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Genetic structure of the forest pest *Hylobius abietis* on conifer plantations at different spatial scales in Europe

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The distribution of genetic variation within and among 20 European sites infested by the large pine weevil, *Hylobius abietis*, was analysed using dominant amplified fragment length polymorphism markers. Analysis of molecular variance was performed at the European, regional and local scales. Most of the genetic variability was found within rather than among populations and the global fixation index averaged over *loci* was low (0.07). We found no evidence of genetic drift, even in relatively isolated sites. This genetic pattern tends to confirm the high dispersal ability of the weevil and the influence of human-mediated expansion of its range through conifer plantations across Europe since the 19th century. Assignment tests demonstrated that the regional forest is a pertinent geographic scale for defining populations in the large pine weevil. Testing the potential influence of the larval host-plant identity (Scot Pine *vs* Norway Spruce) on the genetic structure revealed a weak but significant effect in two of the three regions tested (in Ardèche and in Limousin but not in Finland). One *locus* varied with host-plant use in the two French regions, indicating a potential role in host-plant adaptation. However, host-race formation is not observed in *H. abietis*; we discuss this result in the light of our current knowledge of this insect's biology. Altogether, this study shows that the use of different host plants for development does not constitute a strong barrier to gene flow for *H. abietis* and confirms the high dispersal ability of this forest pest.
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Introduction

The large pine weevil, *Hylobius abietis* (Coleoptera: *Curculionidae*, L.), is the most economically important pest of young conifer plantations in commercial forestry throughout western Europe (Leather *et al*, 1999; Lempérière and Julien, 2003; Grégoire and Evans, 2004). Adults are attracted to stumps of felled trees where the females lay eggs in the bark. The larvae feed under the bark of stumps and roots, where they do no economic damage. In contrast, adults feed around the root-collar of seedling trees (Lempérière and Julien, 2003). When the feeding damage on newly replanted seedlings is severe, the trees die.

In their attempt to summarize the crucial issues and the research priorities for Bark and Wood Boring Insects in Living Trees in Europe (BAWBILT organisms), Lieutier *et al* (2004) raise the point that *H. abietis* are highly damaging to forest trees, but also difficult to track in time and space. Knowledge of the population dynamics and of its driving forces is crucial for forest management. For this purpose, molecular tools are increasingly and successfully used in population genetic studies of insect pests to infer ecological characteristics that are crucial for establishing management strategies, as, for example, the elucidation of long-range movement in the boll-weevil (Kim and Sappington, 2004), or the clarification of the host-plant's role as a potential barrier to colonization and spread in a Chrysomelid pest of willows (Batley *et al*, 2004). Moreover, identifying ecotypes or host races within the target pest species is central to the establishment of successful control programmes (Bourguet *et al*, 2000). For *H. abietis* demonstrating the occurrence of host races could be important for predicting pest response to environmental changes such as the introduction of new conifer cultivars, or the success of a biological control agent such as entomopathogenic nematodes (Salinas, 2002).

Owing to their wide distribution across the Eurasian continent, populations of the large pine weevil are expected to undergo variable selection pressures due to the climatic conditions and the nature and availability of the host plant. This spatial variation in selection creates the potential for local adaptation, but its outcome depends on the magnitude of selection relative to effects of genetic drift and gene flow (Slatkin, 1973). These evolutionary forces do not act similarly on the whole genome: genetic drift affects all loci in the same way, whereas natural selection acts only on those genes involved in adaptation (Campbell and Bernatchez, 2004). Simultaneously analysing multiple *loci* scattered throughout the genome allows us to disentangle those effects. For this study, we therefore chose the amplified fragment length polymorphism (AFLP) technique which

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generates a large number of markers distributed throughout the genome without prior knowledge about it (Vos *et al*, 1995). Genome-wide analysis of population differentiation allows not only inferences about the relative importance of genetic drift and migration among populations but also assessment of the adaptive differentiation of populations by searching for atypical *loci* or 'outliers', that is, *loci* that exhibit exceptionally high or low values of F_{st} between groups of individuals in different environments (Beaumont and Nichols, 1996; Luikart *et al*, 2003).

