Life History Consequences of Host Plant Choice

in the Comma Butterfly

Lina Söderlind

Department of Zoology
Stockholm University
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Doctoral dissertation 2012

Lina Söderlind
Department of Zoology
Stockholm University
SE-106 91 Stockholm
Sweden
lina.soderlind@zoologi.su.se

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To science – that amazing search for knowledge that (no matter how carefully you plan your experiments) usually makes you wind up at a completely different place than you originally thought you were heading for.
ABSTRACT

There is much evidence that herbivory is a key innovation for the tremendous success of insect. In this thesis I have investigated different aspects of host plant utilization and phenotypic plasticity using the polyphagous comma butterfly, *Polygonia c-album*. Even though external conditions affect a phenotypic plastic response, the outcome is often influenced by a genetic background which may differ among populations. In Paper I we suspected the genetic background to seasonal polymorphism to be X-linked. However, results from interspecific hybridization between two populations suggested that diapause response is instead inherited in a mainly autosomally additive fashion, with a possible influence of sexual antagonism on males. In Paper II we showed that female oviposition preference is not a plastic response influenced by larval experience, but has a genetic background coupled to host plant suitability. Further, there is a strong individual correlation between larval host plant acceptance and female host plant specificity (Paper III). We believe this to be a larval feedback genetically linked to female host specificity: offspring to ‘choosy’ specialist mothers benefit by remaining on the original host while offspring to less discriminating generalist mothers should risk inspecting the surroundings, thus compensating for potential poor female choice. In the larval mid-gut, genes are differentially expressed depending on host plant diet (Paper IV). Therefore, we expected to find fitness consequences of host plant switch. However, although growth rate was affected in a few treatments, larvae were generally surprisingly good at adjusting to new diets (Paper V). To conclude, host plant choice in both female and larval life stage is connected to performance. Combined with increased understanding about the plastic response to diet intake and seasonal polymorphism we have gained further insights into the processes of local adaptations and speciation in the Lepidoptera.
LIST OF PAPERS


III. Söderlind L, Janz N, Gamberale-Stille G & Nylin S “When mother does not know best: Contrasting host plant choice across life stages in individuals of the comma butterfly” *Submitted Manuscript*


V. Söderlind L, Janz N & Nylin S “Effects of sequential diets in the comma butterfly, *Polygonia c-album*: testing predictions from gene expression” *Manuscript*

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Preface: A History of Insect-Plant Interactions

Plant-feeding insects are estimated to constitute approximately a quarter of the total number of species on Earth (Strong et al., 1984). Since an additional quarter is represented by their host plants on which they feed and lay eggs on, roughly 50% of diversity of life can be explained by insect-plant interactions (Bernays & Chapman, 1994). The association between arthropods and vascular plants is indeed very old and probably originated in the Early Devonian (approximately 400 million years ago) (Labandeira, 1998). Successful as it is, insect herbivory is believed to have intensified majorly during the Late Pennsylvanian (later part of carboniferous period) and Early Permian period (Labandeira & Seposki, 1993; Labandeira & Phillips, 1996). This association with plants enabled extensive diversification and the number of families within the insect orders beetles, flies and lepidopterans (butterflies and moths) is estimated to be twice as many today as 100 million years ago (Labandeira & Seposki, 1993). During the same 100 million years insect herbivory has probably evolved independently 50 times or more (Bernays, 1998). Today, the diversity of phytophagous insect groups is greater than among their sister groups that do not feed on plants (Mitter et al., 1991). Taken together, the interaction between insects and their hosts is probably a direct reason for the success of herbivorous insects (Ehrlich & Raven, 1964), signifying that insect distribution, abundance and response to habitat and climate change therefore are likely to be essentially determined by the life-history consequences of host plant association and oviposition choice.

Butterflies are one of the most studies invertebrate groups (Boggs et al., 2003). They represent a special case since adults almost exclusively feed on nectar from flowering plants, limiting the acquirements of nitrogen and other important resources to the larval stage. Since life history consequences may vary extensively among hosts, female oviposition choice to a great extent determines offspring future fitness. Butterflies probably evolved sometime between 70 (Vane-Wright, 2004) and 140 (Shields, 1976) million years ago, possibly predating the angiosperms (Viloria, 2003). Considering that the family Nymphalidae (including species such as the tortoiseshell, the peacock butterfly, and the comma butterfly) is possible more than 70 million years old, butterflies as a whole is likely to be older than 70 million years old (Wahlberg, 2006). Since related butterfly species tend to feed on related plants (Ehrlich & Raven, 1964; Janz & Nylin, 1998) it is possible that co-evolution between butterflies and their hosts have caused much of this diversity. Another explanation is relatedness as a result of sequential evolution, i.e. that the diversification of insects has followed the evolution of plants (Jermy, 1976). One estimates that there are approximately 15 000 species of butterflies on Earth, of which 4 500 are Nymphalids. Sadly enough, as an effect of landscape fragmentation and monoculturization, several butterfly species have become endangered or even extinct while unwanted agricultural pests prosper. Increased knowledge on how host plants effect life-history is necessary to preserve species as well as for protecting crops from caterpillars that may destroy whole harvests without extensive pesticide use. Due to their tremendous success and variability, lepidopterans make excellent model organisms for the study of aposematism, polymorphism, mimicry, speciation, and - the central topic of this thesis - insect-plant interactions.
CHALLENGES

Around the world, butterflies symbol happiness and fragile beauty. Denoted for their extraordinary transformation from larva to imago, this ‘ugly duckling’ of invertebrates has a tendency to fascinate even people that generally dislike bugs. However, the life of butterflies is (literally) not a bed of roses. Each life stage is associated with challenges. Adults need to feed, find mates, find and appreciate host plants for the offspring, avoid predators etc. Larvae may be exposed to predators, parasitoids and competition from other larvae. During moulting and pupation the soft and relatively immobile larvae are even more vulnerable. Another risk larvae face is the possibility of being hatched upon a poor host. In temperate areas, the importance of matching developmental pathway to season is yet another challenge. The environmental demands shape the life-history of individuals and therefore several traits (physiological as well as behavioural) can be described as responses to phenotypic plasticity. These environmental variables can be illustrated by differences in temperature and day-length as well as the variety of host plants. To study these consequences of host plants utilization and seasonal variation I in this thesis apply the comma butterfly, Polygonia c-album (Figure 1) as my model organism.

![Figure 1. Adult and 5th instar larva of the comma butterfly, Polygonia c-album. Adult photographed by Niklas Janz.](image)

ADAPTATIONS TO TEMPERATE AREAS

Insects in temperate areas annually face the challenge of surviving winter. The cold temperature affects flying negatively, many body functions seize to work properly, and food supplies and host plants become difficult to obtain - especially if the ground is covered in snow. While a few species (e.g. the monarch butterfly and the red admiral) are known to migrate to other latitudes according to season, others escape the problems by hibernating. Butterfly species may enter diapause as eggs, different instars larvae, pupae or as adults. In Sweden, the Nymphalid butterflies that are first spotted in spring are those species that hibernates as adults. The comma butterfly is one of these species.

