

**Genetic differentiation of populations of the green oak leaf roller (*Tortrix viridana* L.)
and its host (*Quercus robur* L.) using nuclear gene markers**

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Abstract: Populationsgenetische Differenzierung beim Eichenwickler (*Tortrix viridana* L.) und seiner Wirtspflanze (*Quercus robur* L.) anhand nukleärer Genmarker

Populationen des grünen Eichenwicklers (*Tortrix viridana* L., Lepidoptera: Tortricidae) und der Stieleiche (*Quercus robur* L., Fagaceae) wurden auf genetische Differenzierungen mit Hilfe von Kern-DNA-Markern untersucht. Insgesamt wurden 14 Eichenbestände in Nordrhein-Westfalen (NRW) und 7 weitere in verschiedenen Regionen Europas in die Untersuchung einbezogen. Weder für die Eiche noch für den Eichenwickler sind genetische Differenzierungen der Populationen auf einer kleinen geographischen Skala (etwa 150 km) nachweisbar, jedoch zeigen die Eichenpopulationen eine zunehmende genetische Distanz mit zunehmender geographischer Distanz. Für den Eichenwickler konnten innerhalb von Populationen in einem Radius von etwa 40 m Familienstrukturen ermittelt werden und auf einer kontinentalen Skala lassen sich die Ergebnisse für den Eichenwickler mit einer „isolation by distance“ erklären.

Key words: *Tortrix viridana*, *Quercus robur*, population genetics, AFLP, gene flow

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In Western Europe pedunculate oak (*Quercus robur* L.) is the forest tree with the highest number of phytophagous insect species (YELA & LAWTON 1997). One of these, the green oak leaf roller *Tortrix viridana* L. is an oligophagous herbivorous moth with a host range limited to the genus *Quercus* (HUNTER 1990, DU MERLE 1999). During outbreaks, *T. viridana* often leads to defoliation of oaks in spring. The abundance of *T. viridana* is subject to the population size fluctuations typical for herbivorous insects, where periods of small population sizes (latent periods) alternate with periods of high population sizes (outbreak) (e.g. SCHÜTTE 1957, HORSTMANN 1984).

Apart from many experimental studies on population dynamics of the moth (e.g. HUNTER 1990, DU MERLE 1999, IVASHOV & al. 2002) so far little attention has been paid to the genetic variation within the species as an important aspect of the genetics of this host-parasite interaction. SIMCHUK & al. (1999) found changes in the heterozygosity level of different isozyme loci during outbreaks in *T. viridana* and molecular markers for *T. viridana* have been developed for analyses of genetic variation within and among populations (SCHROEDER & SCHOLZ 2005). But, investigations of genetic variation within and among populations of forest pest species are important to predict future pest outbreaks. So far the processes outbreaks based on are not entirely clarified, however it is known that migration plays a major role. Using molecular markers investigations of the genetic variation are possible and offer the opportunity to analyse distribution events.

In this paper first results are presented concerning the genetic variation of the green oak leaf roller at three geographic scales: (1) among trees within a population, (2) among populations at a small spatial scale of about 150 km and (3) among populations at a broader geographic scale up to 3000 km. Furthermore results of the genetic variation of oaks at the small spatial scale are represented.

Material and Methods

Study Sites and Sampling

In an area of approximately 150 x 150 kilometres in North Rhine-Westphalia (NRW) living caterpillars and pupae of *T. viridana*, and oak leaves were collected in May in the years 2003 to 2005 from approximately 25 marked oaks (*Quercus robur*) per stand in a total of 14 oak stands. The immature stages of the moths were reared in the laboratory and the adults were identified. The adults were frozen at -20°C until DNA extraction. Neither larvae nor pupae have directly been used for DNA extraction to avoid a contamination of the moth DNA with parasitoid DNA. A total of 332 leaf rollers and leaves from 379 oaks from NRW were used for AFLP (Amplified Fragment Length Polymorphism) analysis. Additionally a total of 67 green oak leaf rollers from one stand each in the East of Germany, in Slovenia, in Poland and in four stands in the Ural Mountains was sampled using pheromone traps.

DNA Extraction

The DNA extraction method was identical for oak leaves and moths. Frozen moth individuals or 1 cm^2 of single leaves, respectively, were ground to powder in liquid nitrogen. Total DNA was extracted, following a CTAB protocol by DUMOLIN & al. (1995) with a slight modification for DNA extraction of the moths: Proteinase K in a final concentration of 0,4 $\mu\text{g}/\mu\text{l}$ was added to the lysis buffer.

