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Large scale geographic clines of parasite damage to *Populus tremula* L.

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According to the geographic mosaic theory of coevolution (GMTC), clines of traits reflecting local co-adaptation (including resistance genes) should be common between a host and its parasite and should persist across time. To test the GMTC-assumption of persistent clinal patterns we compared the natural prevalence of two parasites on aspen *Populus tremula* trees: mining moths of the genus *Phyllocnistis* and leaf rust *Melampsora* spp. Damage data were collated from the Swedish National Forest Damage Inventory (2004–2006). In addition, occurrence of the parasites was scored in field conditions in two common gardens in the north and south of Sweden over five growing seasons (2004–2008), then related to biomass (stem height and diameter) and to concentrations of eleven leaf phenolics. *Phyllocnistis* mainly occurred in the northern garden, a distribution range which was confirmed by the countrywide inventory, although *Phyllocnistis* was more abundant on southern clones, providing evidence for possible local maladaptation. *Melampsora* occurred all over the country and in both gardens, but built up more quickly on northern clones, which suggests a centre of local clone maladaptation in the north. Stem growth also followed a clinal pattern as did the concentration of three phenolic compounds: benzoic acid, catechin and cinnamic acid. However, only benzoic acid was related to parasite presence: negatively to *Phyllocnistis* and positively to *Melampsora* and it could thus be a potential trait under selection.

In conclusion, clines of *Phyllocnistis* were stronger and more persistent compared to *Melampsora*, which showed contrasting clines of varying strength. Our data thus support the assumption of the GMTC model that clines exist in the border between hot and cold spots and that they may be less persistent for parasites with an elevated gene flow, and/or for parasites which cover relatively larger hot spots surrounded by fewer cold spots.

Most host species are thought to coexist with specific pests and parasites only across part of their geographical range. Therefore, at a landscape level, hot spots of ongoing co-adaptation between host and parasite are likely to be surrounded by cold spots where the parasite is absent (Thompson 1997, 1999, Nuismer et al. 2003). Parasites may potentially occur in cold spots, if they are unimportant as a selective factor due, for example, to the presence of alternative hosts or enemies. The geographic mosaic theory of coevolution (GMTC, Thompson 1997, 1999) is a framework for understanding how local selective pressures between host and parasite may determine the distribution of coevolutionary traits at the scale of both single populations and the landscape. In recent models two factors have been highlighted as determinants for coevolutionary strength: 1) the size of a hot spot relative to the size of surrounding cold spots; and 2) the strength of gene flow in both host and parasite populations (Gomulkiewicz et al. 2000, 2003, Nuismer et al. 2003). More specifically, Nuismer et al. (2003) hypothesized that long-term clines of resistance traits, indicating local maladaptation in the host-parasite interaction, should be common only when host and parasite partially overlap in their distribution, and that patterns of local parasite adaptation exhibiting such clines should be constant across several years. Gomulkiewicz et al. (2007) suggested the use of several approaches to test for the presence of the patterns predicted by the GMTC, including epidemiological surveys in natural populations combined with both experimental approaches and trait matching.

The first evidence supporting the presence of stable clines of resistance over a range of ~100 km was provided by Springer (2007), who compared clone-specific rust resistance in Californian dwarf flax with the prevalence of the rust and resistance to it in natural flax populations. A common problem with large scale epidemiological surveys is that the absence of biotic damage at one site during one or a few seasons may not necessarily be the result of host resistance. Reciprocal transplantation is one way to challenge ecotypes by placing them in new environments; this introduces the possibility of separating genotypic and environmental impact (Clausen et al. 1940, Joshi et al. 2001, Fritz et al. 2006). This approach has also been used to map the genetic components of plant resistance traits (Graham et al. 2001, Schweitzer et al. 2005), although, the transplantation may itself interact with the clone properties and thus cause over- or under-estimation of clone effects.

Forest trees, in particular fast-growing clonal species, provide a unique system for testing the assumptions of the GMTC: they are exposed to different kinds of damage over many growing seasons, and genetic factors strongly govern their resistance to biotic stress (Osier et al. 2000, Osier and Lindroth 2006, Philippe and Bohlman 2007). European aspen *Populus tremula* is dioecious, fast growing, wind pollinated, and disperses its seeds by wind; not surprisingly, despite its widespread asexual clonal reproduction mode, this species is one of the most outbreeding and consequently genetically diverse trees. In a collection of 116 Swedish aspen ecotypes (the SwAsp collection; Luquez et al. 2008) this diversity is reflected at the molecular level, and thus as a high level of nucleotide diversity in the coding regions of genes (close to 1%) (Ingvarsson et al. 2006, Hall et al. 2007). Previous studies have shown that, despite this high diversity and supposed lack of structure at the molecular level, the SwAsp collection expresses strong phenotypic clines with respect to phenological and morphological traits, correlating with the geographical origin of the clones (Hall et al. 2007, Luquez et al. 2008). In natural habitats, aspen is associated with a high diversity of parasites. In poplars' constitutive and induced defence against different parasites, phenolic metabolites, such as the low molecular weight glycosides, appear to be important (Osier et al. 2000, Kopper and Lindroth 2003), although the role of phenolics in tree resistance may not be straightforward either spatially or temporally (see for example Kant and Baldwin 2007, Philippe and Bohlman 2007, Witzell and Martin 2008).

In this study, we tested the presumptions of the GMTC by studying the large scale ecological structure of resistance in European aspen to two abundant parasites: leaf rust *Melampsora* spp. with a high gene flow due to a complex life cycle with several generations per year, and leaf mining moths *Phyllocnistis* spp. with a comparatively low gene flow. We predicted that *Phyllocnistis* should show stronger and more persistent clines than *Melampsora* because of this difference in gene flow, and thus a difference in co-evolutionary potential. We also wanted to relate clinal patterns to the relative size of hot spots covered by the two parasites because relatively smaller hot spots are also predicted by the GMTC to result in a higher cline persistance.

