GENOMIC MAPPING OF REPRODUCTIVE ISOLATION IN THE LARCH BUDMOTH
29 July 2002 (updated 2004)

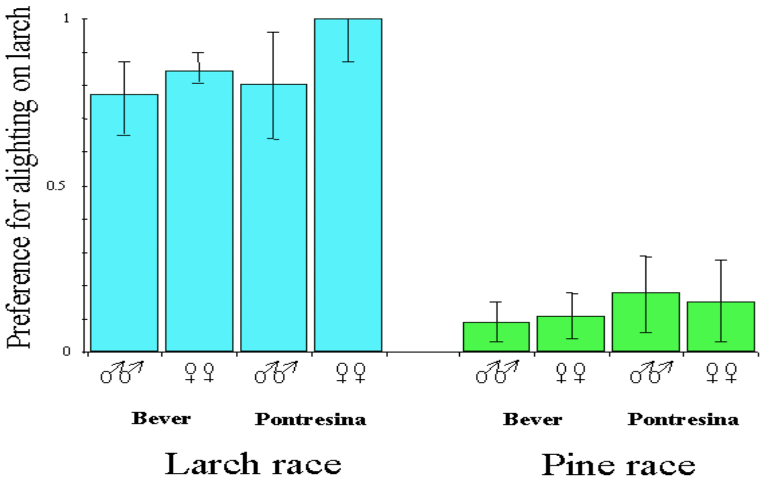
Work carried out on *Zeiraphera diniana* (Lepidoptera: Tortricidae) over the last four years has led to this moth joining the two or three best-studied systems of host races world-wide. We review the host race phenomenon in phytophagous insects, and discuss its relevance to adaptive and sympatric speciation (1). We have studied pre-mating isolation between these host races in detail, and used this work to obtain direct estimates of hybridization of about 3% in the wild (2-4), and we have shown that genomic incompatibility, or post-mating isolation, is minimal (5). Finally, we have shown using abundant AFLP markers (amplified fragment length polymorphisms) and mitochondrial DNA that hybridization actually occurs in wild populations, and that it produces a mosaic pattern of divergence across the genome, as expected under models of "divergence-with-gene-flow" (6, 7).

1) Host races in plant-feeding insects and their importance in sympatric speciation

Host races in plant-feeding insects have often been cited as evidence for sympatric speciation. We provide operational criteria to distinguish host races from other biotypes. In brief: *host races are genetically differentiated, sympatric populations of parasites that use different hosts, and between which there is appreciable gene flow*. We view host races as kinds of species that regularly exchange genes with other species at a rate of more than about 1% per generation, rather than members of a fundamentally distinct kind of taxon. Host races provide a convenient, although admittedly somewhat arbitrary intermediate stage along the speciation continuum between polymorphism and species.

Our review of the data from many studies of host races in phytophagaous insects is perhaps the most complete to date (see also Berlocher & Feder 2002 Ann. Rev. Ent., whose review takes a different approach). Our review favours the idea of sympatric speciation via host shift for three major reasons: **1**. The evolution of assortative mating as a pleiotropic by-product of adaptation to a new host seems likely, even in cases where mating occurs away from the host. **2**. Stable genetic differences in half the cases attest to the power of natural selection to maintain multilocus polymorphisms with substantial linkage disequilibrium, in spite of probable gene flow. **3**. This linkage disequilibrium should permit additional host adaptation, leading to further reproductive isolation via pleiotropy, and also provides conditions suitable for adaptive evolution of mate choice (reinforcement) to cause still further reductions in gene flow (Drès & Mallet, 2002).

