journal of the Linnean Society* 38, 155–171.
- Nylin, S., Wiklund, C., Wickman, P.-O. and Garcia-Barros, E. (1993) Absence of trade-offs between sexual size dimorphism and early male emergence in a butterfly. *Ecology* 74, 1414–1427.
- Nylin, S., Wickman, P.-O. and Wiklund, C. (1995) An adaptive explanation for male-biased sex ratios in overwintering monarch butterflies. *Animal Behaviour* 49, 511–514.
- Nylin, S., Gotthard, K. and Wiklund, C. (1996a) Reaction norms for age and size at maturity in *Lasiommata* butterflies: predictions and tests. *Evolution* 50, 1351–1358.
- Nylin, S., Janz, N. and Wedell, N. (1996b) Oviposition plant preference and offspring performance in the comma butterfly: correlations and conflicts. *Entomologia Experimentalis et Applicata* 80, 141–144.
- Nylin, S., Bergström, A. and Janz, N. (2000) Butterfly host plant choice in the face of possible confusion. *Journal of Insect Behavior* 13, 469–482.
- Nylin, S., Nyblom, K., Ronquist, F., Janz, N., Belicek, J. and Kallersjö, M. (2001) Phylogeny of *Polygonia*, *Nymphalis* and related butterflies (Lepidoptera: Nymphalidae): a total-evidence analysis. *Zoological Journal of the Linnean Society* 132, 441–468.
- Nylin, S., Nygren, G.H., Windig, J., Janz, N. and Bergström, A. (2005) *Biological Journal of the Linnean Society* 84, in press.
- Pianka, E.R. (1983) *Evolutionary Ecology*, 3rd edn. Harper and Row, New York.
- Piersma, T. and Lindstrom, A. (1997) Rapid reversible changes in organ size as a component of adaptive behaviour. *Trends in Ecology and Evolution* 12, 134–138.
- Pigliucci, M. (2001) *Phenotypic Plasticity: Beyond Nature and Nurture*. Johns Hopkins University Press, Baltimore, Maryland.
- Pigliucci, M. and Murren, C.J. (2003) Genetic assimilation and a possible evolutionary paradox: can macroevolution sometimes be so fast as to pass us by? *Evolution* 57, 1455–1464.
- Pratt, G.F. (1994) Evolution of *Euphilotes* (Lepidoptera, Lycaenidae) by seasonal and host shifts. *Biological Journal of the Linnean Society* 51, 387–416.
- Roff, D.A. (1980) Optimizing development time in a seasonal environment: the 'ups and downs' of clinal variation. *Oecologia* 45, 202–208.
- Roff, D.A. (1983) Phenological adaptation in a seasonal environment: a theoretical perspective. In: Brown, V.K. and Hodek, I. (eds) *Diapause and Life Cycle Strategies in Insects*. Dr W Junk, The Hague, pp. 253–270.
- Schluter, D. (2000) *The Ecology of Adaptive Radiation*. Oxford University Press, Oxford.
- Scott, J.A. (1986) *The Butterflies of North America*. Stanford University Press, Stanford, California.

- Scriber, J.M. and Lederhouse, R.C. (1992) The thermal environment as a resource dictating geographic patterns of feeding specialization of insect herbivores. In: Hunter, M.R., Ohgushi, T. and Price, P.W. (eds) *Effect of Resource Distribution on Animal-Plant Interactions*. Academic Press, New York, pp. 429–466.
- Shapiro, A.M. (1976) Seasonal polyphenism. *Evolutionary Biology* 9, 259–333.
- Singer, M.C. and Lee, J.R. (2000) Discrimination within and between host species by a butterfly: implications for design of preference experiments. *Ecology Letters* 3, 101–105.
- Stearns, S.C. (1989) The evolutionary significance of phenotypic plasticity. *BioScience* 39, 436–445.
- Stearns, S.C. (1992) *The Evolution of Life Histories*. Oxford University Press, Oxford.
- Strong, D.R. (1988) Insect host range. *Ecology* 69, 885.
- Sultan, S. (2003) The promise of ecological developmental biology. *Journal of Experimental Zoology B (Molecular and Developmental Evolution)* 296B, 1–7.
- Tammaru, T., Nylin, S., Ruohomäki, K. and Gotthard, K. (2004) Compensatory responses in lepidopteran larvae: the role of growth rate maximisation. *Oikos* 107, 352–362.
- Tauber, M.J., Tauber, C.A. and Masaki, S. (1986) *Seasonal Adaptations of Insects*. Oxford University Press, Oxford.
- Thomas, J. and Lewington, R. (1991) *The Butterflies of Britain and Ireland*. Dorling Kindersley, London.
- Thompson, J.N. (1988) Evolutionary ecology of the relationship between oviposition preference and performance of offspring in phytophagous insects. *Entomologia Experimentalis et Applicata* 47, 3–14.
- Thompson, J.N. (1994) *The Coevolutionary Process*. Chicago University Press, Chicago.
- Thompson, J.N. (1998) The evolution of diet breadth: monophagy and polyphagy in swallowtail butterflies. *Journal of Evolutionary Biology* 11, 563–578.
- Waddington, C.H. (1953) Genetic assimilation of an acquired character. *Evolution* 7, 118–126.
- Wahlberg, N. and Nylin, S. (2003) Morphology versus molecules: resolution of the positions of *Nymphalis*, *Polygonia* and related genera. *Cladistics* 19, 213–223.
- Wedell, N. (1996) Mate quality affects reproductive effort in a paternally investing species. *American Naturalist* 148, 1075–1088.
- Wedell, N., Nylin, S. and Janz, N. (1997) Effects of larval host plant and sex on the propensity to enter diapause in the comma butterfly. *Oikos* 78, 569–575.
- West-Eberhard, M.J. (1989) Phenotypic plasticity and the origins of diversity. *Annual Review of Ecology and Systematics* 20, 249–278.
- West-Eberhard, M.J. (2003) *Developmental Plasticity and Evolution*. Oxford University Press, Oxford.
- Via, S. and Lande, R. (1985) Genotype–environment interaction and the evolution of phenotypic plasticity. *Evolution* 39, 505–522.
- Wiklund, C. and Fagerström, T. (1977) Why do males emerge before females? A hypothesis to explain the incidence of protandry in butterflies. *Oecologia* 31, 153–158.
- Wiklund, C. and Tullberg, B.S. (2004) Seasonal polyphenism and leaf mimicry in the comma butterfly. *Animal Behaviour* 68, 621–627
- Wiklund, C., Wickman, P.-O. and Nylin, S. (1992) A sex difference in reaction norms for direct/diapause development: a result of selection for protandry. *Evolution* 46, 519–528.

-
- Wiklund, C., Gotthard, K. and Nylin, S. (2003) Mating system and the evolution of sex-specific mortality rates in two nymphalid butterflies. *Proceedings of the Royal Society, Series B* 270, 1823–1828.
- Voigt, W. (1985) Zur Induktion und Termination der reproduktive Diapause des kleinen Fuchses, *Aglaia urticae* L. (Lepidoptera, Nymphalidae). *Zoologische Jahrbücher, Abteilung für Systematik, Ökologie und Geographie der Tiere* 112, 277–298.

