



Genetic Architecture of Host Use in Yponomeuta
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Summary of Results and Conclusion

The intent of this thesis is to provide empirical data on the genetic architecture of host specialization in phytophagous insects. A detailed understanding of the genetic architecture underlying diversification in host use can help to estimate the likelihood of ecological speciation. The theory of ecological speciation has been reviewed by various authors and from different perspectives. Attempts to evaluate the likelihood of these theories by mathematical modelling are likewise abundantly available (e.g., reviewed by Gavrillets & Losos 2009; Smadja & Butlin 2011). The focus of these models is on speciation, which is defined as the evolution of reproductive isolation between two diverging populations. This thesis, however, focuses not on reproductive isolation but rather on genetic adaptations to the new niche – in our case the new host plant. The nature of these adaptations determines how habitat-dependent selection can act to increase specialization. The genetic architecture of host use, in relation to how diverging host plant adaptation can cause a restriction in gene flow between the two specializing populations (e.g., Via 2009), takes a central position in the current thinking about ecological speciation. However, models of ecological speciation have been validated by few empirical studies.

In order to place our empirical data on genetic architecture into this perspective, we divide the theories roughly into two main categories. In the first category, the genetic architecture of host use is simple, and the diversification in host use of specialized insect herbivores is based on habitat-dependent selection against maladapted individuals. Under this hypothesis, we expect to find a limited number of genes that differ between individuals utilizing different hosts. Disruptive, habitat-dependent selection on the traits controlled by these genes acts on phenotypic variants that are adapted to a new resource, leading to divergent evolution (e.g., Doebeli et al. 2005; Mallet 2008; Smadja & Butlin 2011; Abbott et al. 2013). If a few genes having a large effect control host use, the selection coefficients on the associated traits will be high, increasing the likelihood of diversification of host use (Rice & Hostert 1993; Gavrillets 2004). In the models, these genes are generally inherited in a Mendelian fashion and their alleles can be expected to show strong pleiotropic effects, because pleiotropy increases the likelihood of the trait associations needed for speciation in the face of gene flow (Smadja & Butlin 2011).

Models in the second category presuppose a more complex genetic architecture underlying host use-traits: in this view, traits are the result of the combined expression of alleles at multiple loci, which can also have additive effects or influence each other's expression (epistatic interactions; Yang et al. 2007). Under this hypothesis, we expect to find many traits differing between specialists on different hosts, and each of these traits may be based on separate genes. Moreover, each trait may be the result of the expression of multiple genes. Models in this category conclude that even if multiple genes define the phenotype of the herbivorous specialist, it is likely that ecological speciation will follow host diversification. In the final stages of evolution of reproductive barriers, disruptive selection is less important than in models of the first category: reproductive isolation through

assortative mating can evolve by “hitchhiking” of alleles for mate choice on the regions in the genome on which selection acts to prevent recombination of host-specific co-adapted loci resulting in speciation (Gavrilets 2004; Smadja et al. 2008; Via 2009).

In this thesis, empirical data is presented on the nature of the inheritance of host use traits that differ between species of small ermine moths (*Yponomeuta* spp.), which are highly specialized on their host plant. The collective evidence on the various aspects of host use indicates that in *Yponomeuta*, speciation is based on a complex genetic architecture.

To study the genetic architecture of host use in *Yponomeuta*, the following questions were addressed:

- How do *Yponomeuta* adults discriminate between hosts? (Chapters 2 and 3).
- Is there evidence for heritable variation in adult host discrimination? (Chapter 5).
- Is there evidence for linkage of host recognition by adults and host acceptance by larvae? (Chapter 4 and 5).
- Is there evidence for heritable variation in larval host use? (Chapter 6).
- Is there evidence for linkage between inheritance of larval host acceptance and larval survival on the host? (Chapter 6).
- Is there evidence for hybrid breakdown due to sterility or genomic incompatibility? (Chapter 7).