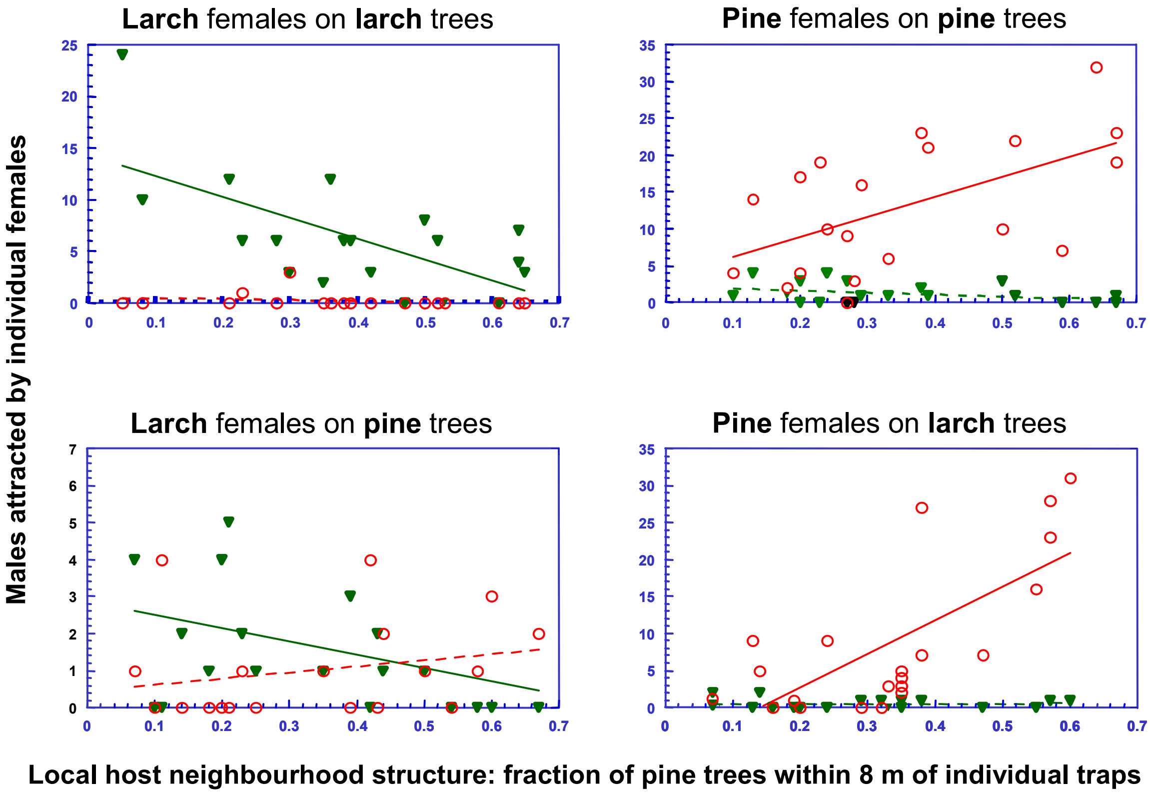
2) Host choice promotes reproductive isolation between host races of the larch budmoth *Zeiraphera diniana* – pre-mating isolation I



We investigated alighting preferences (above; error bars represent 95% confidence limits) using a novel likelihood analysis of genotypic clusters. Moths of both sexes were collected from trees in natural mixed forest, and analysed for genotypes at three semi-diagnostic allozyme loci. Both sexes of larch and pine races show realised alighting preferences for their own host of 79-86%. These preferences are also detectable in small-scale laboratory experiments: alighting preferences of larch and pine races towards their own hosts were 63-69%. Laboratory crossing experiments showed that these alighting preferences were heritable and approximately additive (Emelianov et al. 2003).

3) Host-induced assortative pheromone attraction in the larch budmoth – pre-mating isolation II

Zeiraphera diniana females attract males ("call") by releasing host-independent long-range pheromones. Pheromone composition differs strongly between host races, but we show in an experimental field study that cross-attraction can occur naturally at a rate of 3-38%. Cross-attraction to larch and pine females increases when they call from the other race's host species or from neighborhoods rich in the others' host. (See figure below. Circles: attraction to pine females. Inverted triangles: attraction to larch females).



Thus, the host plant plays a strong role in pre-mating isolation; if females and males choose particular hosts on which to alight, as shown in (2) above, this host choice will have a pleiotropic effect that causes pre-mating isolation. In the larch budmoth today, pheromone specificity determines most of the mating barrier between the races. However, our results also show how host choice alone can also have a strong effect during the initial stages of host race divergence (Emelianov et al. 2001).