AFLP Protocol for *Q. robur* and *T. viridana*

The AFLP protocol (Vos & al. 1995) was used to study the genetic variation of *T. viridana* and *Q. robur*. For analysing of *T. viridana* two primer-enzyme combinations (*Eco*-ACG + *Mse*-ATG; *Eco*-AAC + *Mse*-AAG) and for *Q. robur* three primer-enzyme combinations (*Eco*-ACC + *Mse*-CAT; *Pst*-CAG + *Mse*-CAA; *Pst*-CAG + *Mse*-GCA) were used. 5 μl of DNA (containing 100 ng DNA per μl) were digested with *Eco*RI and *Mse*I restriction enzymes and ligated to specific adapters. 5 μl of each restricted and ligated sample were used for preselective and selective amplification. AFLP products were electrophoresed on 6 percent denaturing polyacrylamide gel by ALF sequencer. Band scoring was performed using an ALF express II (Amersham Pharmacia Biotech). AFLP profiles were recorded in a matrix as presence (1) and absence (0) of bands with the software ALF win Fragmentanalyser 1.02 (Amersham Pharmacia Biotech). Only polymorphic bands were used for the population genetic analysis.

Genetic data analysis

Using the program SPAGeDi (HARDY & VEKEMANS 2002) spatial genetic structure was analysed with a multilocus estimator of the pairwise relatedness coefficient between individuals. This is an estimator of the kinship coefficient that is specifically adapted to dominant genetic markers. Average multilocus kinship coefficients per distance interval were computed for six distance classes (20, 40, 60, 80, 100, 120 m) and were plotted against distance. A permutation procedure was applied to test significant deviation from a random spatial distribution of the moths. Each permutation consisted of a random redistribution of the AFLP profiles over the spatial co-ordinates of the samples. Gene diversity was calculated and an AMOVA was carried out for populations of *Q. robur* and *T. viridana* using ARLEQUIN 2.0 (SCHNEIDER & al. 2000). Values of gene diversity were obtained by averaging across loci. Specific SAS macros (STAUBER & HERTEL 1997) were used to calculate genetic distances according to GREGORIUS (1978). The subsequent UPGMA cluster analysis and drawing of dendrograms were also carried out with SAS (SAS Institute Inc. 2002). Correlations between genetic and geographic distances within *T. viridana* and *Q. robur* were calculated and tested using the Mantel nonparametric test calculator (LIEDLOFF 1999).

Results and discussion

SGS within populations

The SGS analysis detected kinship coefficients that were significantly larger than expected for a random distribution among the insects within a radius of 20 m to 40 m (Fig. 1). The correlograms showed the expected tendency of decreasing kinship with increasing distance typical for family structures. We explain our findings with a different mobility for both sexes and the mating behaviour of *T. viridana*. Results with regard to the mobility of *T. viridana* were equivocal. According to SCHNEIDER (1984) the adult moths have

a low flight activity, whereas DU MERLE (1999) and HORSTMANN (1984) found a high dispersal activity for the species. In the laboratory we observed for *T. viridana* a low flight activity for females and a high flight activity for males. Similarly the males of other lepidopteran species have been reported to be more mobile than the females (SALVATO & al. 2002, WAHLBERG & al. 2002). SIMCHUK & al. (1999) observed that males of *T. viridana* mate first with females that developed on the same tree and then start flying over some greater distance (up to a few kilometres). Thus, this behaviour explains our findings of family structure within a radius of about up to 40 m.

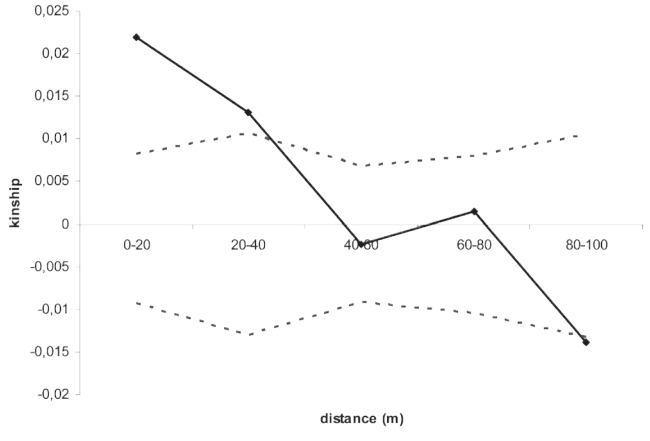


Fig. 1: Spatial genetic structure for one of the observed populations. Dotted lines represent the 99% confidence interval of the permutations. Distances above the zero-line indicate a spatial structure in which genotypes are more similar than expected from a random distribution and values below the zero-line indicate greater genetic differences than expected for a random spatial genetic structure.