The present study addresses the following questions on the biology of the insect through examination of its population genetic patterns:

Is the genetic structure shaped by outbreak dynamics?

Perhaps because of the ephemeral nature of its habitat, *H. abietis* is a moderately outbreaking species (Speight and Wainhouse, 1989). This means that it periodically undergoes eruptive outbreak events that are followed by population size decreases when conditions become unfavourable. Depending on the minimum population size reached, those crashes may create genetic bottlenecks. The genetic composition may also be strongly influenced by founder effects when new populations are established by few migrants. In this context, genetic drift may play an important role in the genetic composition of a given population. Thus, genetic drift may be responsible for decreasing genetic variation within geographically isolated populations and increasing genetic variation among geographically isolated populations, without causing a pattern of isolation by distance.

Alternatively, is the structure shaped by high population mixing?

This insect became a major pest as a consequence of human forestry activity (Leather *et al*, 1999). Indeed, clear-felling provides numerous favourable breeding sites, and consequently the number of sites no longer represents a population limiting factor (Örlander *et al*, 1997). It might therefore be predicted that the populations of this insect will remain dense in managed forest regions where breeding sites are always available. This pattern could promote high levels of population mixing depending on the weevil dispersal behaviour. Under this scenario, we might expect high genetic variation to be found within populations and reduced genetic differentiation among populations.

Does the larval host-plant act as a selective factor

In phytophagous insects, distribution, availability, longevity and chemistry of the host plant are major factors affecting the genetic differentiation of populations (Mopper, 1996; Zangerl and Berenbaum, 2003). Recent studies of *H. abietis* highlight the importance of considering the influence of the tree species on the larval performance (von Sydow and Birgersson, 1997; Thorpe and Day, 2002). There was considerable variation in larval mortality, development time and weight of adults at emergence, depending on the host species (Thorpe and Day, 2002), which can affect the potential of the insect to damage its hosts. If differential selection on the alternate hosts is strong enough to cause local adaptation to each host species, it can also prevent gene flow, even locally.

Among the many conifer species cultivated throughout the Eurasian continent, Norway spruce (*Picea abies* Karst) and Scots pine (*Pinus sylvestris* L.) are of primary importance for *H. abietis*. Their current distribution in Europe is mainly the result of the relatively recent plantations for commercial forestry and they overlap in managed forests such as in the regions of Limousin and Ardèche, France. In contrast, the forest of Landes (southwest of France) has comprised a monoculture of *Pinus pinaster* (Ait.) trees since the 19th century. If the use of several host plants by the larvae were a source of diversifying selection, we would expect to find a lower proportion of neutral genetic variation in populations established in monocultures than in more diversely planted forest regions.

Experimental procedures

Study sites and sampling

The 367 weevils (305 larvae and 62 adults) were collected in 20 sites across Europe in Estonia, Finland, France, Ireland and Poland. Sampling sites are detailed in Table 1 and the locations are shown in Figure 1. Collecting sites mainly consisted of exploited forest parcels where larvae were collected on stumps. In order to avoid sampling other Curculionid species, we sampled only the late instars of *H. abietis* larvae, which can be unambiguously identified by their size and cephalic capsule characteristics. We also collected larvae on trees fallen after storms (Les Quatre Vios in Ardèche and Royère in Limousin, France). Forest parcels in which we collected larvae both on pine and spruce stumps are designated as 'mixed' localities because the two host plants were available at the same time for the females laying eggs. Thus we obtained three forest region replicates for comparing the host-plant influence: two in France (Ardèche and Limousin) and one in Finland. To test for the effect of the host plant on the genetic diversity of the insects, adults were excluded from the tests because it was not possible to know the host plant they developed on as larvae. Insects were immediately killed at sampling by putting them in absolute ethanol for optimal DNA preservation.