***Yponomeuta* adults discriminate between plants, using non-volatile plant-surface compounds**

During oviposition, *Yponomeuta cagnagellus* adult females most likely use contact chemoreceptors located on their antennae and/or tarsi. Moths are able to recognize their host plant *Euonymus europaeus* (Celastraceae) solely by its surface compounds. These compounds were washed from the twigs and applied to artificial oviposition substrates. Methanolic extracts of the twig surface, applied onto an artificial stem, were sufficient to stimulate the complete sequence of behavioural steps leading to oviposition, and this response was dose dependent. Moreover, adults were able to distinguish between the methanolic extract of their host plant and the methanolic extracts of the non-hosts *Crataegus monogyna* and *Prunus spinosa* (both of which are in the Rosaceae, and host plants for the related *Y. padellus*). The exact adult chemoreceptors involved and the compounds on which adult host discrimination is based remain to be identified.

There is a strong heritable component in adult host acceptance in *Yponomeuta*

F1 hybrids of *Y. cagnagellus* × *Y. padellus* deposited on average more than 80% of their egg masses on *E. europaeus* when given a choice between this host and *P. spinosa*. F1 hybrids of *Y. cagnagellus* with another species, *Y. malinellus*, displayed a similar behaviour in the choice between *E. europaeus* and *Malus domestica* (host plant of *Y. malinellus*), suggesting a semi-dominant inheritance of the acceptance of *E. europaeus*. This mode of inheritance was confirmed in oviposition tests with backcross hybrids: backcross hybrid F1

× *Y. cagnagellus* oviposited mainly on *E. europaeus* and F1 × *Y. padellus* were more likely to accept *P. spinosa*, but still deposited more than half of their egg masses on *E. europaeus*. The trait is not sex-linked: host acceptance did not differ between reciprocal hybrids.

Host acceptance of hybrids was shown to be dependent on chemical compounds present in the methanolic extract of host twig surface. This led us to conclude that host discrimination is encoded by genes that are involved in the chemosensory detection of relatively polar, non-volatile compounds present on the plant surface.

We found that *Y. padellus* was most likely to accept the non-host *E. europaeus*, whereas *Y. cagnagellus* rarely accepted its non-host. Whether this is due to an inherently lower host fidelity (*Y. padellus* is an oligophagous species) or due to sensitivity to phytochemical components common to both *E. europaeus* and *P. spinosa*, remains to be elucidated. The observation suggests either a predisposition to accepting *E. europaeus* in the species now utilizing rosaceous hosts or the occurrence of phenotypic plasticity, allowing oviposition mistakes (Thoming et al. 2013).

Our data suggests that adult host acceptance is based on more than one gene, involved in processing chemosensory neural input from phytochemical compounds that characterise the host plants of these species. The data does not allow us to conclude whether host acceptance is driven by host-specific stimulants (Resh & Cardé 2009), by deterrents causing rejection of the non-host (Chapman 1993), or a combination of the two. Further work on the identification of host-specific compounds and the associated behavioural response of adult moths is necessary, and this can be followed by the identification of the genes coding for the receptor proteins controlling acceptance behaviour.

There is only limited evidence that dulcitol stimulates oviposition in *Y. cagnagellus*

The ancestral host shift of European *Yponomeuta* from Celastraceae to Rosaceae may have been facilitated by the presence of small amounts of dulcitol in some Rosaceae (Fung & Herrebut 1988; Menken et al. 1992; Menken & Roessingh 1998). Dulcitol is the main sugar alcohol found in the Celastraceae, and sugar alcohols are known to act as phagostimulants in *Yponomeuta* (Herrebut et al. 1987, Peterson et al. 1990). We found that the sugar alcohol dulcitol is also present on the surface of its host, *E. europaeus*. However, we found only limited evidence that dulcitol stimulates acceptance of a plant for oviposition to adult *Y. cagnagellus*. In addition, when dulcitol was removed from host plant extracts, oviposition stimulatory activity of this extract was retained. High concentrations of dulcitol, in combination with the main stimulatory fraction of the host extract, can enhance oviposition acceptance, but this effect was modest.

This result indicates that more than one compound is involved in host acceptance behaviour but does not preclude a pleiotropic relationship between the various alleles of the genes that control larval feeding behaviour and adult oviposition. Acceptance behaviour may still be based, in addition to the sugar alcohols, on as-yet unidentified compounds, that also act

as species-specific larval phagostimulants. A test of this hypothesis would benefit from the development of a phytochemically neutral artificial diet for *Yponomeuta* caterpillars. Attempts to do this have not yet been successful.