The comma butterfly exists in two different seasonal morphs, suited for two different developmental pathways. The dark autumn morph hibernates and then mates in spring while...
the light summer morph does not overwinter but instead mate already in summer (i.e. they produce an additional generation, without needing to diapause first) (Figure 2). The morphs show slightly different allocating patterns depending on developmental path. The hibernating morph allocates more nitrogen to thorax compared to the summer morph, which put more resources into the abdomen (Karlsson & Wickman, 1989), as a function of trade-offs regarding longevity v.s. egg production (Karlsson et al., 2008). In northern Europe (e.g. in Sweden) only the hibernating autumn morph exists while the summer morph is common in southern Europe (e.g. in Spain), where it is not unusual for comma butterflies to manage an additional second or third generation during the same season.

![Figure 2](image)

**Figure 2.** The life-cycle of the comma butterfly. Depending on the photoperiodic conditions during development, larvae can become of either the dark diapausing autumn morph or the light directly developing summer morph.

The developmental pathway is a plastic response mainly decided by photoperiod, where increasing day-length above a certain threshold results in light morph individuals whereas decreasing day-length results in the dark morph (Nylin, 1989). Changes in day-length indicates how much of the season is still left: increasing day-length signals that summer solstice (midsummer) has not been reached yet and that there is thus sufficient time left of the summer for producing additional generations, while a decrease in the number of sunlit hours suggests that autumn is approaching and larvae better prepare for diapause (Nylin, 1989). Seasonal polymorphism is also influenced by host plant use, since hosts resulting in smaller final body size increase the proportion of the darker diapausing morph (Wedell et al., 1997). Moreover, also temperature seems to affect development pathway. Development time is strongly dependent on temperature and there seems to be a temperature-regulated threshold
for the proportion of each morph, where the dark morph is produced to a higher extent in lower temperatures (Nylin, 1992). Also in other butterfly species both temperature and photoperiod is important for the determination of whether to diapause or develop directly (Shreeve, 1986). Thus, developmental pathway is a plastic response, although genetic variation exists among populations concerning differences in critical photoperiod. English comma butterflies for instance require shorter day-length change than Swedish ones and thus have a lower light threshold for inducing the light morph (Nylin, 1989). The ecological explanation to this is that summer days in southern Europe are shorter compared to northern Europe, although the summer season is longer. Hence, the critical photoperiod for development into the direct developing morph is reached at a shorter day-length in southern Europe compared to the Scandinavian population. In Paper I the genetic background of such population differences in seasonal polymorphism is investigated.

The X-chromosome is linked to several population differences

Local adaptations may result in larger patterns of geographical differences and play an important role in speciation. In butterflies, females are the heterozygous sex (XY or ZW), while males are the homozygous sex (XX or ZZ). Previous studies have suggested that the X-chromosome host a disproportionate high number of genes of importance for species differences in the Lepidoptera (Sperling, 1994; Prowell, 1998). This pattern has been further supported by other studies that have shown that female host preference is determined by the preference of their male parents’ populations, suggesting X-linkage (Thompson, 1988b; Scriber et al., 1991; Janz, 1998; Nygren et al., 2006). In Papilio glaucus and P. Canadensis there is evidence for differences in host plant preference and diapause patterns to be correlated with genes for both traits located on the X-chromosome (Rockey et al., 1987; Scriber, 1994). Hybrids of these two taxa nowadays constitute a third population with a false second generation (P. Appalachiensis), which is isolated in time from the two mother populations (Scriber & Ording, 2005). In the comma butterfly, host plant specificity varies according to latitude and is probably also associated to voltinism (Nylin, 1988; Nylin et al., 2009). Critical photoperiod and host plant preference could therefore both be X-linked and inherited together as a co-adapted gene complex, as in the P. glaucus species group. Sexually linked seasonal polymorphism would fit quite well with other X-linked features that are important for population differences, such as host plant specificity. But in order to understand the relationship between seasonal polymorphism and host plant specificity we first need to take a closer look at phytaphagous insect patterns of host plant use.

Patterns of Host Use

As mentioned above, seasonal morph is somewhat affected by host plant (Wedell et al., 1997). However, host plant choice has the potential to influence life-history in several other perspectives than seasonal morph determination. The diet of adult butterflies generally consists of nectar from flowering plants, which are basically sugars. Females are able to use the nitrogen-rich spermatophores gained from males during mating for their own somas as a resource compliment (Boggs & Gilbert, 1979; Wedell, 1996), but the main supply of nitrogen and other important categories of nutrition needs to be collected during the larval stage (Nylin & Janz, 1999; Stjernholm & Karlsson, 2006). The properties and quality of larval diet is therefore crucial for an individual’s future fitness. Since newly hatched butterfly larvae are limited in their ability to move to other hosts female oviposition preference to a great extent determine offspring fitness.
Phytophagous insects are commonly defined from the width of their host plant repertoire. Consequentially, ‘monophagous’ insects usually feed on a single host plant, although the term also includes species that feeds on a small number of plants within the same genus. In comparison, ‘polyphagous’ insects utilizes a larger number hosts from several families. Sometimes also the term ‘oligophagous’ is used for the description of insects that are able to use host from different genera but within the same family (Bernays & Chapman, 1994). In polyphagous insects, possible host plans may differ in their suitability, and to deal with this females have often developed an oviposition preference hierarchy of hosts that may correlate quite closely with larval fitness on those plants (Wiklund, 1974; 1981; Nylin, 1988; Janz et al., 1994; Nylin & Janz, 1996; Nylin et al., 1996; Poykko, 2006). Although larvae might be polyphagous with the potential of feeding on several plants, different oviposition strategies may exist within species, viz. generalists and specialists (Wiklund, 1981; Futuyma & Moreno, 1988). As the word indicates, specialists tend to specialize on one host species or genera (often the one providing offspring with the best conditions) while generalists distribute their eggs on a number of hosts. A specialist oviposition strategy is advantageous when the optimal host is abundant since offspring are then provided with a diet that maximizes their fitness. In addition, specialists are usually better at distinguishing good host plant quality from poor and consequentially oviposition mistakes are rare (Wiklund, 1981; Bernays & Chapman, 1994; Nylin et al., 2000). Due to cognitive constrains, a generalist oviposition strategy is on the other hand associated with difficulties to appreciate host plant quality and suitability (Janz & Nylin, 1997; Nylin et al., 2000; Egan & Funk, 2006). However, individual fitness is not only determined by offspring fitness and the consequence of a specialist strategy is that the realized fecundity of females is relatively low compared to species that apply a generalist oviposition strategy (Jaenike, 1990). Generalists benefit from spreading their eggs on several plants, maximizing fecundity while simultaneously spreading the risks of particular hosts being associated with predators, competition, low abundance etc (Jaenike, 1990). Although both strategies are beneficial under respective conditions, most phytophagous insects appear to be specialists (Futuyma & Gould, 1979; Cates, 1980; Chapman, 1982; Price, 1983; Bernays, 1984; Futuyma, 1991; Thompson, 1994), and it has been argued that specialization evolves at a faster rate than generalization does (Nosil, 2002).