AFLP protocol

The protocol is known to be sensitive to contamination, especially from bacterial or fungal DNA (Savelkoul *et al*, 1999), which can be abundant in the digestive system of the insect. To avoid such contaminations, muscular tissue was dissected and isolated from both larvae and adults. Total DNA was extracted using the Dneasy Tissue Kit (Qiagen) following the manufacturer's instructions. Genomic DNA was first digested with 2 U of *Taq*I at 65°C for 1 h, and secondly with 6 U of *Eco*RI at 37°C for 1 h. Double-stranded *Taq*I and *Eco*RI adapters were ligated to restriction fragments for 3 h at 37°C. The *Eco*RI adapters were 5′-CTCGTAGACTGCGTACC-3′ and 5′-AATTGGTACGCAGTCTAC-3′. The *Taq*I adapters were 5′-GACGATGAGTCCTGAC-3′ and 5′-CGGTCAGGACT CAT-3′. The preselective PCR amplification parameters were as follows: 2 min at 72°C, 30 cycles of 30 s denaturing at 94°C, 30 s annealing at 56°C and 2 min extension at

Table 1 Geographic location, collector name and characteristics of *Hylobius abietis* sites collected

Site	*Region-country*	*Longitude/latitude*	*Sample size (and life stage: 'A' for adults and 'L' for larvae)*	*Host plant (for larvae) (s for spruce, p for pine)*	*Collector*
1. Jalkala	Finland	27°15′E/62°33′N	10 L	Spruce	H Viiri
2. Kalakukkokangas	Finland	27°15′E/62°43′N	73 L	Mixed (35 s, 38p)	H Viiri
3. Mooste	Estonia	27°08′E/58°10′N	15 A	—	K Voolma
4. Celestynów	Poland	21°14′E/51°18′N	25 A	—	I Skrzecz
5. Les Quatre Vios	France–Ardèche	4°13′E/44°28′N	56 L	Mixed (38 s, 18p)	C Conord
6. Lachamp Raphaël	France–Ardèche	4°18′E/44°49′N	8 L	Spruce	C Conord
7. Mézilhac	France–Ardèche	4°21′E/44°48′N	10 L	Pine	C Conord
8. S^{t} Etienne de Lugdares	France–Ardèche	3°57′E/44°39′N	4 L	Spruce	C Conord
9. Annouillards	France–Limousin	2°12′E/45°40′N	28 L	Mixed (23 s, 5p)	C Conord
10. Basville	France–Limousin	2°24′E/45°52′N	10 L	Pine	C Conord
11. Bellechassagne	France–Limousin	2°13′E/45°39′N	7 L	Pine	C Conord
12. Ebraly	France–Limousin	2°22′E/45°34′N	29 L	Pine	C Conord
13. Maussac	France–Limousin	2°09′E/45°28′N	19 L	Pine	C Conord
14. Pontgibaud	France–Limousin	2°52′E/45°49′N	10 L	Pine	C Conord
15. Puits de la Blanche	France–Limousin	2°01′E/45°17′N	7 L	Spruce	C Conord
16. Royère	France–Limousin	1°54′E/45°49′N	5 L	Pine	C Conord
17. Le Sen	France–Landes	1°30′W/44°07′N	6 L	Pine	C Conord
18. Pontenx	France–Landes	2°52′W/44°15′N	14 L	Pine	C Conord
19. Callan Forest	Ireland	7°37′W/52°42′N	9 L	Pine	D Ward
20. Galway	Ireland	9°03′W/53°16′N	22 A	—	G Lempérière