The genetic basis of adult host acceptance is subject to phenotypic plasticity, as evidenced by the difference in adult host choice of hybrid siblings reared on each of the parental hosts in a full-sib split-family design. Larval diet influenced oviposition in hybrids of *Y. padellus* × *Y. cagnagellus*, reared on the host of *Y. padellus*, *P. spinosa*. However, the heritable component affects adult acceptance more than larval experience does: larvae with no prior experience of *E. europaeus* still deposited more than half of their egg masses on *E. europaeus*. Even so, phenotypic plasticity can contribute to host-plant adaptation: for those individuals that are able to survive on the new host, the effect of larval experience on adult host choice could contribute – be it modestly- to maintenance of the host association. Further study of the effect of the larval host on the expression of genes underlying adult host acceptance may well increase our understanding of how the inherited component can be modified by previous experience.

With certainty, we thus identified two independent heritable traits that need to become co-adapted in the course of host specialization in *Yponomeuta*: the sensitivity to sugar alcohols, important for larval host discrimination, and the sensitivity to host-surface compounds, which shapes adult host discrimination. In a recent review, Matsubayashi et al. (2010) summarized empirical data on host-use traits in the context of ecological speciation. They concluded that studies focusing on both larval host preference and adult preference are rare. Studies investigating the relationship between host acceptance (as a measure of 'preference') and host avoidance ('deterrence') are particularly lacking (Forbes et al. 2005). In the few studies that are available, differences in host choice between closely related taxa usually appear to be under simple genetic control, but examples of polygenic control, and therefore more complex interactions, also exist (e.g., Henniges-Janssen 2011; Oppenheim et al. 2012). More work elucidating the physiological and genetic mechanisms underlying both larval and adult acceptance behaviour is required before generalizations can be made as to the impact of these traits on ecological speciation.

Multiple loci are involved in acceptance of and survival on the host

Larval host use is a combination of host acceptance for feeding and the capability of the larvae to develop on the host plant, that is, to reach adulthood. Obviously, the two traits are mutually dependent. Larval development is based on the capacity of individuals to successfully digest the plant and to cope with unpalatable and/or toxic secondary plant compounds. But being able to develop on a host is of little consequence to the individual that does not accept that host for feeding. Although it seems to be a chicken-or-egg problem, there is evidence that suggests that the evolution of acceptance behaviour precedes physiological adaptation to the host (Gassmann et al. 2006).

F1 hybrids of *Y. cagnagellus* and *Y. padellus* carry a full co-adapted complement of genes for use of the host plants of both parents, much like heterozygotic individuals in a hypothetical

ancestral population with dual host use. These first generation hybrids fed and developed well on both host plant species. The highest pupal weights – and corresponding fecundity – were always achieved on *E. europaeus*, the host of *Y. cagnagellus*.

Backcrosses of the F1 with either parent rarely survived on the host of the least-contributing parental species. Offspring of two F1 parents (F2 hybrids) could not be reared to adulthood on either host: more than 90% died before reaching the third instar, irrespective of the host plant. This may be attributed to the breakdown of the co-adapted gene complex for host use by recombination of the parental genomes in the F1 hybrids: a large proportion of the gametes produced by the F1 does not transmit the full set of co-adapted host-use genes to the next generation. Backcrosses need a full complement of the relevant genes for their development. These results support the conclusion that there are multiple loci involved in the 'larval survival on the host' trait.

We now may ask how many genes are involved in larval survival on the host? Based on the fraction of surviving backcrosses on the diet of the least-contributing parent, we estimate that at least three independently inherited genes have become co-adapted for larval development on *E. europaeus*. We cannot rule out that these traits are subject to non-Mendelian inheritance (Pfennig & Servedio 2013), which complicates the estimation, and considerable theoretical and empirical work remains to be done to identify the cause(s) of host-induced mortality of non-adapted individuals.