The definitions of generalist/specialist strategies are however not as straight-forward as one might think (Dennis et al., 2011). For example, the model organism for this thesis, the polyphagous comma butterfly, although it is able to utilize a number of distantly related hosts is to be considered a pollyspecialist rather than a true generalist (Nylin & Janz, 1999). This signifies that the butterfly utilizes plants in four different orders, but specializes on only a few species in each order. The mixed terminology of monophagous vs. specialist and polyphagous vs. generalist may appear confusing. It is therefore important to note that generalist/specialist refers to host plant utilization – what is actually being used. Hence a specialist strategy is commonly defined by female oviposition specificity regardless of what larvae are able to feed on since larvae might never come in physical contact with a potential host if females refuse to lay eggs on it. As a result, larval and female host ranges are not necessarily identical. The hosts that larvae are able to survive and develop upon are included in the potential host range, while female oviposition constitutes the realized host range. It is not uncommon for the potential host range to be broader than the realized one. Neither is it rare for females to rank a good larval host low. Preference-performance correlations is a useful tool for determining host plant suitability (Jaenike, 1978; Thompson, 1988a). Close correlations signifies that females base their oviposition decision on plant intrinsic quality, which will maximize larval growth and survival. Poor correlations on the other hand suggest that environmental factors such as abundance, predation risk etc. influence female host range (Nylin & Janz, 1996; Nylin
et al., 1996). Poor correlations may also imply that a host-range is undergoing change (Thompson, 1988a). In such case the realized host range might not have had sufficient time to adapt to the potential host range or vice versa, e.g. in events of invasive species.

Host plant suitability differs both among and within plant species and depending on the diet, larval performance (and thus also future individual fitness) is to a large extent determined by their mothers’ oviposition choice (Thompson, 1988a; Nylin et al., 1996; Awmack & Leather, 2002). In case of oviposition mistakes, e.g. if females lay eggs on a non-host, offspring may face severe fitness drawbacks, since larval mobility is somewhat restricted. Still, comma butterfly larvae are able to move short distances from non-hosts to hosts (Nylin et al., 2000) and on top of this they seem to be able to distinguish between hosts and display the same preference hierarchy as their ovipositing mothers (Nylin & Janz, 1996). Larval host plant choice is therefore something that deserves a deeper investigation and should be assigned a more vital part of host plant utilization theory.

**HOST PLANT PREFERENCE**

Female oviposition preference ensures females to receive the highest fitness possible, by making trade-offs between plants intrinsic and extrinsic traits. However, instead of considering preference as an in advance set proportion of eggs on each plant, it may more correctly be viewed as sequential acceptance/rejection since a female cannot decide whether or not to oviposit on a host until she encounters it (Courtney et al., 1989). Despite the extensive support for the genetic background of female host plant preferences in *P. c-album* and other phytaphagous insects there is one question that always tends to pop up during oral presentations and peer reviews: should not ovipositing females prefer their juvenile host plant species? This idea, first proposed rather vaguely by (Hopkins, 1917), has later been named Hopkins Host Selection Principle (HHSP) and can be viewed as a type of phenotypic plasticity where female oviposition preference is determined by larval experience. According to HHSP one may assume that juvenile host plant experience provides reliable information about host quality and availability for ovipositing females to base their choice on since they themselves evidently have survived to adulthood. Although repeatedly rejected (Wiklund, 1974; van Emden et al., 1996; Barron, 2001), HHSP is a theory that has been surprisingly resistant (Anderson et al., 1995; Rietdorf & Steidle, 2002; Akhtar & Isman, 2003; Gandolfi et al., 2003; Chow et al., 2005; Hora et al., 2005; Olsson et al., 2006; Facknath & Wright, 2007). Regardless of what mechanisms are expected to be responsible for HHSP, it is difficult to imagine during what natural conditions juvenile experience should be a more reliable source than genetically determined oviposition preference. Taken to its extreme HHSP would doom offspring of such females to always remain on this inferior host, a strategy that will hardly be favoured by natural selection. Therefore, we should not expect HHSP to be favourable if host plants vary greatly in their effects on larval performance. Because of the great variety in how different host plants influence life-history in *P. c-album* it is nevertheless improbable for HHSP to apply for this species. In **Paper II** we empirically tested this prediction and reasoned under what circumstances (if any) HHSP might be a strategy superior to genetic correlations between female preference and larval performance.

In **Paper II** female host plant preference was found to be genetically determined, in accordance with previous studies (Nylin, 1988; Janz et al., 1994; Nylin & Janz, 1996; Nylin et al., 2009) and the preference hierarchy mirrored larval performance on those plants. Since
larvae have been found to show a preference in accordance to their parental population (Nylin & Janz, 1996) we in Paper III wanted to put this preference hierarchy in relation to genetic correlation between life stages. The possibility that preference is preserved from larva through metamorphosis to adult is an intriguing thought, but should not be confused with HHSP, which presumes similarities according to experience and not according to genetic background. It is appealing to believe (although not yet clearly demonstrated in butterflies) that larvae would prefer host that are optimal for them as individuals. A genetic correlation in preference between larval and adult life stage would then imply that realized host range would adapt rapidly to any change in the potential host range. In the evolution of host range, natural selection could then work on both life stages. In an experiment P. c-album larvae have indeed abandoned suboptimal plants in attempts to find more suitable hosts and managed to establish themselves on stinging nettle (Urtica dioica (Nylin et al., 2000). Thus larvae are able to appreciate host plant suitability and move some distance in case they find themselves situated on suboptimal plants.

**HOST PLANT PERFORMANCE**

Host plants may vary greatly in quality and suitability, even over season (Nylin et al., 2009). This is especially true for polyphagous species where hosts may differ in both nutritional content and chemical defences. In P. c-album stinging nettle (Urtica dioica) result in a high growth rate whereas individuals generally become larger on Salix-species such as sallow (Salix cinerea) (Nylin, 1988; Janz et al., 1994). Downy birch (Betula pubescens) is not a very good host since both growth rate, final size and survival rate is low compared to other hosts. To some extent, host-plant also affects diapause propensity (Wedell et al., 1997).