4) Hybridization rates between host races of the larch budmoth *Zeiraphera diniana* in laboratory choice tests – pre-mating isolation III

Using a quartet mate choice design (one male and one female of each of two biotypes per cage), we have shown that the overall degree of hybridization between larch and pine biotypes of *Z. diniana* at close range is approximately 28%. This is a measure of pre-mating isolation between host races after individuals have been brought into close proximity, for example via pheromone attraction (Drès 2000).

These laboratory estimates of hybridization, when combined with field studies of host choice and cross-attraction, and adult alighting preferences in the wild (studies 2-3), yield an estimated rate of natural hybridization between sympatric host races of 2.2-3.8% per generation in the wild (Emelianov et al. 2004).

5) Relative fitness of larch budmoth *Zeiraphera diniana* host races and their hybrids – evidence for lack of post-mating isolation

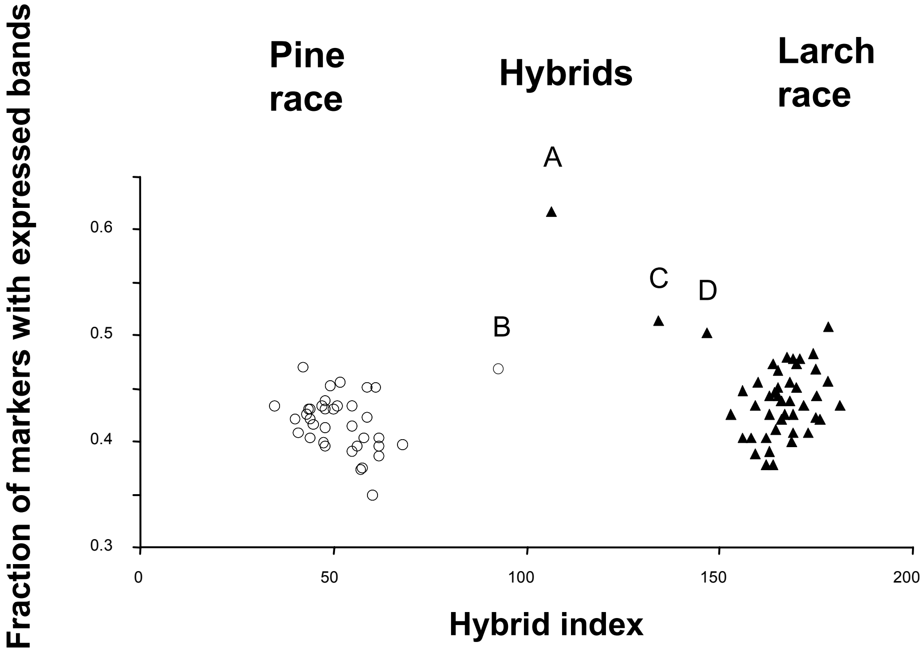
Larch and pine host races of *Zeiraphera diniana* maintain genetic differences at several traits while sustaining actual gene flow of approximately 3% per generation. Given the extent of gene flow, these differences must be maintained via disruptive selection, and might be operating against hybrid forms in the wild. However, we found no evidence that larch, pine, hybrid or backcross broods differ in their percentage of hatching eggs, or larvae surviving to final instar in the laboratory. As with previous studies, we found no evidence for deviation from 1:1 sex ratios in any brood type. Thus, ecological factors apparently play a predominant role in maintaining differences between biotypes (Drès 2000).

6) Lack of mitochondrial DNA divergence between host races of the larch budmoth

Larch and pine associated races of *Zeiraphera diniana* cannot be distinguished on the basis of nucleotide sequence across an 811 bp region of mitochondrial COI (partial), tRNA-leu, and COII (partial). This lack of differentiation is expected if there is no disruptive selection on mitochondria, if the host races hybridize, and if at least some of this gene exchange is female-mediated (Drès 2000). The work adds to existing allozyme data (Emelianov et al. 1995: Heredity 75: 416-424), and to the AFLP genomic survey (7, below).