More specifically, we tested patterns of resistance extracted from large-scale epidemiological screenings and damage data for the 116 aspen clones of the SwAsp collection, transplanted into two common gardens 1200 km apart (Luquez et al. 2008). In the common gardens we examined patterns associated with distance to the clone's origin. Elevated damage levels on local clones were defined as local adaptation of the parasite and maladaptation of the host. Models built on latitudinal distance were used to test if the distance from the home environment might express a pattern of possible gradual change in the interaction between host and parasite corresponding to a potential gradual change in selection pressure which might correspond to range and intensity of gene flow for each of the parasites. Latitudinal clines have previously been found for traits related to growth and phenology in the SwAsp collection (Ingvarsson et al. 2006, Hall et al. 2007). Therefore, as an alternative or complementary explanation, we related damage patterns to growth data and, in a subset of the SwAsp clones, concentrations of phytochemicals.

Materials and methods

Natural large scale epidemiology

Countrywide damage data for aspen from 2004 to 2006 were retrieved from the Swedish National Forest Damage Inventory (SNFDI) data base. This programme records damage to randomly selected individual trees across the whole Swedish forest area (Wulff et al. 2006). A tree species will thus occur in the data set at a frequency reflecting its abundance in an area. Only data pertaining to damage to spruce and pine, the most common tree species in Sweden, have previously been published from this survey (Thor et al. 2005, Wulff et al. 2006). Aspen is a far less abundant forest tree in Sweden. It is a primary coloniser and was chemically eradicated in many Swedish forests over a few decades in the mid twentieth century because of its association with *Melampsora pinitorqua*, a rust that has pine as its alternate host (Quencez et al. 2001). The surveys were conducted in all parts of the country, and sampling naturally decreased with remoteness and increased with *P. tremula* density. To minimise the effect of sampling errors, the frequency of aspen individuals that were damaged by *Melampsora* spp. and *Phyllocnistis* spp. were each averaged for the three years, as follows:

$$\hat{K} = \frac{1}{3}(p_{\hat{2004}} + p_{\hat{2005}} + p_{\hat{2006}})$$

where p is the proportion of damaged trees for each individual year and parasite. The averaged proportions were then related to mean latitude of the sampled area, density and tree trunk diameter. When fewer than ten trees represented a site across sampling years (or an average of three trees per year), data from this site was excluded to minimize the risk of false negatives. Thus, data points that represented a mean of 11–33 trees were included for latitudes north of 60°N, while all data points south hereof represented 63–161 trees.

SwAsp collection used for damage scoring

The SwAsp collection consists of a selection of 116 *P. tremula* genotypes that originate from 12 sites located east and west, at every second degree of latitude up through Sweden (from 56°18′N, 12°54′E to 66°12′N, 22°12′E) (Fig. 1). In 2004 four root-propagated replicates of each clone were planted into each of two common gardens: Ekebo (55°54′N, 13°06′E) and Sävar (63°54′N, 20°36′E). Two additional replicates of each clone were overwintered in 7.5 l pots in a cold chamber (4°C) and in spring 2005

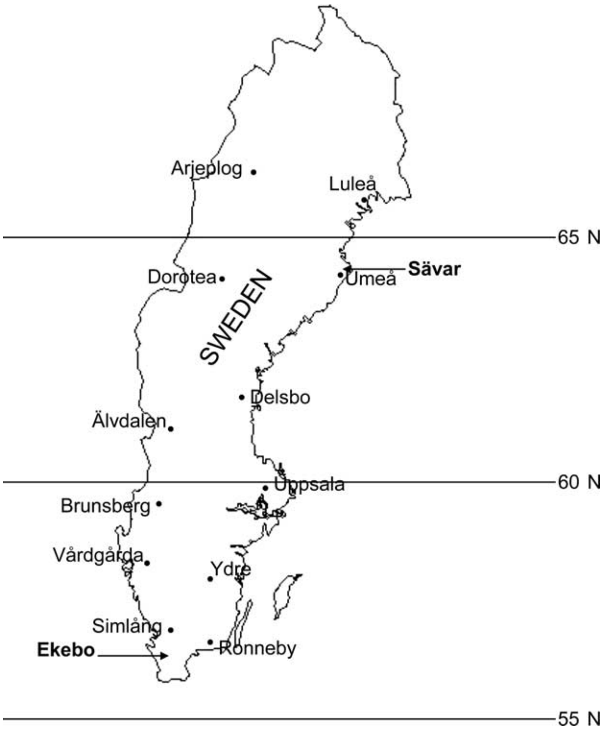

Figure 1. A map showing clone origin of the SwAsp collection of Swedish *P. tremula*. Ten clones were collected from each of twelve named locations (six clones from Arjeplog). In 2003 each clone had been multiplied by root propagation and four replicates were planted into two common gardens indicated with arrows: Ekebo (55°54′N) and Sävar (63°54′N).

they were transferred to continue growing in a glasshouse at the Swedish Univ. of Agricultural Sciences, Umeå (63°49′N, 20°18′E), as described by Ingvarsson et al. (2006). At the end of four growing seasons (2005–2008) survival, stem height (from soil surface to tip of the longest branch) and stem diameter (measured with a slide calliper, 20 cm above the soil surface) were recorded.

Parasites

We followed damage patterns by two parasites (*Phyllocnistis* spp., Lepidoptera: Gracillariidae, and the rust *Melampsora* spp.) on *P. tremula* plants from the SwAsp collection from 2004 to 2008 in the two gardens, except for in Ekebo in 2007 when no recording was possible. Measurements were repeated between 2005 and 2008, and all data from 2006 are presented to illustrate seasonal patterns; for between-year comparisons a representative survey was selected for each year and garden.