Genetic variation and differentiation

The AFLP analysis for *Q. robur* was carried out with three primer-enzyme combinations resulting in a total of 104 fragments in an interval of 50 to 600 bp, of which 76 were polymorphic (Tab. 1). For *T. viridana* two primer combinations used in AFLP analysis revealed a total of 74 bands in the interval of 60 to 300 bp, of which 59 were polymorphic. All analyses have been done with these polymorphic fragments only. For *T. viridana* gene diversity was slightly lower than for *Q. robur* and the F_{st} value was clearly lower for *T. viridana* than for *Q. robur* at the small spatial scale of NRW (Tab. 1).

	Gene diversity	F_{st}	p-value
<i>Q. robur</i>	0,164 ± 0,085	0,086	0,218
<i>T. viridana</i>	0,145 ± 0,076	0,045	0,362

Tab. 1: locus-by-locus AMOVA and gene diversity of *Q. robur* and *T. viridana* populations using nuclear DNA markers.

Nevertheless we neither could differentiate between the oak nor between the leaf roller populations. For oaks this is due to higher pollen-mediated gene flow revealed using nuclear gene markers and the life-cycle characteristics of long-living trees (AUSTERLITZ & al 2000) and for the green oak leaf roller the explanation for our observation of low genetic differentiation among populations is the above mentioned different mobility of the two sexes manifested in biparental inherited gene markers.

A dendrogram, created by the AFLP genetic distance matrix, does not reflect the geographic order within NRW, but at the continental scale a gradient from West (NRW) to East (Ural Mountains) has been revealed (Fig. 2). On this larger geographic scale, including distances of about 500 km up to 3000 km, geographic distances are correlated with genetic distances. Here, isolation by distance was proven by a significant result of the Mantel test ($r = 0,6795$; $p = 0,001$). This might be due to reduced levels of gene flow within and among sample sites resulting in a subpopulation structure as ascertained for other lepidopteran species (SALVATO & al 2002, WAHLBERG & al. 2002).

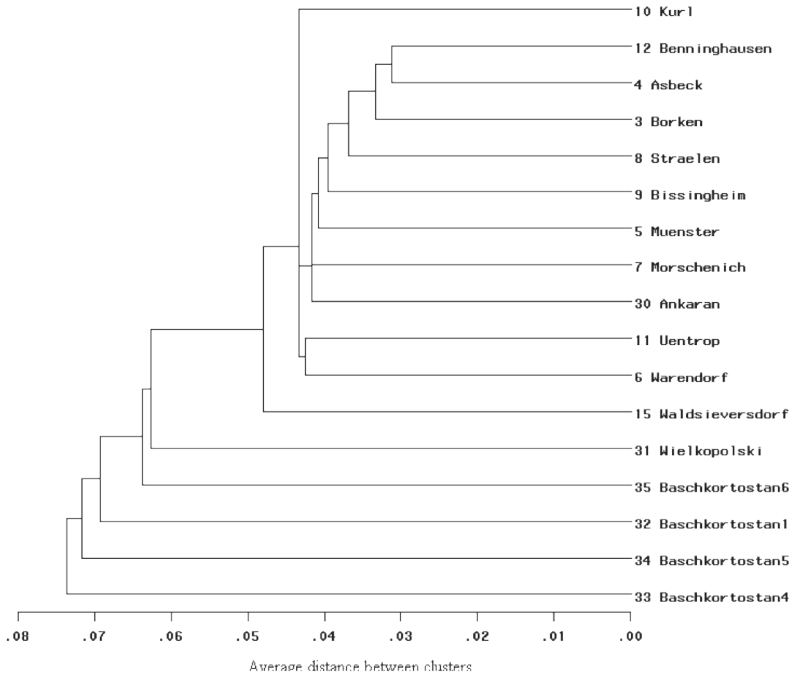


Fig. 2: Dendrogram created with SAS for *T. viridana* populations based on AFLP data. Populations numbered 3 to 12 belonging to North Rhine-Westphalia, 15 Eastern Germany, 30 Slovenia, 31 Poland, 32-35 Ural Mountains.

Genetic distance vs geographic distance

A Mantel nonparametric test (LIEDLOFF 1999) for the populations of *T. viridana* resulted in no correlation between genetic and geographical distance ($r = -0,0644$; $p = 0,35$) due to high gene flow. For *Q. robur* a Mantel test resulted in a significant positive correlation ($r = 0,3424$; $p = 0,04$). Although we could not clearly differentiate between the oak populations we nevertheless find an increasing genetic distance with an increasing geographic distance already at a small spatial scale of up to 150 km in NRW. This might be due to a clearer spatial structure in oaks than in associated insects because of postglacial re-colonisation routes and men made seed transfers, which gave rise to the current distribution of the genetic variation within the genus *Quercus* (KÖNIG & al. 2002).

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