Genes controlling larval host acceptance and larval survival are unlinked

There is ample intraspecific variation in host acceptance behaviour, as can be seen from the considerable individual variation and plasticity in chemosensory responses to plant compounds (Schoonhoven & van Loon 2002). This variability can facilitate host shifts of insect herbivores to new resources. Host acceptance, therefore, is not expected to be the main hurdle for a host shift. Our results indicate that physiological adaptation of the larva to the new host, which needs to occur once the variation for acceptance of the new host has become established in a population, is a more difficult step to take before the host shift becomes evolutionarily successful. Furthermore, if the traits are inherited independently, genes coding for acceptance of and development on the new host must reach a linkage disequilibrium in the population (Barton & de Cara 2009). In chapter 6, an estimated minimum of three co-adapted genes for survival on *E. europaeus* were reported in *Yponomeuta*. The relationship between host acceptance and host adaptation in the resulting trait, host survival, was further studied. Is physiological adaptation associated with larval host acceptance, either through pleiotropy or genomic linkage of the genes underlying these traits? As described above, backcross hybrids rarely survived on the host of the least-contributing parent. However, they may yet accept this host when given the choice, pointing to independent inheritance of survival and acceptance. Choice tests clearly showed that in backcrosses, acceptance of *E. europaeus* was more pronounced than acceptance of *P. spinosa*. As a result, backcrosses with *Y. padellus* did not survive when offered both hosts simultaneously: larvae chose to feed on *E. europaeus*, but did not manage to develop completely, because the majority of the larvae did not inherit the full complement of co-

adapted genes for performance. Backcrosses with *Y. cagnagellus* were able to reach adulthood on a mixed diet, and we observed that this is probably caused by avoidance of the *P. spinosa* leaves in the diet.

In the context of ecological speciation through host shifts, the extent to which host choice involves preference for one host, avoidance of alternative hosts, or both, is an unresolved question. In most studies, it is unknown whether avoidance of non-host plants is caused by a lack of physiological adaptation or by behavioural restrictions (Nyman 2010). Moreover, the genetic bases of host plant preference and of performance have rarely been investigated in the same herbivore-plant combination (Matsubayashi et al. 2010). Further studies on quantification of food intake of hybrid larvae in response to phytochemicals, together with studies on the physiological tolerance to host-specific plant compounds, are recommended.

There is no decrease in hybrid fitness due to incompatibility of the parental genomes

The fertility of the F1 and backcross hybrids was only slightly less than that of the offspring of intraspecific crosses. Nevertheless, cytogenetic study of hybrid meiosis showed evidence of chromosomal rearrangements between the karyotypes of the parental species. These rearrangements were responsible for irregularities in the meiotic chromosome pairing observed in hybrids. Even though chromosomal rearrangements between the species were detected, we did not find a direct selective disadvantage of hybridizing individuals resulting from the rearrangements. The limited evidence for hybrid sterility indicates that postzygotic reproductive isolation between the sister species is still in its infancy.

The chronology of the evolution of different types of reproductive barriers is important for understanding speciation. Of course, we have been studying the genetic differences between the species retrospectively, including those that evolved after the establishment of the pre-zygotic reproductive barrier that separates the species today. The relative importance of barriers that exist between current species at this moment does not necessarily reflect their importance at the time that divergent host use led to reproductive isolation (Coyne & Orr 2004; Via 2009).

From our data it is clear that for *Yponomeuta*, the current pre-zygotic reproductive barrier, based on differential sex-pheromonal blends, is the main barrier separating present-day species in sympatry (Hendrikse 1979; Löfstedt & Van der Pers 1985; Löfstedt et al. 1991). Direct selection against hybridizing individuals due to intrinsic hybrid incompatibility is not to be expected from the observed genomic structural differences.

It is interesting to explore whether the rearrangements we have observed may have facilitated co-adaptation of host-use genes during the divergence of the populations. Theoretically, the regions observed on the chromosomes that cause disturbance in synapsis during meiosis, could serve to locally reduce the effect of recombination between co-adapted genes, if these genes are located in those regions. The inhibitory effect on

recombination from structural differences such as inversions must be very strong to result in a linkage disequilibrium in a population. Even low levels of recombination are quite effective at breaking down associations between genes in a randomly mating population (Feder & Nosil 2009).

Conclusion

Our main conclusion is that host use in *Yponomeuta* is controlled by a complex of co-adapted genes that need to be present in the right combination. Formation of gametes in F1 hybrids breaks down these complexes and results in offspring that may oviposit on a host that the larvae may not accept as food, or the larvae may lack the required alleles for survival on the host they are likely to accept. The fitness of second-generation hybrids is therefore strongly influenced by the covariance between three factors: chance recombination of alleles in the hybrid parent, parental mate choice, and maternal choice of larval host plant.