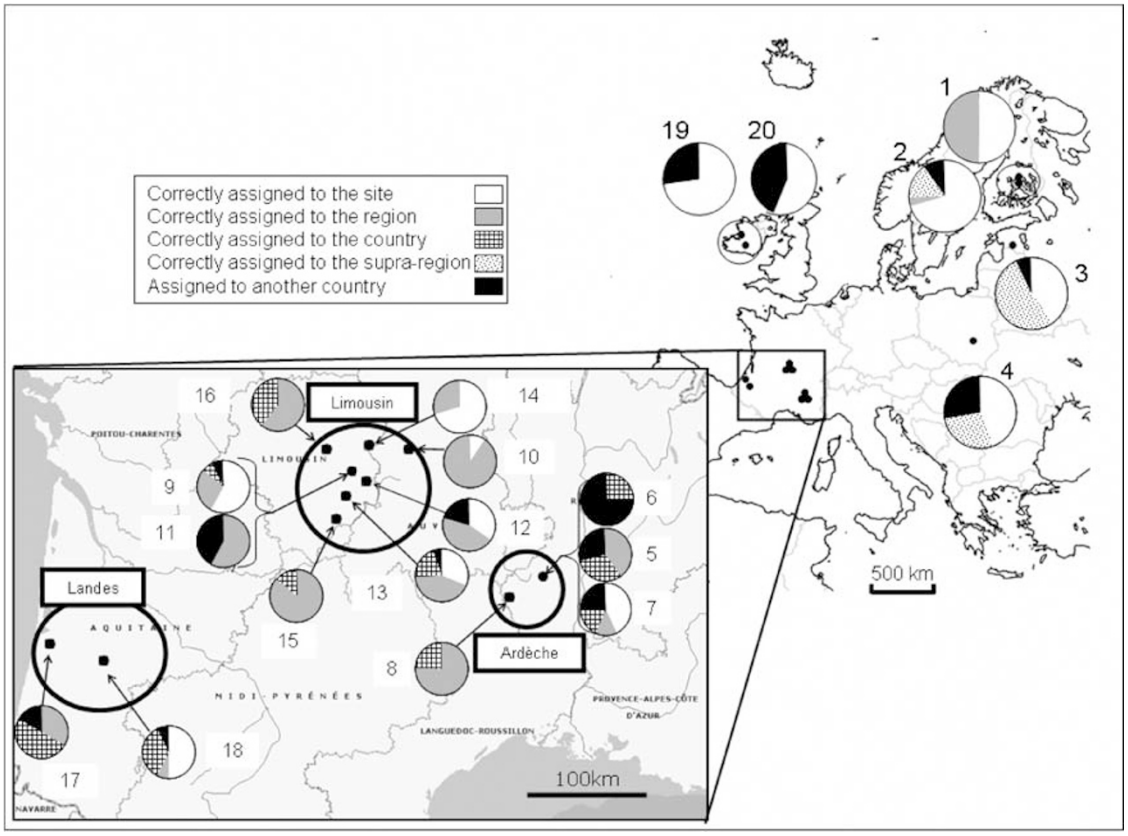

Figure 1 Geographic location of the 20 sampled sites of *H. abietis* and results of the assignment tests. For each site, the diagrams show the proportion of individuals according to the geographic level they were assigned to.

72°C, ending with 10 min at 72°C for complete elongation, using *Taq*I primer T/A (5′-GATGAGTCCTGACCG AA-3′) and *Eco*RI primer E/A (5′-GACTGCGTACCAA TTCA-3′). For the selective amplification, touchdown PCR parameters were 10 min at 95°C followed by 36 cycles of 30 s denaturing at 94°C, 30 s annealing and 1 min extension at 72°, ending with 10 min at 72°C for complete extension. Annealing was initiated at 65°C and then reduced by 0.7°C for the next 12 cycles and maintained at 56°C for the remaining 23 cycles. Three selective PCR primer pairs were selected over 17 tested for the quality of the produced bands (ie an even distribution of bands with relatively homogeneous intensity): E/ATC-T/ACA, E/ATC-T/AAG and E/AGT-T/AAG.