Phyllocnistis moths

In Sweden, two *Phyllocnistis* species (*P. labyrinthella* and *P. unipunctella*) oviposit on young aspen leaves soon after bud break. The caterpillars are specialist miners that feed on epidermal cells of the leaf surface, leaving the cuticle layer intact as a barrier to the exterior. This activity results in a characteristic labyrinth shaped mine that may be located on either the adaxial or abaxial leaf surface. Detailed studies in North America by Wagner et al. (2008) have demonstrated how *Phyllocnistis populiella* miners restrict net photosynthesis in leaves of *Populus tremuloides*, cause premature abscission, and slow down shoot elongation and diameter increase. In our surveys *P. labyrinthella* was consistently the more abundant of the two species, being responsible for >90% of the mines. Damage due to the moths was scored by counting the number of mines of the two *Phyllocnistis* species on every plant.

Melampsora infection

Populus tremula is often infected by leaf rusts belonging to the genus *Melampsora* (Quencez et al. 2001). These rusts are typically macrocyclic and heteroecious, i.e. they produce five different spore stages on two alternate host species over their complete life cycle. The stage that results in the characteristic symptoms on aspen leaves is the uredial stage, where uredospores are produced within uredia, yellow or orange pustules usually on the lower side of leaves, in several cycles. The taxonomy of the *Melampsora* species is complex (Pei and Shang 2005). For the purpose of this study we did not determine the species composition of rusts across the geographic area. Rust incidence was estimated as the relative proportion of leaves with symptoms to those without symptoms on each plant. An index divided into 10% intervals and with zero as one class was used to assess differences in rust resistance among the plants.

Chemical analyses

To determine the phenolic profiles, we sampled leaves for analyses from a subset of 48 clones (two plant replicates per clone) grown in a glasshouse. The fourth leaf below the first fully expanded leaf on branches in a similar position on each plant, was sampled on 12–13 September, 2005, between 11.00–13.30 h. The leaves were air-dried at room temperature for about two weeks and ground to a fine powder using a Retsch Mixer Mill (Model MM301, Retsch, Haan, Germany). Approximately 10 mg of the resulting homogenous powder was extracted in methanol and the extract analysed by HPLC, as described by Witzell et al. (2003) and Srivastava et al. (2007). Two samples of the powder were extracted to obtain two replicates for HPLC analysis. Individual phenolics were identified by comparing their retention times and UV-spectra (200–400 nm) to those of standard compounds. Tremulacin and tremuloidin were tentatively identified by comparing the retention times and UV-spectra to those recorded in the literature (Shao 1991) and to a tremulacin standard obtained from R. Lindroth. Salicylates (salicin, salicortin and tremulacin) were quantified as salicin equivalents, phenolic acids as chlorogenic acid or cinnamic acid equivalents (Sigma, St Louis, MO, USA) and flavonoids as quercetin-3-glucoside (Extrasynthese, Genay, France) equivalents. Peaks were measured at 220 and 320 nm.

Statistical analyses

Plant fitness responses were assessed by analysing survival and growth with respect to genotype and environment. Using logistic regression analysis, the probability of clone survival (log odds, logit) was related to the effects of: 1) origin (latitude of home environment); 2) common garden (latitude of transplantation environment); and 3) the interaction between the two. The reported R^2 for logistic regression fits are pseudo-R^2, representing the portion of the total uncertainty attributed to the model fit, and computed as the ratio between the log-likelihood of model difference divided by the log-likelihood of the reduced model (JMP ver. 5.1).

We examined consistency in susceptibility of plants among clones and years, and between parasites. In order to allow a comparison of parasite damage among years the data were transformed to presence and absence (logit) of symptoms on single plants in the field. The relatedness between *Phyllocnistis* abundance and *Melampsora* infection levels was tested using Cohen's Kappa statistics (Landis and Koch 1977). A value close to one indicated a perfect match of susceptibility to the two parasites.

The linearity of clinal patterns was tested with an ANOVA model with contrasts with origin as the independent variable and phenology and damage traits as quantitative responses. Two models were compared:

H1) a clinal model where the contrasts explain a constant trend from north to south (sites indicated by S(1–12) and contrast in bold): S1 **−0.29**; S2 **−0.24**; S3 **−0.19**; S4 **−0.14**; S5 **−0.1**; S6 **−0.05**; S7 **0.05**; S8 **0.1**; S9 **0.14**; S10 **0.19**; S11 **0.24**; S12 **0.29**.
H2) a step model that divides the clones into a southern and a northern group: ≤S6 **−0.167**; ≥S7 **0.167**, respectively.

Akaike's information criterion (AIC with small sample bias correction) was used to select the model that fitted the data better. All statistical analyses were performed using the statistical package JMP ver. 5.1.

Results

Natural large scale epidemiology

Data from the national damage inventory (SNFDI) showed that *Phyllocnistis* were confined to latitudes between 57 and 61° and were not present in detectable amounts at 55°54′N and 63°54′N, the latitudes of the two common gardens. *Melampsora* was most abundant in the southern part of Sweden and followed a clinal pattern, with the exception of latitude 57°N where it only occurred in low densities (Fig. 2a). Both *Phyllocnistis* and *Melampsora* infections were positively related to tree density across the sampling area (Fig. 2b), however, for both species there was a positive relationship between detection level and sampling effort. The severity of *Melampsora* infection was related to tree size (affected trees, i.e. those with >10% infection, tended to be in the diameter class 21–30 cm: $\chi^2 = 12.39$; $p > \chi^2 < 0.003$) whereas *Phyllocnistis* presence was not related to tree size category ($\chi^2 = 1.03$; $p > \chi^2 < 0.60$) (Fig. 2c).