Our findings suggest a genetic architecture of host use, characterised by quantitative inheritance, based on multiple, independently segregating genes. This causes breakdown of fitness in second-generation hybrids. This provides an advantage for individuals to select mates with a similar set of adapted genes, and can favour the evolution of assortative mating (Via 2009). In this way, host specialization in *Yponomeuta* might have been facilitated rather than opposed by this relatively complex genetic architecture. Our empirical findings support the two-stage model described by Via (2009). However they may not fit this model exactly. The two-stage model supposes quantitative trait loci QTL underlying a single key trait in host use, whilst our data show that host specialization in *Yponomeuta* seems dependent on adaptation of multiple independent loci underlying different traits, and the phenotype of some of these traits is determined by more than one locus.

In first generation hybrids, we found no evidence for a direct decrease in fitness of these heterozygous individuals, neither because of hybrid genomic incompatibility (Coyne & Orr 2004), nor because of host plant-dependent hybrid inviability caused by mismatch between the hybrid genotype and the parental hosts (Rundle & Whitlock 2001; Matshbayashi et al. 2010). However, there is evidence in support of indirect selection against hybridization. Due to recombination of the independent alleles for host use in the hybrid genome, hybrids are likely to produce gametes that do not carry the full set of co-adapted alleles. This breaking up of the co-adapted alleles causes a host plant-dependent hybrid breakdown, visible in second-generation hybrids (Felsenstein 1981). This also suggests a historical fitness advantage for individuals that do manage to select their mate on the basis of similarity of their co-adapted alleles: they will produce well-adapted, homozygous offspring.

The challenge in predicting the probability of the evolution of assortative mating in sympatry lies in the correct estimate of the loss of hybrid fitness as a basis for disruptive selection, caused by the observed architecture for host use (Rundle & Whitlock 2001).

Although our data are strongly indicative of a role for this loss of hybrid fitness, they unfortunately still lack sufficient resolution to provide such an estimate.

Implications of genetic architecture of host specialization for speciation in *Yponomeuta*

Considering the phylogenetic history of the Western European *Yponomeuta* species, does the empirical evidence presented in this thesis shed light on the host shifts that occurred within the European clade of *Yponomeuta*? As suggested by Nyman (2010), we must consider that ecological speciation may have occurred in different geographic contexts: even for species that are now completely sympatric, the current geographic distribution of the hosts may not reflect the ancestral situation. Also, the mechanism(s) for speciation may differ between various branching points in the phylogeny. Indeed, in phylogenetic studies of 'recent' changes in host relationships in phytophagous insects, it has been shown that associations can involve both allopatric and sympatric elements (Feder et al. 2003; Xie et al. 2007)

In the phylogenetic history of *Yponomeuta* in Europe, two major host shifts between Celastraceae and Rosaceae have occurred: first from Celastraceae to Rosaceae at the base of the European clade, and second, a shift back from Rosaceae to Celastraceae in the common ancestor of *Y. padellus* (feeding on Rosaceae) and *Y. cagnagellus* (feeding on Celastraceae) (Turner et al. 2010; Ulenberg et al. 2009).

The empirical data gathered by crossing *Y. cagnagellus* and *Y. padellus* do not allow us to identify the nature of the genetical architecture that has led to the specialized host associations from the ancestral populations at the base of the Western European clade of *Yponomeuta*. We can only speculate on the selection pressures that are likely to have shaped those ancestral host associations. We cannot rule out that the shift from Celastraceae to Rosaceae may have been caused by geographical isolation of a population in a region in which *Euonymus* was rare or even completely absent. Unfortunately, the historical biogeography of both Rosaceae and Celastraceae in Europe is basically unknown, because insect-pollinated species are hardly represented in the pollen record (Faegri et al. 1989). However, assuming the current low number of species of Celastraceae (*E. europaeus* is the only species native to much of Europe) compared to the large number of rosaceous species in Europe is comparable to the numbers at the time the host shift from Celastraceae to Rosaceae occurred, this might have led to a greater ecological opportunity for a shift to Rosaceae on the European continent (Araujo et al. 2011).