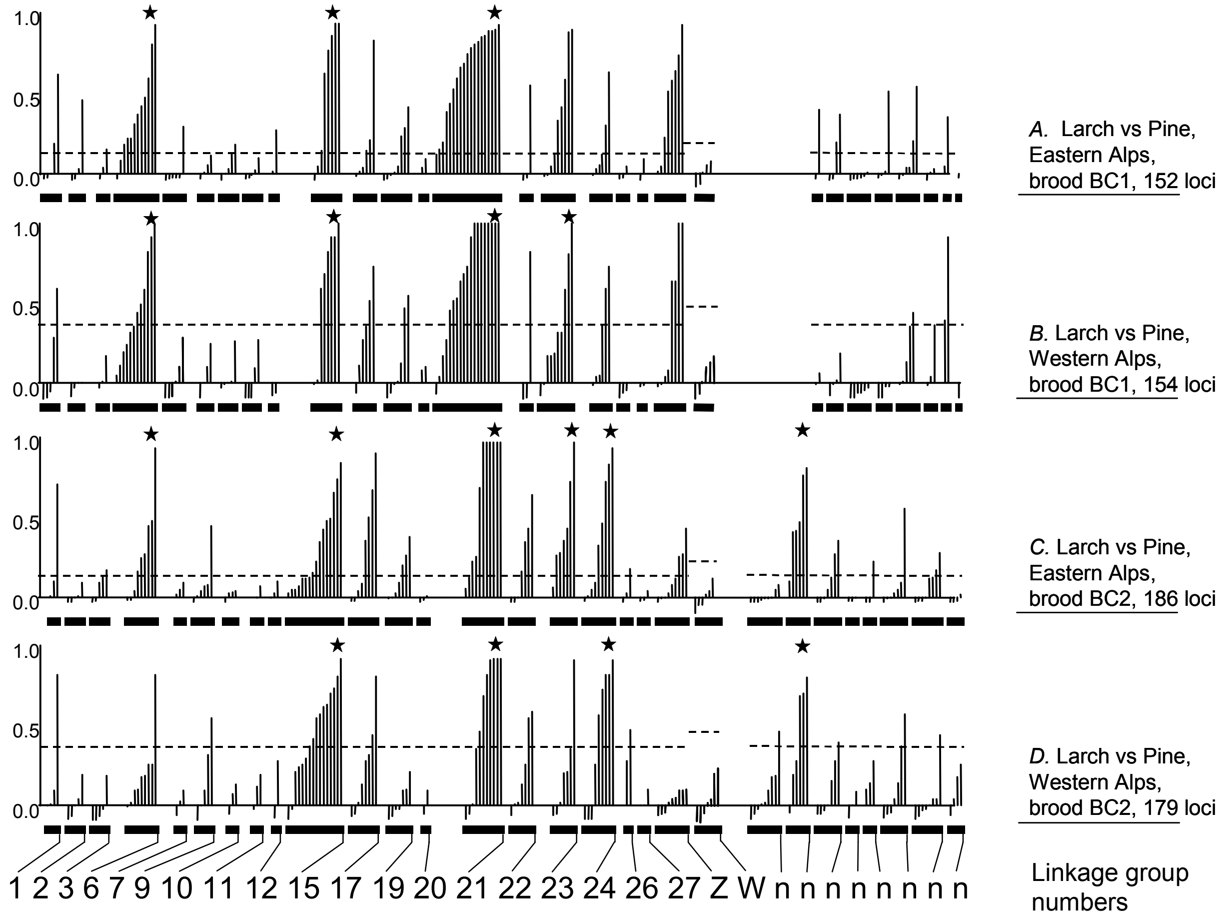
7) Genomic signature of adaptive divergence in host races of the larch budmoth

A key expectation of divergence in the presence of gene flow is evidence for heterogeneity of differentiation across the genome. This pattern is expected because QTLs (quantitative trait loci) or single-gene differences that contribute to adaptive divergence or reproductive isolation are expected to carry with them other, perhaps neutral marker loci, by means of hitch-hiking. In contrast, recombination and gene flow should homogenize differences at marker loci in regions of the genome not under divergent selection.