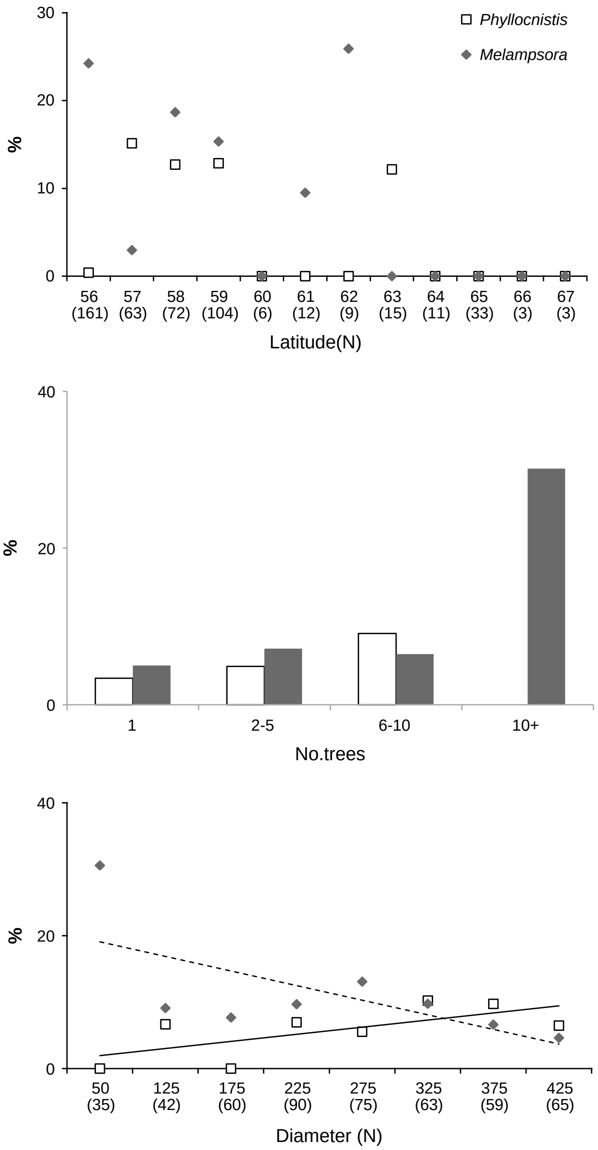

Figure 2. Epidemiological data from the National Forest Damage Inventory Project, Sweden, showing frequencies of trees with leaf damage averaged for 2004–2006 relative to (a) tree origin (expressed as latitude), (b) tree density (number of trees per area), and (c) tree diameter group (measured in breast height, in cm). *Phyllocnistis* damage is shown as white squares, white bars, and full line and *Melampsora* damage is indicated with black diamonds, black bars, and dashed line.

Clone mortality and stem growth traits in common gardens

In 2006, two years after transplantation, northern clones had suffered from higher mortality in both gardens. In a logistic ANCOVA model, latitude of origin explained most of the variation in clone mortality (Log regression, latitude effect: $\chi^2 = 29.91$; $p > \chi^2 < 0.0001$), and there was no single effect of transplant garden (garden effect: $\chi^2 = 1.18$; $p > \chi^2 < 0.28$); however, an interaction effect confirmed differences between the common gardens which resulted from a steeper mortality curve for clones in the northern Sävar garden (interaction effect: $\chi^2 = 4.57$; $p > \chi^2 < 0.03$,

Table 1. Logistic regression analysis of *Populus tremula* clone survival as a function of clone origin, common garden locality (site into which the clones were transplanted), and their interaction. Odds ratios between gardens are presented in Table 3.

Variable	DF	Par Est	SE	χ^2	$p > \chi^2$
Intercept (β_0)	1	−13.28	2.08	40.92	<0.0001
Origin (β_1)	1	−0.18	0.03	29.91	<0.0001
Garden	1	−0.12	0.11	1.18	0.28
Origin × Garden	1	−0.07	0.03	4.57	0.03

Table 1). Almost half of the clones from the Arjeplog site (66°06′N), which had the shortest growing season (130 d with the average temperature above 5°C), died in Sävar, compared to one only one quarter of the clones when planted in Ekebo. Genotypes from the southern subpopulations (220 d above 5°C) had a survival rate close to 95% in both gardens.

Growth followed parallel patterns in the two gardens, with genotypes from southern latitudes growing more vigorously in both gardens (Table 2). At the end of the 2006 growing season, the average tree height in Ekebo was 140.22 ± 2.97 cm (mean ± SE) and the average stem diameter was 10.49 ± 0.24 mm. In Sävar, the corresponding values were 104.24 ± 1.94 cm and 9.18 ± 0.18 mm. Both origin and common garden explained the differences in plant height, whereas stem diameter was only significantly related to origin (Table 2). The lack of interaction in this analysis confirmed that growth displayed the same clinal pattern in the two gardens. Stem height and diameter were always highly correlated (Pearsons r: 0.96, $p < 0.0001$).