The ancestral lineage of the Western European *Yponomeuta* species must have acquired genetic variation that enabled this taxon to include Rosaceae in its diet. The presence of phytochemically similar hosts encountered after the geographic expansion to Europe (Nyman 2010), in combination with a low availability of the original host, could have selected for individuals that could utilize rosaceous plants. The absence, or low availability, of Celastraceae in the habitat, could lead to a fitness benefit of individuals capable of utilizing the more abundant rosaceous host plants. This benefit would enable an increase of

individuals in the population adapted to Rosaceae, even if fitness is lower than on the original Celastraceae host (Janz & Nylin 2008; Agosta et al. 2010). Because of absence or low availability of Celastraceae in the habitat, some of the ability to utilize Celastraceae could have been retained, without disturbing the incipient adaptation on rosaceous plants. The genetical architecture in host use described in this thesis can explain how the more recent back-shift from Rosaceae to a celastraceous host (resulting in what we now know as *Y. cagnagellus*), could have occurred in complete sympatry. The (semi-)dominant inheritance of several host use traits favors both fitness on, and larval and adult acceptance of, *E. europaeus*. It is not hard to see how genetic variability for inclusion of Celastraceae in the diet could spread in a population. The shift back from Rosaceae to Celastraceae would have been facilitated if the genes for use of Celastraceae were still retained in the population that had expanded to the European continent. This assumption is supported by *Y. padellus*, which still occasionally accepts *E. europaeus* twigs for oviposition and by the sensitivity of Rosaceae-specialists for dulcitol that is only found in a few representatives of the Rosaceae, in particular *Prunus* species (van Drongelen 1979).

After inclusion of the celastraceous host in the diet, reproductive isolation apparently evolved. There is at present no evidence of a pleiotropic coupling between host use and mate choice in *Yponomeuta*, although a closer investigation of the effect of larval diet on production of the male sex-pheromone is a point of interest. Without a pleiotropic effect, assortative mating requires the selection of individuals that mate within their own host-specific population. This is a prerequisite for the evolution of reproductive barriers (e.g. Kirkpatrick 2001; Nosil et al. 2003, Smadja & Butlin 2011). Our results point to a selection for assortative mating based on host plant-dependent survival of the second generation of hybrids. Survival on a particular host depends on the partner chosen by the F1 hybrid, which determines the genetic make-up of the progeny and thereby determines survival on one of the two alternative hosts.

To explain in more detail how the evolution of current pre-zygotic reproductive barrier between *Y. padellus* and *Y. cagnagellus* may have been affected by the host use architecture, we have made two assumptions: 1. The rosaceous and celastraceous hosts of the –oligophagous - ancestral taxon at the base of the segregation between *Y. padellus* and *Y. cagnagellus* resembled *P. spinosa* and *E. europaeus*, respectively. 2. The genetic variation in host use between present-day *Y. padellus* and *Y. cagnagellus* reflects the variation present in the ancestral population.

The observed dominance in inheritance for host use of *E. europaeus* causes different consequences for the fitness of offspring of heterozygous females of choosing a mate adapted either to the Rosaceae or the Celastraceae. Mating with a male adapted to Celastraceae is not disadvantageous, regardless of the composition of host-use alleles of the female: they have inherited the coadapted alleles of survival on the preferred celastraceous host from their father. However, mating with a male homozygous for use of Rosaceae compromises the chance of survival of the offspring of any heterozygous female. Such a female may choose to oviposit on Celastraceae, and a significant part of her

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offspring will prefer feeding on Celastraceae, but most are not equipped with the full complement of genes for survival on Celastraceae.

Selection is in favour of those heterozygous females that reject males adapted to the rosaceous host plant. This may give rise to a linkage-disequilibrium, favouring the combination of alleles for host use on Celastraceae with alleles supporting female mating preference for males that are themselves adapted to Celastraceae. This behavioural adaptation of females will increase inbreeding in the population specializing on Celastraceae, allowing fixation of co-adapted genes in the population. With further gustatory and physiological adaptations to the new host, fitness on this host increases, correspondingly increasing the number of alleles underlying the phenotype of the celastraceous specialist, leading to the (complex) genetic architecture of host use that we see today.