Insect herbivory can cause extensive damages on plants. Therefore, plants usually have different chemical defences to avoid being eaten. These chemical defences are important for shaping insect-host associations. Related host plants may present larvae with similar chemicals and larvae mid-gut needs to be able to cope with these chemicals in order for the larva to utilize a specific plant. Phenotypic plasticity is advantageous in situations of variable environments since phenotypes match environmental demands. In cases of host plant use, differential gene expression depending on food would be beneficial since individuals would only need to express those that are necessary for consuming a specific plant.

**CHANGES IN HOST PLANT RANGE**

Most herbivore insects appear to be specialists (Futuyma & Gould, 1979; Thompson, 1994) and there are indications that specialisation evolves faster than generalization does (Nosil, 2002). This does however not imply that species will inevitable end up in an evolutionary dead-end as suggested by (Futuyma & Moreno, 1988; Thompson, 1994; Kelley & Farrell, 1998). There are several possible mechanisms for increasing host plant range. For example, in some cases the narrowing of host plant repertoire may only occur in the realized host plant range although larvae are still able to feed hosts that are no longer oviposited. The oscillation hypothesis suggests that increases in host range may often actually be re-colonisations on hosts that have previously been part of the repertoire (Janz & Nylin, 2008). Much of the
utilization of closely related host plants could be the result of historic associations where insects of plants have coevolved together.

A few of the plants that *P. c-album* utilizes are closely related, but others are distantly related. How distantly related hosts are included in an insect's host range has been a subject of wide discussion. Feeny (Feeny, 1976) suggested that more apparent plants (like trees and bushes), because of being physical large and long-lived, should be subjected to more extensive insect herbivory. Because of this continuously high level of insect attack, constitutive chemicals would be used for defences. This in turn would explain the confirmed similarities in chemical defences in plants that share a common growth form (i.e. among trees and shrubs). Plant-feeding insects that are able to utilize one tree would then potentially express a detoxification system with the by-effect of being able to feed also from another tree. Trees might therefore work as bridges between the utilization of different plant genera (Janz & Nylin, 1998).

**METHODS**

**Study organism**
The comma butterfly is widely distributed over Eurasia. *P. c-album* is to be considered a poly-specialist rather than a true generalist as its host range includes a small number of relatively unrelated plants. Egg-laying females display a preference hierarchy of host plants that correlates well with larval performance (Nylin, 1988; Janz et al., 1994). The host plants, approximately in order of female preference, are: hop (*Humulus lupus*), stinging nettle (*Urtica dioica*), elm (*Ulmus glabra*), gooseberry (*Ribes uva-crispa*), black currant (*Ribes nigra*), red currant (*Ribes rubrum*), alpine currant (*Ribes alpinum*), sallow (*Salix caprea*), grey sallow (*Salix cinerea*), tea-leaved willow (*Salix phylicifolia*), downy birch (*Betula pubescens*) and hazel (*Corylus avellana*). When ovipositing, females usually lay only one egg per landing occasion and thus distributes eggs among plants.

**Methods in common**

**Rearing jars**
In the papers, 3 types of rearing jars were used (Figure 3). Due to withering or feeding, plants were changed when needed, except in experiments when diet was required to be replaced every day.

*Type 1* (Figure 3a): In these jar larvae were provided with host plants in a watered culture. Jars were big enough (Ø11.5 cm, height 12 cm) to support the whole development, pupation and moulting to adults. However, the jars takes up a lot of space and the set-up and maintenance of jars takes longer time than the other two types used in these papers.

*Type 2* (Figure 3b): These petri-dishes (Ø9 cm, height 2 cm) are very practical for massive rearings in climate chambers and for host plant switching experiments. They are also suitable for larval host plant choice trials. The bottom of each petri-dish is covered with a moist filter paper, on which host plant leaves can be placed. However, they may only support larvae until the beginning of the 5th instar since larvae thereafter tend to eat more than can be fitted into the dishes and fully grown larvae need to suspend themselves from the lid in order to pupate.

*Type 3* (Figure 3c): When larvae become too large for petri-dishes they are transferred to these jars (Ø6 cm, height 6.5 cm). Since they are higher than the petri-dishes larvae are able to pupate. Jars can also contain higher quantities of plant, which is required in the last larval stage when feeding is more extensive. In the bottom of each jar a piece of moist dishcloth provides hosts with enough water to keep them from withering for at least 24 hours.
Seasonal morph induction
Some of the experiments performed in this thesis have required the dark morph while the light morph has been used in others. Usually changing photoperiod has been obtained by simply moving larvae to other room, although special cabins were used for maintaining a stable photoperiod in Paper I. In cases when the Swedish comma butterflies were desired to induce the light directly developing morph, larvae were initially reared in a temperature of 17°C and a photoperiod of LD 12:12. Shortly after larvae had moulted into the 3rd instar they were transferred to a room with the temperature of 26°C and LD 22:2 to give the impression of increasing day length. In cases where the dark morph was desired larvae carried out their complete development in the room with LD 12:12.

Mating cages and oviposition cages (0.5m · 0.5m · 0.5m) are very similar in appearance. The floor of the cages are covered with paper towels, that are moisturized a few times each day. The back and sides are covered with cloth, the front is covered by a removable net and the top is covered with a see-through plastic ceiling. Over the cage hangs a 100W light bulb that provides butterflies with light and heat. The day length in mating cages is usually LD 17:7 and the temperature 20°C. At least 2 sponges with diluted sugar solutions are placed at different height in the mating cages to feed the butterflies. Occasionally sets of flowers are added in the cages as well to further motivate mating. Couples are usually removed in copula and after mating males are released into mating cages again while females are put into separate flight cages for oviposition. In oviposition cages (Figure 4), the day length is set to LD 8:16 and temperature to 20°C. In the high centre of each cage a sponge with diluted sugar solution is present and host plants are usually arranged around this sponge. Since butterflies tend to fly upwards towards the light host plants are placed at equal heights, near the ceiling of the cage, in all experiments but Paper III.

Figure 3. Three types or rearing jars. (a) Type 1, jar (b) Type 2, petri-dish (c) Type 3, jar

Figure 4. A typical cage used for oviposition
Sexually linked seasonal polymorphism would fit quite well with other X-linked features that are important for population differences, such as host plant specificity. In an intraspecific hybridization event between Spanish and Swedish comma butterflies in Paper 1 females were expected to follow the diapause pattern of their father. For the population crossings, light morph individuals of both populations were divided among mating cages accordingly: two cages containing only Swedish males and females (Sw); two cages containing a mixture of Spanish females and Swedish males (Sp♀ · Sw♂); two cages containing Swedish females and Spanish males (Sw♀ · Sp♂); one cage containing only Spanish butterflies (Sp). Offspring were reared individually on U. dioica in rearing jar type 1. Jars were kept in climate chambers (temperature 23°C, LD 17:7). These light and temperature conditions induce dark morph in the majority of pure stock Swedish individuals and light morph in most Spanish ones. Individuals were sexed after pupation and the morph of adults were checked a couple of hours after eclosure. Females did however not follow the diapause determination thresholds of their fathers (Figure 5). The threshold for diapause critical photoperiod is therefore unlikely to be inherited by X-linked genes. Since females of both crosses tended to show a light morph propensity in between those of their parental populations, we instead propose that the critical photoperiod threshold is controlled by several autosomal genes. In both population crosses the propensity to enter diapause was higher for hybrid males than for females. Also, the direction of cross was important for male seasonal morph decision. 22% of the males in the Sp♀ · Sw♂ cross developed into the light morph compared to 48% of the males in the reciprocal cross. We suggest this significantly lower propensity to become of the directly developing light morph to be a result from higher selection for diapause in Northern Europe where direct development never occurs naturally.