Temporal and spatial patterns of parasite presence

Both *Phyllocnistis* and *Melampsora* were more frequent in the Sävar common garden; there was almost no damage by miners in Ekebo (Table 3). To study the annual variation in parasite damage, we scored damage by *Phyllocnistis* and *Melampsora* in both gardens over five consecutive years. In order to allow for comparisons among years, quantitative damage data were transformed into presence and absence (logit) of the pests on individual plants. In 2004 only a subset of plants was examined, but in 2005–2008 all plants in the gardens were included and recording was conducted on several occasions. Consequently, the number of plants examined (N) varied between 139 and 465 depending on year, sampling event and garden (Table 3). Logistic regression analyses showed that northern clones in Sävar consistently experienced a lower risk of being attacked by *Phyllocnistis*. However, the explanatory effect of clone origin was generally low (with R^2 values ranging from 0.01 in 2005 to 0.14 in 2007). Likewise for *Melampsora*, an among-year consistent clinal pattern was found in Ekebo and Sävar from 2005 to 2008, with highly significant enhanced risk of infection to northern clones. In this case, the explanatory effect of clone origin (R^2) varied between 0.03 (2005) and 0.18 (2006). The reverse pattern, i.e. a lower risk of rust infection in northern clones, was however recorded in Sävar in 2004 (the year in which the common gardens were established and only a subset of the plants were surveyed). The relatedness between the two types of damage was tested using Kappa statistics. A value close to zero indicated the absence of any relationship between damage by rust and miners in any year or garden (Table 3).

To study the variation in damage within seasons, we scored parasite symptoms in the common gardens from two to five times each year. In 2006 in Sävar, *Phyllocnistis* started to appear after 20 June and were most abundant on the 11 July survey. *Phyllocnistis* displayed a remarkably consistent attack pattern over the season with the number of mines on a plant decreasing with latitude of origin (Fig. 3). Thus, the largest number of mines was recorded for the southernmost clones in the Sävar garden (linear regression #mines = 43.79 − 0.64 × latitude, F = 73.6***, N = 380, R2 = 0.16), a pattern that remained if adjustments were made for plant height (linear regression: #mines/plant height (cm) = 17.74 − 0.24 × latitude, F = 30.7***, N = 380, R2 = 0.08). In 2006, from 26 June onwards, the number of mines on each tree was highly positively correlated between scoring events (Perssons correlation r ranging from 0.47 ± 0.03 (26 June) to 0.71 ± 0.09 (11 July)).

Symptoms of *Melampsora* became visible earlier in Sävar than in Ekebo. In Sävar *Melampsora* reached a level where every plant was damaged in August; in Ekebo infections did not reach such high levels. At extremely high or low infection levels the scoring failed to detect differences in *Melampsora*. In both gardens, when there were low to moderate levels of *Melampsora* infection, a clinal pattern was detected, with higher levels of infection in the northern clones (Fig. 4, Table 3).

Table 2. ANOVA table evaluating a) height and b) diameter of *Populus tremula* clones after two years of growth in relation to latitude, common garden locality, and their interaction.

Response	Variable	R^2	$F_{23,\ 795}$	p > F	DF	F	p > F
a) Height	Model:	0.54	40.32	<0.0001			
	Intercept				2	136.07	<0.0001
	Origin				11	64.61	<0.0001
	Garden				1	9.91	0.0017
	Origin × Garden				11	1.08	0.38
b) Diameter	Model:	0.42	25.10	<0.0001			
	Intercept				2	53.64	<0.0001
	Origin				11	48.12	<0.0001
	Garden				1	0.35	0.55
	Origin × Garden				11	1.23	0.26

Table 3. Overview of the spatial and temporal risk of infection of *Populus tremula* clones by *Melampsora* spp. and *Phyllocnistis* spp. The frequency of uninfected (0) and infected (1) trees are given for each parasite, garden and year, which is the basis of the odds ratio (odds); β_0 = intercept, β_1 = slope after logistic regression with parasite as the response variable and latitude as the effect. Parasites are most frequent on northern clones if $\beta_1 < 0$, and on southern clones if $\beta_1 > 0$. ***p <0.0001; **p <0.001; *p <0.05; n.s. p >0.05.

		Melampsora spp.						*Phyllocnistis* spp.						Comparison	
	Date	0	1	Odds	R^2	$\beta_{0\ (intercept)}$	$\beta_{1\ (slope)}$	0	1	Odds	R^2	$\beta_{0\ (intercept)}$	$\beta_{1\ (slope)}$	Kappa	SE
Ekebo															
2004	18 Aug	188	39	0.21	0.16	4.03***	−0.35***	222	5	0.02	0.01	3.39***	0.13^{ns}	0.01	0.04
2005	16 Aug	66	399	6.05	0.03	0.98**	−0.15**	465	0	–		–	–	–	–
2006	21 Aug	335	98	0.29	0.18	3.87***	−0.37***	429	4	0.01	0.03	3.73***	0.17^{ns}	−0.2	0.01
2008	17 July	409	24	0.06	0.12	24.07***	−0.34***	427	6	0.01	0.00	7.05^{ns}	-0.05^{ns}	0.05	0.06
Sävar															
2004	16 Aug	29	110	3.79	0.09	−3.12***	0.26***	103	36	0.35	0.07	0.08^{ns}	0.15*	0.12	0.03
2005	14 Sep	45	320	7.11	0.03	−1.73***	−0.16*	323	41	0.13	0.01	1.47***	0.11^{ns}	−0.002	0.01
2006	11 July	81	294	3.63	0.01	−1.04***	-0.03^{ns}	81	299	3.69	0.08	−16.57***	0.23***	0.19	0.06
2007	30 July	223	185	0.83	0.14	2.10***	−0.30***	221	183	0.82	0.06	−2.58***	0.20***	−0.12	0.04
2008	27 July	.	.	.	.	.	.	225	117	0.52	0.01	−5.17*	0.09***		
	5 Sep	104	304	2.92	0.03	7.34**	−0.14**	.	.	.	.		.	0.00	0.00

Secondary chemical profiles

The qualitative and quantitative phenolic profiles of the 48 analysed genotypes were similar to those previously reported for aspen (cf. Palo 1984, Osier et al. 2000, Kopper and Lindroth 2003), with salicin (9.16 ± 0.54 mg g^{-1} leaf weight ± SE), salicortin (8.83 ± 1.05 mg g^{-1}) and tremulacin (6.67 ± 0.79 mg g^{-1}) dominating the average profile. There were pronounced quantitative differences between individual genotypes. Phenolic glycosides displayed individual patterns, which could be related to clone origin (see Geographic model comparison below) and the relationship between damage and specific phenolics was examined for those that followed the same patterns (see Relationship between damage and phytophenolics below).