References

- Abbott, R, D Albach, S Ansell, JW Arntzen, SJE Baird, N Bierne, JW Boughman, A Brelsford, CA Buerkle, R Buggs, R K Butlin, U Dieckmann, F Eroukhmanoff, A Grill, SH Cahan, JS Hermansen, G Hewitt, AG Hudson, C Jiggins, J Jones, B Keller, T Marczewski, J Mallet, P Martinez-Rodriguez, M Most, S Mullen, R Nichols, AW Nolte, C Parisod, K Pfennig, AM Rice, MG Ritchie, B Seifert, CM Smadja, R Stelkens, JM Szymura, R Vainola, JBW Wolf & D Zinner 2013. Hybridization and speciation. *Journal of Evolutionary Biology* 26: 229-246.
- Agosta, SJ, N Janz & DR Brooks 2010. How specialists can be generalists: resolving the "parasite paradox" and implications for emerging infectious disease. *Zoologia* 27: 151-162.
- Araujo, MS, DI Bolnick & CA Layman 2011. The ecological causes of individual specialisation. *Ecology Letters* 14: 948-958.
- Barton NH & MA de Cara 2009. The evolution of strong reproductive isolation. *Evolution* 63: 1171-1190.
- Coyne JA & HA Orr 2004. *Speciation*. Sinauer Associates, Sunderland, MA, USA.
- Doebeli M, U Dieckmann, JAJ Metz & D Tautz 2005. What we have also learned: adaptive speciation is theoretically plausible. *Evolution* 59: 691-695.
- Drongelen, W 1979. Contact chemoreception of Host plant specific chemicals in larvae of various *Yponomeuta* species (Lepidoptera). *Journal of Comparative Physiology* 134: 265-279.
- Faegri K, PE Kaland & K Krywinski 1989. *Textbook of Pollen Analysis*. 4th edition. Alden Press, London.
- Feder JL & P Nosil 2009. Chromosomal inversions and species differences: when are genes affecting adaptive divergence and reproductive isolation expected to reside within inversions? *Evolution* 63: 3061-3075.
- Feder JL, SH Berlocher, JB Roethele, H Dambroski, JJ Smith, WL Perry, V Gavrilovic, KE Filchak, J Rull & M Aluja 2003. Allopatric genetic origins for sympatric host-plant shifts and race formation in *Rhagoletis*. *Proceedings of the National Academy of Sciences of the USA* 100: 10314-10319.
- Felsenstein J 1981. Skepticism towards Santa Rosalia, or why are there so few kinds of animals? *Evolution* 35: 124-138.
- Forbes AA, J Fisher & JL Feder 2005. Habitat avoidance: Overlooking an important aspect of host-specific mating and sympatric speciation? *Evolution* 59: 1552-1559.
- Fung SY & WM Herrebut 1988. Sorbitol and dulcitol in some celastraceous and rosaceous plants, hosts of *Yponomeuta* spp. *Biochemical Systematics and Ecology* 16: 191-194.
- Gassmann AJ, A Levy, T Tran & DJ Futuyma 2006. Adaptations of an insect to a novel host plant: a phylogenetic approach. *Functional Ecology* 20: 478-485.
- Gavrilets S 2004. *Fitness Landscapes and the Origin of Species*. Princeton University Press, Princeton, NJ, USA.