Figure 5. The proportion light morph among adult pure-stock Swedish and Spanish individuals and reciprocal hybrids. Females did not follow the development pattern of their fathers and thus the threshold for critical photoperiod is not inherited on the X-chromosome. However, there was an effect of sex since significantly fewer males of both population crossings developed into the light morph compared to females.
**Paper II**

The aim of the empirical part of Paper II was to test if Hopkins’ Host Selection Principle (HHSP) applies for *P. c-album*. The F1 generation larvae of wild-caught females from Åkersberga, Sweden, were reared individually in type 1 rearing jar on either *U. dioica*, *S. cinerea* or *R. uva-crispa* to the light directly developing morph. After pupation, sex, weight and development time was recorded. Adult butterflies were mated. A total of 41 mated females were individually presented with pairwise trials of two of the three host plants. Each female was tested with all three possible combinations of pair (*U. dioica – S. cinerea; U. dioica – R. Uva-crispa; S. cinerea – R. uva-crispa*). The plant combination was changes each day and fresh plants were used. The order of the trials was randomized between females. Eggs were counted and removed after each day. Days with a total less than 10 eggs were excluded and the host plant set-up repeated until the 10-eggs criteria was met. Oviposition preference data was analysed both to test a preference hierarchy of one host over another and also for testing HHSP. To test HHPS, the difference in oviposition preference of a specific host plant was set in relation to whether that particular host species had been used as larval host plant or not. Previous studies have found that larval growth rate differs between host plant and that larvae grow faster on *U. dioica* than on other hosts (Janz et al., 1994). This finding was reconfirmed in the current study since the growth rate was highest for *U. dioica*, followed by *S. cinerea* and *R. uva-crispa*.

In the oviposition preference experiment, *U. dioica* was significantly more preferred over both *S. cinerea* and over *R. uva-crispa*. However, there was no difference in preference between *S. cinerea* and *R. uva-crispa*. Females did not oviposit significantly more on their juvenile host (Figure 6). In fact, females instead seemed to prefer to lay eggs on *U. dioica* – the plant which support the highest growth rate – regardless of juvenile host plant. Thus we found no support for HHSP in this species.

![Figure 6](image-url)  
**Figure 6.** Female preference for larval host plant did not differ from how females that had been reared on any of the other hosts preferred it. For example, females reared on stinging nettle (U-same) did not differ in preference for nettle compared to females that had been reared on elm or grey sallow (U-different). Instead, the overall preference turned out to correlate quite closely to larval performance on these plants.
**Paper III**

Correlation in preference and performance is a classical tool to appreciate host plant patterns. In **Paper III** we have altered this method for investigation of the consistency in host plant preference and acceptance between life stages. By comparing life stages within individuals possible genetic correlations in host plant utilization is easier found. Since preference is hard to appreciate in the larval stage (larvae might have just stayed at the first host they encountered), the experiment was modified to test acceptance for *Salix* between 1st instar larvae and ovipositing females. When larval acceptance for *Salix* was investigated, larvae were put in petri-dishes with *Salix* and *Urtica*. All larvae were placed on the piece of *Salix* leaf. When 10% of a plant had been consumed, larvae were reared separately in type 1 rearing jars. Adults were mated and females were put in oviposition cages where *Salix*, *Urtica* and *Betula* were presented at unequal heights (*Urtica* was placed further down in the cage than the other two hosts) (Figure 7). By this set-up females, that tend to orient themselves upwards towards the light bulb above the cage, would encounter the low and medium ranked hosts more often than the more optimal *Urtica*. Since females would then make a sequential lay/withhold egg acceptance more often on *Salix* and *Betula* this is a good set-up for testing acceptance. In the acceptance trial there was a significant negative correlation in *Salix* acceptance between life stages. Females that accepted *Salix* as larvae tended to avoid laying eggs on it as ovipositing females, while females that had abandoned *Salix* in the larval stage laid a high proportion of eggs on it as adults (Figure 8).

**Figure 7.** The oviposition flight cage set-up for the female host plant acceptance experiment. The better and generally more preferred stinging nettle was placed low while the sub-optimal hosts grey sallow and downy birch were placed high up in the cage. By this set-up, females encounter the low-ranked hosts more often than they encounter stinging nettle.

**Figure 8.** The negative correlation of acceptance for sub-optimal plants between individuals as feeding larvae and ovipositing adults. Individuals that accepted sallow as larvae tended to avoid to lay eggs on the sub-optimal hosts sallow and downy birch later in life as ovipositing females. Larvae that abandoned sallow instead grew up to become females that discriminated less strongly against the sub-optimal plants during oviposition.
Larvae were reared on *U. dioica*, *S. caprea* or *U. glabra* until the 4\textsuperscript{th} larval stage. Then individuals were dissected, separating the mid-gut from the rest of the body. By using differential gene expression techniques, the genes expressed in the mid-gut of larvae from each rearing was compared among diets. Larvae were shown to have greater similarities in gene expression when feeding on related hosts (*U. dioica* and *U. glabra*) or hosts that belonged to the same growth form (*U. glabra* and *Salix caprea*). Also, with a diet of *U. dioica* or *S. caprea* there were more uniquely upregulated genes in the mid-gut than when larvae had fed on *U. glabra* (Figure 9). Several of the induced genes were found to be involved in metabolism and digestion.

**Figure 9.** Differential gene expression in mid-gut among diets. Blue color represents qualitative differences or similarities (present/absent), while red represents quantitative difference or similarities (more/less). (a) The number of uniquely upregulated genes on the three hosts, showing that significantly fewer genes were upregulated in elm. (b) Pair-wise similarities in upregulation between two of the three host plants. More genes were upregulated on plants that are either closely related or share growth form.

![Figure 10](image-url)  
**Figure 10.** Schematic representation of host plant relation and host switch experiment. Negative effects were predicted to be more pronounced when switching between stinging nettle and sallow (1. NS) than between the other combinations, since the (2. SE) switch occurs between two hosts that share a growth form and (3. EN) between two closely related species.
**Paper V**

Based on the results from **Paper IV**, larvae might experience difficulties when switching between diets if induced gene expression is irreversible or if there is a significant time-lag between host switch and altered gene expression. Because of the larger differences in differential gene expression between *Salix* and *Urtica* negative effects would be expected to even more pronounced when switching diet between those plants than between *Urtica* and *Ulmus* (that are both urticalean rosids) or between *Salix* and *Ulmus* (that are both trees) (Figure 10).