Geographic model comparison

We compared two models to analyse the relationship between latitude and different variables that described growth and resistance: a clinal, and a step model. Strong and consistent fits were generated for growth characteristics and for *Phyllocnistis* damage. Stem growth characters could be fitted most closely to the step model, although the clinal model provided a strong alternative (Table 4). *Phyllocnistis* presence could be related to both models, however the clinal model provided the preferred fit (AIC comparison) with southern clones being more attractive to *Phyllocnistis*. *Melampsora* was also, although not always significantly, best described by the clinal relationship. However, in all cases the slope suggested a positive relationship, with northern clones being less resistant (Table 4).

Eleven phenolic compounds were identified and quantified from glasshouse plants. Individual phenolic compounds represented all types of patterns when related to the two geographic models. The occurrence of only three phenolics, benzoic acid, catechin and cinnamic acid, was significantly described by the clinal model. Cinnamic acid (R^2: 0.09, $p = 0.04$) and catechin (R^2: 0.07, $p = 0.06$) decreased with latitude of clone origin and they co-varied positively (Pearsons r: 0.82, $p < 0.0001$). In contrast, benzoic acid was negatively correlated with cinnamic acid and catechin (Pearsons r: −0.41, $p = 0.004$, and r: −0.51, $p = 0.0002$, respectively) and expressed a clear negative relationship with latitude of clone origin (R^2: 0.17, $p = 0.0037$), and thus benzoic acid concentrations were higher in northern clones. The relationships between phenolic content and origin were generally weaker than those between growth or *Phyllocnistis* damage and origin recorded in the field.

Relationship between damage and phytophenolics

Because *Phyllocnistis* and *Melampsora* occurrence fitted the clinal models best, they were also related to those phytophenolic compounds exhibiting a clinal relationship. Thus, levels of benzoic acid, catechin and cinnamic acid were related to damage by *Melampsora* and *Phyllocnistis* to plants in Sävar scored on 11 July 2006. This scoring event was considered to be representative because this was when the moth damage peaked and the highest differentiation among clones with respect to rust incidence was recorded.

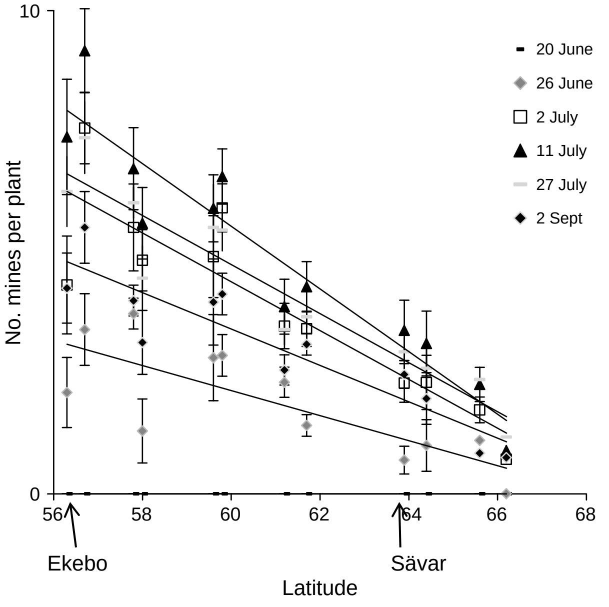

Figure 3. Repeated counts of miner damage by *Phyllocnistis* spp. to *P. tremula* clones in the Sävar common garden on six occasions in 2006. Clone mean values ± 1 SE are shown per site.

Benzoic acid was significantly and positively related to *Melampsora*: (R^2: 0.10, $p = 0.0268$) and negatively related to damage by *Phyllocnistis* (R^2: 0.10, $p = 0.0285$). Neither catechin nor cinnamic acid could be related to damage.

Discussion

Local selection acts to produce locally adapted ecotypes (Turesson 1922, Joshi et al. 2001). A growing body of studies also suggests that resistance genes are heterogeneously distributed in natural plant populations (Burdon et al. 1999, Ericson et al. 2002, Laine 2004, Osier and Lindroth 2006, Wimp et al. 2007). However, at a broader geographic scale we still have a limited knowledge of how local selective pressures may result in varied patterns of adaptation and maladaptation (Burdon 1987, Springer 2007). In the current study, we examined trends in damage to *Populus tremula*, caused by mining *Phyllocnistis* moths and *Melampsora* rust, over a five year period in Sweden, both under natural conditions and in two common gardens that were located 1200 km apart. To our knowledge, this study is unique as it combines large scale damage inventories with common garden data, and focuses on two different parasite species, mining moths and a pathogenic fungus. This approach allows us, for the first time in a forest tree species, to compare selection pressure with resistance patterns and to test assumptions about long term damage clines suggested by the geographic mosaic theory of coevolution (Thompson 1997, 1999, Nuismer et al. 2003).