Symbols at left represent wild-collected genotypes of *Zeiraphera diniana* from larch (triangles), and pine (circles). The fraction of markers with expressed AFLP bands in each individual is plotted against a hybrid index based on AFLP differentiation between larch and pine races. Two genotypic clusters are clearly evident, consisting of larch and pine races. Individual A is a clear F1 hybrid; B, C & D are putative backcrosses. Hybrids are expected to have more expressed bands, as shown here, because they are more heterozygous for divergent loci than the parental forms.

Our AFLP genotype data clearly shows that hybrids occur in nature (see individuals A-D above). The same data also provides perhaps the first evidence for genomic heterogeneity of adaptive divergence expected when gene flow is present. Between-race differentiation is purged from the genomes of larch and pine moths, except for markers on some highly differentiated chromosomes, which presumably contain divergently adapted QTLs. This mosaic genomic pattern is observed only in geographic areas of sympatry and natural hybridization, and is not present in geographic comparisons between populations diverging in the absence of gene flow, for example between Kamchatka and the Alps (Emelianov et al. 2004).



The figure shows differentiation at 152-186 AFLP loci in 74 wild-caught individuals, based on maps obtained from two backcross broods – BC1, BC2. Each needle shows between-host-race differentiation (F_{ST}) at an individual locus; needles attached to each solid horizontal bar show differentiation at loci mapped to a single linkage group (chromosome). Between-map homology was established for all chromosomes designated by numbers, and Z and W sex chromosomes. Eight "non-homologised" chromosomes are designated by "n". Dashed horizontal lines show the level above which F_{ST} becomes significant ($P<0.05$; W-linked markers can be scored only in females, leading to lower significance levels). Loci within groups are sorted by F_{ST} value, not linkage order. Negative estimates (Weir-Cockerham) of F_{ST} evident here can occur by chance. Stars indicate linkage groups contributing significantly to overall genomic heterogeneity.

Conclusion

This work provides what is arguably the first genomic picture of adaptation in the face of gene flow, and has resulted in a remarkably complete picture of pre- and post-mating isolation in the host races of *Zeiraphera diniana.* There are still gaps in our understanding of this system. In particular, preliminary work on mapping genes for sex pheromone production and male response is still based on smaller sample sizes than we would like (Plepys et al. in prep.). We had also hoped to map QTLs for host choice, but the lab bioassay for host choice was not promising. There is strong individual variation in host alighting choice (Emelianov et al. 2003), with the means only 10-20% different from random (50%), and many insects also showed "null choice" (they ignored the host and settled on the wall of the cage), making QTL studies on host choice impossible with reasonable sample sizes. In addition, we still do not yet fully understand the nature of the selection in the wild that maintains genetic differences in spite of gene flow.

Nonetheless, the results obtained here represent an important advance in the understanding of speciation. We have combined traditional experimental analyses of speciation with a new genomic approach. This work also provides insight into genomic patterns expected between other closely related species that exchange genes, even though rates of hybridization in such taxa may be much less than the few percent measured here for *Zeiraphera.* The understanding of similar adaptive systems with gene flow are vitally important for understanding how pests adapt to crops, including novel transgenic insect-resistant varieties.

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Relevant publications

Drès, M.A. (2000): Gene flow between host races of the larch budmoth *Zeiraphera diniana* (Lepidoptera: Tortricidae). Ph.D. Thesis, University of London.

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Drès, M. & Mallet, J. (2002): Host races in plant-feeding insects and their importance in sympatric speciation. Phil. Trans. Roy. Soc. Lond. B 357: 471-492.

Emelianov, I., Simpson, F., Narang, P., & Mallet, J. (2002a). Host choice promotes reproductive isolation between host races of the larch budmoth *Zeiraphera diniana*. J. Evol. Biol. 16: 208-218.

Emelianov, I., Marec, F., & Mallet, J. (2004). Genomic evidence for divergence with gene flow in host races of the larch budmoth. Proc. R. Soc. Lond. B. 271: 97-105.