To test this prediction, 1\textsuperscript{st} instar larvae were placed on one of the three hosts in type 2 rearing jars and then subjected to sequential diets. In the first experiment, larvae were given a new host plant species when they reached the third instar. In the second experiment, host plant was switched at the 4\textsuperscript{th} instar, to represent the larval stage when the differential gene expression experiment was performed. In a third experiment, diet was switched between two of the hosts on a daily basis from the 1\textsuperscript{st} instar until pupation. However, there were few negative effects from host plant switch in any of the treatments. Following predictions, there was an effect of switching from *Salix* to *Urtica* in the 3\textsuperscript{rd} instar as well as when switching hosts every day, but these results were not consistent. We also found that a single switch to *Ulmus* resulted in negative effects (Figure 11). Generally, individuals were surprisingly good at adjusting to new hosts considering the differential gene expressions found in **Paper IV**.

![Figure 11. Single host switch from either stinging nettle or sallow to elm resulted in negative effects on growth rate.](image)

**DISCUSSION:**

There is a strong connection between host plant utilization and life-history consequences in the comma butterfly. In this thesis I have covered the Hopkins Host Selection Principle (**Paper II**), how female host plant specificity is correlated to larval acceptance (**Paper III**), the host-induced differential mid-gut gene expression and how it affects larval performance...
(Paper IV and Paper V). I have also studied the genetics of diapause between two populations with different degrees of host plant specialization since if the traits were inherited together at the X-chromosome, as a co-adapted gene complex, this would affect the host range and distribution of the species (Paper I).

**Diapause response is not X-linked, but is affected by sex**

Since in Paper I females did not follow the photoperiod response of their paternal population no support for X-linkage of critical photoperiod threshold was found. Nevertheless, there were still sex effects on daylength response. In both crosses hybrid males had a higher propensity to enter diapause than females. Such a sexually induced effect has been found previously in *P. c-album* (Wiklund et al., 1992; Wedell et al., 1997) and might be explained by sexual conflicts. Males and females may differ in optimal lifecycle responses where females profit from larger body size, since size is correlated with fecundity (Gilbert, 1984; Roff, 1992; Honek, 1993; García-Barros, 2000) while males obtain higher fitness if development time is short (Abrams et al., 1996). One explanation for higher male light morph induction is that slow growing males, that would emerge too late for protandry in the direct developing generation, instead diapause for a better chance to compete for females in spring (Wiklund et al., 1992; Wedell et al., 1997). The sex-effect on diapause control is probably a result of sexual antagonism and sexually antagonistic genes might eventually become so strongly connected to sex that they become sex linked (Bergero & Charlesworth, 2009).

Further, male hybrids experienced an effect of direction of cross: hybrid males with Swedish fathers followed the diapause propensity of Swedish pure stock males while males of the reciprocal cross displayed a diapause propensity somewhat intermediate to the parental populations. This pattern is difficult to explain by classical genetics but could result from some form of parental effect. Independent of the mechanism of inheritance, it is not unlikely that one underlying reason could be the greater importance for the northern population to really enter diapause compared to butterflies from the warmer southern Europe. In Sweden, direct development almost certainly equals no surviving offspring since adults stand a very insignificant chance to survive winter and offspring will not have sufficient time to develop before the arrival of winter. Selection for diapause in males might therefore be even stronger in Sweden than in Spain, resulting in partial dominance of genes inherited from the male parent in hybrids.

This study has shown that inheritance of diapause behaviour in *P. c-album* seem to be inherited in an autosomally additive fashion but with some influence of sex. As already mentioned, closely related Lepidopteran species often have sex-linked differences. These differences have most probably originated by sexually antagonistic traits and therefore it is possible that sexually antagonistic traits are important for speciation.

**Female preference is not affected by larval experience**

Previous results have shown that host plants vary in their effect on *P. c-album* performance and this finding was reconfirmed in the current study: nettle-reared larvae tended to have a higher growth-rate than larvae reared on grey sallow or gooseberries. This difference in larva suitability would make HHSP a poor strategy for ovipositing females since offspring reared on suboptimal plants would attain a low performance, resulting in low female fitness. In turn the linage would continue to be associated to a poor plant. In line with this assumption, we in Paper II found no effect of larval host plant on adult oviposition. Instead, females tended to prefer hosts on which offspring performance is high, a genetically based behavior that is
clearly favored by natural selection. Therefore, HHSP does not seem to be applicable on \( P. c-album \).

Possibly, there are situations when observations are mistaken for a ‘Hopkins effect’, e.g. when maternal effects cause increased oviposition on hosts suboptimal for offspring but favorable for a female in stress. To evaluate the applicability of HHSP we must ask ourselves under what circumstances larval feeding environment can provide a more accurate source of information on future host quality and availability than the genome. The conditions that should be most likely to favor HHSP are: (a) low variation in host quality among hosts, (b) predictable temporal heterogeneity, (c) high spatial heterogeneity (i.e. local host availability varies greatly between patches) and intermediate gene flow between spatial patches, and (d) high costs for adult butterflies to gather information on host availability and suitability. The comma butterfly does not correspond to these conditions, and they are likely to be rare in nature. HHSP should therefore not be invoked as a general hypothesis for host selection in plant feeding insects.

**Mother may not always know best**

In Paper III females tended to show an opposite pattern of \( Salix \) acceptance as ovipositing adults compared to the pattern as feeding larvae. This probably genetic negative correlation is likely to be a result of feed-back between female host plant specificity and larval acceptance. Females with high host specificity discriminate stronger against medium-ranked hosts and thus withhold eggs when encountering them. This may imply a lower realized fecundity, but offspring to more specialized females have a high probability to be hatched on suitable hosts of good quality, enhancing offspring growth rate and survival. Therefore it is would be advantageous for larvae to ‘trust’ mothers choice and remain on the plant. In other words, ‘mother knows best’. If the mother instead is of a more generalist non-discriminating type, being less choosy in her oviposition choice, there is a greater risk for offspring to be distributed on less optimal plants. In case that mother does not know best, larvae would benefit from rebelling against mothers choice and not at once accept the primal host plant but to search for possible better hosts in the near vicinity of the hatching location. This feed-back strategy would function as a sort of back-up system for larvae in cases of females-larvae conflict.

This proposal is supported by the fact that larvae of more specialist populations (English) have a tendency to remain on the original hosts compared to more generalist ones (Swedish) (Nylin et al., 2000). The feed-back mechanism would decrease the disadvantages of generalist oviposition strategy since fecundity can still be high without offspring necessarily suffering from very high consequences. There is a need to consider larval host plant choice with greater importance for host plant utilization also in species where female oviposition choice is distinct and larvae less mobile.