Phyllocnistis exhibit consistent large scale patterns

In the large-scale national inventory, *Phyllocnistis* were detected only between latitudes 57 and 61°N. This suggests the existence of a hot spot, where there may be stronger co-selection between the host and the parasite (Nuismer et al. 2003). We found, however, that while this pattern was valid in Ekebo in the south, where there were extremely few *Phyllocnistis* mines present (56.12°N), it was not supported by data from the supposed cold spot in Sävar common garden (63.9°N) where attack risk to trees varied between 0.10 (in 2005) and 0.70 (in 2006). Thus, our data suggested that the exposure to *Phyllocnistis* differed between the southern and the northern "cold spots" and that clones from the south, where the *Phyllocnistis* were less common and mostly absent, became more attractive when transplanted to the northern garden, thus exhibiting a potential maladaptation of the parasite. The clinal pattern was repeated both qualitatively among years and quantitatively across the growing season. The enhanced susceptibility of southern clones in the north could be caused by a relaxed defence due to the absence of the *Phyllocnistis* in the home area or some change due to the transplantation event. However, clones from the core area also showed to be more susceptible at the country scale (in the epidemiologic study) thus suggesting maladaptation of the average clone in this area.

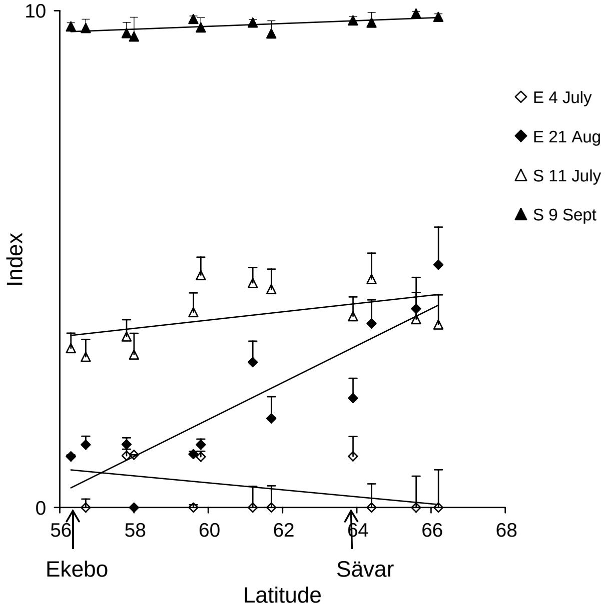

Figure 4. Index of *Melampsora* spp. on *P. tremula* clones, scored at two occasions in common gardens Sävar and Ekebo, in 2006 in relation to clone origin. Clone mean values + 1 SE are shown per site.

Melampsora spp. exhibit strong dynamics

At the country scale, *Melampsora* was most frequent in the south. In the common gardens the converse was consistently true, but the pattern varied in strength. During the study period *Melampsora* infection increased in the northern garden from early July and often reached extremely high levels late in the season. In Ekebo, pustules became visible later, in August, and never reached the same levels of infection. At extremely low and high infection levels we failed to demonstrate latitude-related damage differences.

Table 4. The effect of distances among clone origins on stem traits and parasite damage to SwAsp clones in the field fitted to H1: a clinal model, and H2: a step model. t values from the ANOVA contrast test and significance levels are stated for each model and in bold if one model was selected as stronger in an AIC comparison. Slope is indicated for significant relationships only and a positive value indicates a higher response for northern clones. Test power and least significant sample size (LSN) are reported.

Response	Slope	H1	H2	$DF_{dn,n}$	N	Power	LSN
Height							
Ekebo	−	15.48***	**16.61*****	445,11	456	1	18
Sävar	−	13.70***	**14.43*****	410,11	421	1	22
Diameter							
Ekebo	−	14.20***	**15.13*****	444,11	455	1	19
Sävar	−	12.44***	**12.88*****	409,11	420	1	25
Phyllocnistis							
Sävar 26 June	−	**4.70*****	4.48***	379,11	380	0.98	130
Sävar 3 July	−	**7.82*****	7.68***	379,11	380	1	52
Sävar 13 July	−	**8.54*****	7.99***	379,11	380	1	45
Sävar 29 July	−	**7.73*****	7.40***	379,11	380	1	53
Sävar 5 Aug	−	**6.67*****	6.15***	379,11	380	1	71
Melampsora							
Ekebo 4 July		-1.40^{ns}	1.51^{ns}	452,11	463	0.24	1264
Ekebo 21 Aug	+	**−11.2*****	**−11.0*****	452,11	463	1	33
Sävar 11 July	+	**−2.32***	**−2.47***	424,11	436	0.52	559
Sävar 9 Sept		-1.38^{ns}	-0.95^{ns}	400,11	411	0.26	1114
Phenolics							
Salicin		1.15^{ns}	-0.58^{ns}	85,11	96	0.56	113
Salicin deriv	+	-0.18^{ns}	-0.17^{ns}	85,11	96	0.70	85
ChlorogenicA		-0.01^{ns}	0.21^{ns}	85,11	96	0.08	1560
Salicortin		-0.45^{ns}	-0.09^{ns}	84,11	95	0.09	1061
BenzoicA	+	**−3.49****	−2.5*	85,11	96	0.92	52
Quercetin	−	1.52^{ns}	**2.18***	84,11	95	0.51	123
Tremulacin	+	-1.18^{ns}	-1.5^{ns}	85,11	96	0.94	47
Luteolin		0.49^{ns}	0.49^{ns}	85,11	96	0.27	247
Tremuloidin		−0.2	0.50^{ns}	85,11	96	0.44	145
Catechin	−	**2.35***	2.13*	85,11	96	0.58	108
CinnamicA	−	**2.49***	2.16*	85,11	96	0.52	122

Infection risk by *Melampsora* in natural *P. tremula* stands was positively related to tree population density and negatively related to tree size, and we expect that plant size and density also affected infection risk in the gardens. A group of *P. tremula* × *tremuloides* hybrids growing adjacent to the northern garden may also have increased the infection risk and speeded up the onset of infection in Sävar. Trees in this garden generally performed less well than those in the southern garden. They suffered from higher mortality and slower growth, suggesting a higher stress level, which could be accompanied by a higher level of susceptibility. The southern garden is mostly surrounded by birch, and all clones performed better here and exhibited a generally higher level of resistance. Thus, our common garden results may be affected by landscape structural factors (Matter et al. 2005) and general plant stress (White 1984).