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- Gavrilets S & JB Losos 2009. Adaptive Radiation: Contrasting Theory with Data. *Science* 323: 732-737
- Hendrikse A 1979. Activity patterns and sex pheromone specificity as isolating mechanisms in eight species of *Yponomeuta* (Lepidoptera: Yponomeutidae). *Entomologia Experimentalis et Applicata* 25: 172-180.
- Henniges-Janssen K, A Reineke, DG Heckel & AT Groot 2011. Complex inheritance of larval adaptation in *Plutella xylostella* to a novel host plant. *Heredity* 107: 421-432.
- Janz N & S Nylin 2008. The oscillation hypothesis of host plant-range and speciation. In *Specialization, Speciation, and Radiation: the Evolutionary Biology of Herbivorous Insects* (ed. K. J. Tilmon), pp. 203–215. University of California Press, Berkeley, CA.
- Kirkpatrick M 2001. Reinforcement during ecological speciation. *Proceedings of the Royal Society of London B: Biological Sciences* 268: 1259–1263.
- Herrebut WM, SY Fung & RE Kooi 1987. Sugar alcohols and host-plant selection in *Yponomeuta*. In: *Proc. 6th. Int. Symposium Insect-Plant Relationships (Pau 1986)*. V Labeyrie, G Fabres & D Lachaise (eds), pp 257-260. Junk, Dordrecht.
- Löfstedt C & JNC Van der Pers 1985. Sex pheromones and reproductive isolation in four european small ermine moths. *Journal of Chemical Ecology* 11: 649-666.
- Löfstedt C, W Herrebut & SBJ Menken 1991. Sex pheromones and their potential role in the evolution of sex reproductive isolation in small ermine moths (Yponomeutidae). *Chemoecology* 2: 20-28.
- Mallet J 2008. Hybridization, ecological races, and the nature of species: empirical evidence for the ease of speciation. *Philosophical Transactions of the Royal Society B-Biological Sciences* 363: 2971–2986.
- Matsubayashi KW, I Ohshima & P Nosil 2010. Ecological speciation in phytophagous insects. *Entomologia Experimentalis et Applicata* 134: 1–27.
- Menken SBJ, WM Herrebut & JT Wiebes 1992. Small ermine moths (*Yponomeuta*): their host relations and evolution. *Annual Review of Entomology* 37: 41–66.
- Menken SBJ & P Roessingh 1998. Evolution of insect–plant associations: sensory perception and receptor modifications direct food specialization and host shifts in phytophagous insects. *Endless Forms: Species and Speciation* (ed. by DJ Howard & SH Berlocher), pp. 145–156. Oxford University Press, UK.
- Nosil P, BJ Crespi J & CP Sandoval 2003. Reproductive isolation driven by the combined effects of ecological adaptation and reinforcement. *Proceedings of the Royal Society of London B: Biological Sciences* 270: 1911–1918.
- Nyman T 2010. To speciate, or not to speciate? Resource heterogeneity, the subjectivity of similarity, and the macroevolutionary consequences of niche-width shifts in plant-feeding insects. *Biological Reviews* 85: 393-411.
- Oppenheim, SJ, F Gould & KR Hopper 2012. The Genetic Architecture of a Complex Ecological Trait: Host Plant Use in the Specialist Moth, *Heliothis subflexa*. *Evolution* 66: 3336-3351.
- Peterson SC, WM Herrebut & RE Kooi 1990. Chemosensory basis of host colonization by small ermine moth larvae. *Proceedings Koninklijke Nederlandse Academie van Wetenschappen* 93: 287-294.
- Pfennig DW & M Servedio 2013. The role of transgenerational epigenetic inheritance in diversification and speciation. *Non-Genetic Inheritance* 1:17-26.

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Chapter 8 – Summary of results and Conclusion.

- Resh, VH & RT Cardé (eds) 2003. Encyclopedia of Insects, Academic Press/Elsevier Science, San Diego, USA, 1024 pp.
- Rice WR & EE Hostert 1993. Laboratory experiments on speciation: what have we learned in 40 years? *Evolution* 47: 1637-1653.
- Rundle HD & M Whitlock 2001. A genetic interpretation of ecologically dependent isolation. *Evolution* 55: 198-201.
- Schoonhoven, LM & JJA van Loon 2002. An inventory of taste in caterpillars: Each species its own key. *Acta Zoologica Academiae Scientiarum Hungaricae* 48: 215-263.
- Smadja, CM & RK Butlin 2011. A framework for comparing processes of speciation in the presence of gene flow. *Molecular Ecology* 20: 5123-5140.
- Smadja C, J Galindo & R Butlin 2008. Hitching a lift on the road to speciation. *Molecular Ecology* 17: 4177-4180.
- Thoming G, MC Larsson, BS Hansson and P Anderson 2013. Comparison of plant preference hierarchies of male and female moths and the impact of larval rearing hosts. *Ecology* 94: 1744-1752.
- Turner, H, N Lieshout, WE Van Ginkel & SBJ Menken 2010. Molecular phylogeny of the small ermine moth genus *Yponomeuta* (Lepidoptera, Yponomeutidae) in the Palaearctic. *PLoS ONE* 5(3): e9933. doi:10.1371/journal.pone.0009933
- Ulenberg, SA 2009. Phylogeny of the *Yponomeuta* species (Lepidoptera, Yponomeutidae) and the history of their host plant associations. *Tijdschrift voor Entomologie* 152: 187-201.
- Via S 2009. Natural selection in action during speciation. *Proceedings of the National Academy of Sciences of the USA* 106: 9939-9946.
- Xie X, J Rull, A Michel, S Velez & AA Forbes 2007. Hawthorn-infesting populations of *Rhagoletis pomonella* in Mexico and speciation mode plurality. *Evolution* 61: 1091-1105.
- Yang J, J Zhu & RW Williams 2007. Mapping the genetic architecture of complex traits in experimental populations. *Bioinformatics* 23: 1527-1536.