**Differential gene expression and the small costs of switching hosts**

In Paper IV we presented evidence that genes in larval mid-gut is differentially expresses according to diet. Similarities between hosts that were either closely related or shared a common growth form suggested that elm might be a link between the utilization on urticalean rosids and trees such as \( Salix \)-species. Since similarities in mid-gut gene expression were fewer between the distantly related herb stinging nettle and the tree sallow negative effects were predicted to larger when switching hosts between those plants in Paper V. So why were there so few costs?
One possibility is obviously that gene expression is altered when larvae starts to consume another host. In that case, there does not seem to be any significant time-lag between diet switch and the induction of another gene complex fitted for the new plant. Another possibility is that larvae are able to express several complexes simultaneously, enabling a swift change of diet or even mixed diets. In such case, mixed diets might even be better than a single host plant diet. There was a near-significant effect (p=0.058) of daily diet switch between Salix and Ulmus where growth rate showed a trend toward actually becoming enhanced by host switch. However, this could just as well be an effect of compensatory increase after exposure to unfavorable conditions – larvae subjected to periods of starvation have been shown to increase their relative growth more than individuals with constant access to food (Tammaru et al., 2004). In order to ascertain how the differential gene expression responds to switching diets one would need to rear larvae on different hosts, perform a host switch and thereafter dissect larvae to analyze gene-expression in the mid-gut at certain times after the switch.

It could be difficult to separate the effects of host plants from effects of the host switch per se. Growth rate differs between the hosts and it is possible that the original host has affected life-history in ways that cannot be altered although host plant is changed – the ‘settings’ of a specific developmental path leading to slower growth or larger final size may perhaps not be modified to fit the new conditions. This has less to do with gene expression and more to do with developmental ‘decisions’ that have already been made before the switch.

Changing from either sallow or stinging nettle to elm did seem to be associated with at least slight negative effects. Since this stands in contrast to what was initially expected we are forced to ask what makes elm so special. However, from another perspective it might be more correct to instead ask what makes elm so unspecial. Fewer genes were uniquely upregulated in the mid-gut when larvae were feeding on elm than on stinging nettle or sallow. Many genes are instead commonly expressed when utilizing elm and either of the other two hosts. We do not yet know what happens to gene expression when diet is changed, but it is possible that the body does not immediately recognize a diet switch to elm and therefore continues with the already expressed gene expression. Due to either incompatibility or time lag between host switch and response, in the form of induction of another set of mid-gut genes to cope with the new host, larval fitness might suffer negative consequences. But in such scenario, how come there were no apparent costs of elm-related host switches in the daily diet change? Several authors have shown that once feeding is initiated on one host, larval preference is induced to the original host (Jermy et al., 1968; Wiklund, 1973; Hanson, 1976; Scriber, 1979; Ting et al., 2002). Unchangeable diet-induced gene expression could explain such a pattern, since it would then be disadvantageous for larvae to change diet. Larvae do not feed continuously. They need to rest and moult into other instars. It is not unlikely that larvae during daily diet switch time those pauses with the presence of the second host. Further studies are needed to find clarity in why host switches to elm is associated with costs when other combinations seem to manage the switch better. According to predictions, a switch including elm should involve smaller costs because of its phylogenetic relatedness to stinging nettle and shared growth form with sallow, however the results from Paper V suggests the opposite pattern. We are not yet certain of which host association is the oldest one of elm and sallow and they may possibly be equally old hosts. The utilization of elm is then perhaps not so much a bridge between two genera as a side effect to the ability to feed on stinging nettle and Salix-species.

Since Paper V did not find a strong tendency towards costs from host plant switch during development, it would not seem difficult for larvae to change host plant half-way through development. Combined with results from Paper III the influence of larval host plant choice
should be regarded to have a greater importance on shaping host plant utilization patterns. Ecologically this signifies that larvae are not as bound to their mothers choice as one might expect. The potential host range defined by larval utilization then changes how we interpret the realized host range. Put in an evolutionary context the establishment of novel hosts in the host plant repertoire might be simpler than previously believed since overlapping patterns of gene expression in larval mid-gut enables utilization on quite different hosts. Larval preadaptation to hosts may then exist long before female oviposition follows.

Regardless of how common it is for larvae to switch host plant in nature the small negative effects of altering diet suggests that the comma butterfly has a great ability to adjust, and together with the differential gene expression that places it somewhere in between a specialist and a generalist. One would expect a generalist to not have differential gene expression depending on host, and the specialist to specialize on only one plant family. The term polyspecialist thus seems to fit the comma butterfly rather well.

CONCLUSIONS

This thesis has described how life-history in the polyphagous comma butterfly is affected by environmental demands, in particular host-plant choice. Female host plant preference has been confirmed to have a genetic background and not be influenced by previous host plant experience in the larval stage. In fact, preference in both the female and life stages is strongly connected to larval performance. In previous studies comma butterflies have been shown to differ in both female specialisation and larval performance depending on their geographic origin (Nylin, 1988; Nylin et al., 2009). Host plant specificity is probably unequally important for different populations of the comma butterfly, either since hosts with higher growth rate increases the probability to manage additional generations or since host plant quality changes throughout season (Nylin et al., 2009). However, the threshold for critical photoperiod and host plant specificity are not inherited together on the X-chromosome and could thus evolve separately. Discovered differences in diapause response between sexes could nevertheless be influenced by some kind of parental affect which could be the result of sexual antagonism.

The individual variation in the acceptance of suboptimal plants reconfirms that differences in host plant specificity exists also within populations. Patterns of host plant utilization are likely to be influenced by both female and larval host plant choice since larvae seems able to compensate for poor female choice by abandoning a suboptimal plant. This could help explain why a generalist oviposition strategy is favored by natural selection even when oviposition accuracy is less than optimal for offspring fitness. Differential gene expression – induced by the ingestion of different plant species – shows larger similarities between closely related species as well as between hosts that share a common growth form. These host-specific responses bring further support to the notion that the comma butterfly is a polyspecialist rather than a true generalist. Despite such a variable gene expression, where mid-gut induced response seems to be tailored to host chemistry, there seem to be small costs in switching diet during development. Consequentially, larvae that are hatched on a sub-optimal plant have the opportunity to change to another (nearby) host if subjected to competition, predators or other problems associated to their original host plant. Results also implies that larval preadaptation to plants affects larval potential host plant range, and that in turn could influence the realized host plant range since it is obviously not determined by female host plant choice alone.
REFERENCES


Ungefär en fjärdedel av alla arter på jorden utgörs av växtätande insekter och ytterligare 25% av de växter som dessa insekter äter av och lägger ägg på. Alltså utgör ungefär hälften av diversiteten idag av insekt-växtinteraktioner och man tror att själva sambandet däremellan kan förklara mycket av båggruppans väldiga framgång.