Phenolic compounds as resistance traits

A common response in stressed plants is elevated levels of phenolic compounds (Witzell and Martin 2008). Three individual phenolics displayed clinal trends; in addition, benzoic acid was negatively associated with damage by *Phyllocnistis* and positively associated with *Melampsora* infection, suggesting interaction-specific roles for this compound in the chemical defence of *P. tremula*. Varying roles for benzoic acid have been reported earlier: it is a precursor of salicylic acid (Chong et al. 2001) and generally induced in response to stress by abiotic and biotic factors (Pausler et al. 1995, Chong et al. 2001, Senaratna et al. 2003). Enhanced tolerance against a range of pathogens has been reported for plants with elevated levels of benzoic acid (Amborabé et al. 2002, Williams et al. 2003, Wang et al. 2005), although in other cases a lack of effect on pathogens has been recorded (Silverman et al. 2005, Santiago et al. 2006).

Testing predictions of the GMTC

The geographic mosaic theory of coevolution (GMTC, Thompson 1997, 1999) suggests mechanisms by which local selective pressures may shape resistance patterns in coevolving systems at the landscape scale. The time frame of selection is often highly un-balanced between a tree host and its parasites. Our system is no different. As a clone-forming tree *P. tremula* even extends its generation time beyond the life time of an individual tree whereas most of its insect and fungal parasites have life cycles that last for only a year or less. Therefore, even though we may not watch ongoing selection, we may still test assumptions of the GMTC in relative terms by comparing pests that differ in reproductive potential and dispersal range. Abundance thus reflects a parasite's hot spot size relative to surrounding cold spots, and its dispersal range and mode mirrors its potential gene flow, which are both suggested to shape the presence and strength of clinal resistance patterns at the landscape level (Gomulkiewicz et al. 2000, 2003, Nuismer et al. 2003).

Clines of *Phyllocnistis* were stronger and more persistent within and across years, compared to *Melampsora* which showed contrasting clines of varying strength. We tested our data for clinal behaviours varying with distance to clone origin or, in a rougher manner, separating the data into a northern and a southern behaviour. The smooth clinal model was preferred in the case of *Phyllocnistis* and benzoic acid which supported the distance related pattern for this parasite and for one phenolic trait. In contrast growth parameters were better fitted by the north-south model and *Melampsora* was either not related to landscape patterns or equally well fitted by both models, thus supporting a less consistent clinal pattern. *Melampsora* spp. have a complex life cycle that includes asexual autoinfection on *P. tremula* and sexual reproduction on its alternative host, and which gives rise to an extremely large production of spores and a high potential for selection and adaptation at both local and regional landscape scales. *Phyllocnistis*, on the other hand, has one to two generations per year within the studied area, and its fecundity and dispersal potential is comparatively low. Thus, our data support the assumption of the GMTC model that clines exist at the border between hot and cold spots and that they should be less persistent for organisms with the elevated gene flow and large hot spots (in this case, *Melampsora* spp.).

Problems in assessment of clines, hot spots, cold spots and resistance traits

The within-seasonal variation in the level of damage makes damage assessments challenging. For instance, the frequency of rust uredia generally increases during the growing season, whereas damage by *Phyllocnistis* peaks in the middle of the season. Thus, repeated assessments of subpopulations need to be performed over several years (Nuismer et al. 2003) and, ideally, they should also be performed several times through a season and in random order, to correct for systematic differences in damage as a result of timing and seasonality. In the assessment of resistance to different parasites, differences in the mode of attack may also complicate the analysis. In our study system, for example, *Phyllocnistis* mines are the result of single successful ovipositions, where moth searching behaviour and abundance are important, whereas infection by rust involves initial colonisation of spores and subsequent autoinfection.

The concept of hot and cold spots is associated with patchy distribution of a natural enemy across the host's range (Thompson 1997, 1999, Nuismer et al. 2003). The problem of detecting empty patches or cold spots has previously been addressed in landscape ecology (Tyre et al. 2003, Gu and Swihart 2004, Mackenzie 2005, Mackenzie and Royle 2005). Pseudo-absence, non detection, imperfect detection or false negatives may bias the reliability of damage assessments in un-manipulated field studies. In the national survey data we found a considerable decrease in sample sizes north of latitude 60°, enhancing the risk of false negatives. When we averaged the data for three years to reduce this risk, the data set still contained no damage records for these latitudes. The scoring levels used in the National Forest Damage Inventory Project thus failed to recognise low damage levels, although the causal agents were, in fact, plentiful. On the other hand, in the middle of Sweden, below latitude 60°, *Phyllocnistis* may cause the foliage to have a silvery appearance, a feature not reported from other parts of the country. This centre of *Phyllocnistis* distribution is confirmed by epidemiological data, which not only support the presence of a hot spot, but further suggest that the hot spot may be much more clearly defined than we have been able to demonstrate with available data. Furthermore, the clines towards the surrounding cooler spots may be even more extensive at the landscape scale than we were able to demonstrate in the common gardens.

In summary, this paper provides evidence that two parasites show different large-scale dynamics on the same host, which may be related to their life cycle and dispersal mode. By combining epidemiologic data and transplant experiments, resistance patterns could be interpreted at the landscape level, and the presumption behind the GMCT that gene flow is likely to determine the distribution of resistance genes in natural plant populations was supported. As possible selective traits in *P. tremula* we included growth and defence compounds. However, these traits may be inter-correlated, and future studies should address the interrelationship between growth parameters, phenolic profile, and other clone attributes that may shape resistance.

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