Populationers anpassning till årstiderna


En hona av den ljusa direktutvecklande morfen. Foto: Niklas Janz

Fjärilar är olika däggdjur så tillvida att det är hanarna som är det homozygota könet (XX) medan honorna är heterozygota (XY). X-kromosomen verkar vara associerad med många gener som har stor betydelse för artskillnader och att X-kromosomen alltså har en viktig roll i artbildning hos Lepidoptera. Mycket tyder på att värdväxtpreferens (se nedan) nedärvs på X-kromosomen hos fjärilar. Den spanska populationen av vinbärsfuks är mer specialiserad än den svenska, vilket

I hybridiseringen var hanar med en svensk far mindre benägna att bli ljusa än hanar med en spansk far. Mekanismen bakom detta mönster är svåra att förutsäga, men vi föreslår att diapausresponsen har influerats av någon form av föräldraeffekt, dvs att egenskapen inte endast påverkas av genotypen eller uppväxtmiljön utan av genotypen och/eller miljön som modern eller fadern bidragit med.

Intressant nog var hanarnas respons i hybridiseringarna inte intermediär till föräldrapopulationerna utan istället mer benägna att utvecklas till den diapauserande formen än honor. Detta kan vara en sexuellt antagonistisk effekt då hanar och honor inte nödvändigtvis har samma optimala livscykel. Honor vinner på att vara stora eftersom de då kan lägga fler ägg. Hanar däremot tjänar på att utvecklas snabbt och fullfölja utvecklingen före honorna eftersom de då kan konkurrera om fler parningar, ett fenomen känt som protandri. Hanar som utvecklas sent eller längstamt gör då ett bättre strategiskt ”val” att utvecklas till den diapauserande morfen eftersom de då har bättre chans att konkurrera om honor på våren istället för att vara för sen till parningarna på sommaren. Skillnader mellan nära besläktade fjärilsarter är ofta könsbundna och har troligtvis sitt ursprung i sexuellt antagonistiska egenskaper. Skillnader mellan honor och hanars diapausrepons kan därmed påverka artbildning hos fjärilar.

**Värdväxtens betydelse**

De larver som hamnat på bra växter skulle visserligen föredra dessa, men på grund av tidsskillingen mellan larvstadium och tiden för äggläggning så kan miljön ha ändrats däremellan. Detta gäller framför allt för arter med fler generationer varje säsong då värdväxterna kan ha ändrats mycket i kvalitet sedan honan själv var larv. HHSP förutsätter också att det borde finnas stora geografiska skillnader i förekomsten av värdväxter, annars skulle en genetisk koppling till värdväxtpreferens vara precis lika tillförlitlig. Att larvmiljön utgör ett bättre underlag till äggläggningsvalet än vad honornas miljö gör måste dessutom betyda att det finns höga kostnader associerade med att honorna samlar information om växternas tillgänglighet i omgivningen. Hos en polyfag art som vinbärsfuks, där värdväxterna varierar kraftigt i lämplighet och där kvalitén hos dessa växter varierar över säsongen, borde således en genetisk koppling mellan äggläggningspreferens och avkommans prestation ha selekterats för och utgöra ett bättre underlag för värdväxtväxter än honornas uppväxtnmiljö. Mycket riktigt så visade resultaten i Papper II att HHSP inte är en giltig teori för vinbärsfuks. Istället för att föredra växten från larvstadiet så överensstämde äggläggningspreferensen med larvernas tillväxthastighet. Således föredrog honorna att lägga ägg på brännässla, som ger högst tillväxthastighet, oavsett vad de vuxit upp på.


**Effekten av olika värdväxter**
Hos vinbärsfuks resulterar olika växter i olika livshistoriekonsekvenser. Om


Det fanns även vissa kostnader i utvecklingshastighet när man flyttade larver endast en gång till alm. Det har ännu inte etablerats utifall vinbärsfuksen och dess släktningar har en äldre relation till alm eller till säl. Det är möjligt att alm inte alls är fungerat som en bro mellan brännässla och säl och utan att användandet av alm snarare är en bieffekt av förmågan att kunna äta de andra två värdarna. I så fall är det möjligt att larverna har förmåga att konsumera växter som ännu inte är del av honornas värdväxtbredd.

Relationen mellan brännässla (Urtica), alm (Ulmus) och säl (Salix). Brännässla och alm är nära släktningar, medan både alm och säl är träd. Genuttrycket i larvernas mittarm skiljde sig mest mellan brännässla och säl och hypotesen var att det skulle vara svårare att flytta mellan dessa växter (1. NS).

Genom att föda upp larver på olika växter och sedan flytta dem i olika larvstadijer visade Papper V att larver generellt sätt anpassar sig bra till de nya omständigheterna. När man flyttade larver varje dag kunde vi hitta en viss effekt av flytten mellan brännässla och säl, vilket skulle kunna bero på att en föda bestående av dess resulterade i så få likheter i genuttryck.

starkt kopplad till sina värväxter och i Storbritannien har man sett att vinbärsfuks ändrat sin preferens för sina värväxter och följer den nordliga utbredningen av dessa. Om miljön förändras, t.ex. till följd av klimatförändringar, är det troligt att värvväxternas utbredning och kvalitet förändras med klimatet. Eftersom diapausrespons inte är direkt kopplad till preferens på samma kromosom så måste båge egenskaper evolvera separat, vilket kan det resultera i ett missförhållande mellan egenskaperna och därmed mellan värvväxtutnyttjande och utbredning.

**Ordlista**

*autosomal* Gen som ligger på en kromosom som inte är sexkromosom, alltså inte på X- eller Y-kromosomen.

*diapaus* Övervintring

*feckunditet* Den potentiella reproduktiva kapaciteten, dvs hur många ungar man får.

*fitness* En individs framgång i termer av överlevnad, storlek, och framförallt hur mycket avkomma individen får och hur levnadsduglig den är.

*fotoperiod* Fördelningen av antalet timmar med dagsljus och mörker på ett dygn, tex innebär 17:7 en fördelning med 17 timmar ljus och 7 timmar mörker.

*kritisk fotoperiod* Gränsen i antalet timmar på ett dygn med dagsljus, under vilken en individ generellt blir den mörka morfen men över den direktutvecklande morfen.

*monofag* En art som äter endast av en värvväxtart, eller av ett fåtal växter inom samma familj.

*morf* En variant med visst utseende av flera möjliga. Vinbärsfuksen finns tex. i två morfer: en ljus direktutvecklande och en mörk diapauserande.

*polyfag* En art som kan äta av flera (avlägset besläktade) värvväxter.

*tillväxthastighet* Genomsnittlig daglig viktökning, alltså slutvikten dividerat med utvecklingsstiden.

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