Selective products were analysed on an ABI Prism 3100 DNA automated sequencer and AFLP patterns were visualized with Genescan® Analysis 3.7 (Applied Biosystem). AFLP profiles were recorded in a matrix as the presence (1) or absence (0) of bands for each individual. A fragment was considered as absent in a given individual if the electrophoregram showed a peak not higher than 10% of the size of the highest peak found among all individuals. Fragments that could not be scored unambiguously were not included in the analysis. Reproducibility of each primer pair was tested by carrying out the whole AFLP protocol twice for each of 35 randomly chosen individuals (9.5%). Using the methodology of Bonin *et al* (2004), we calculated a reproducibility rate of 92% as the ratio between observed number of phenotypic differences and total number of phenotypic comparisons.

Data analysis

Independence of markers was assessed by calculating their pairwise linkage index $D_{A,B}=1/n \sum i \mid V(A,i)-V(B,i) \mid$, where $V(A,i)$ is the allele value of individual i for the marker A and n, the total number of individuals analysed. D-values were calculated using the program DDM (disequilibrium between dominant markers) version 0.1 (P Berthier, personal communication).

Genetic diversity within populations was quantified using POPGENE version 1.32 (Yeh *et al*, 1997) in three ways: (i) the percentage of polymorphic *loci*, (ii) Nei's (1978) unbiased expected heterozygosity (H_E) and (iii) Shannon's index of phenotypic diversity (I, Lewontin, 1972). Values of H_E and I were obtained by averaging across *loci*.

Genetic differentiation among populations and groups was calculated as the unbiased F_{st} estimator θ of Weir and Cockerham (1984) and its 95% confidence interval, obtained by bootstrapping 1000 replicates over *loci* using TFPGA Version 1.3 (Miller, 1997). In order to test for population differentiation, exact tests (Raymond and Rousset, 1995) were performed with TFPGA. These tests use a contingency table (Fisher's RxC test) and a Markov Chain Monte Carlo approach to determine if significant differences in allele frequencies exist among groups of individuals.

The genetic structure was examined by an analysis of molecular variance (AMOVA) using the ARLEQUIN 2.011 software package (Excoffier *et al*, 1992; Schneider *et al*, 2000). This method was used to partition the genotypic variance among groups, among populations within groups and within populations. The individuals were grouped either by geographical location or by host species (for larvae only) or both. Levels of significance were determined through the computing of 1000 random permutations replicates. For host-plant effect, we also considered the F_{st} values that ARLEQUIN provided for each individual *locus*. *Loci* with $P<0.05$ for F_{st} were considered significantly differentiated (Emelianov *et al*, 2004).

In order to test for a correlation between genetic distances (pairwise $F_{st}/(1-F_{st})$ according to Rousset (1997) and geographical distances (in km) among populations, Mantel tests were performed using GenAlEx V5 computing 1000 permutations (Peakall and Smouse, 2001).

Assignment tests were performed using AFLPOP 1.1, the population allocation software based on AFLP markers, of Duchesne and Bernatchez (2002). We used the reallocation procedure that estimates the expected allocation success rate for each of the source populations. Each individual was withdrawn from its population and allelic frequencies for this population were computed anew (the 'leave one out' procedure). The likelihood that the individual genotype was found in each of the tested populations was calculated. Each individual was then allocated to the population showing the highest likelihood for the genotype.

Results

AFLP polymorphism

Using three primer combinations, we unambiguously scored 83 polymorphic markers. The 367 genotyped insects gave 367 different haplotypes. The mean linkage index was 0.45. No marker pairs had D-values lower than 0.01 or higher than 0.99, meaning that all 83 markers were unlinked. Consequently, they were all retained for further analyses.

High within-population genetic diversity

The percentage of polymorphic loci ($P\%$) and the Nei (H_E) and Shannon (I) indices within each of the 20 sites were highly correlated (Table 2, pairwise Spearman's rank correlation coefficient $P\%$ *vs* H_E: $r_s=0.650$ with $P=0.002$ and I *vs* H_E: $r_s=0.959$ I *vs* $P\%$: $r_s=0.813$ both with $P<0.0001$). The percentage of polymorphic *loci* was the only diversity index to be significantly positively correlated to the sample size (Spearman's rank correlation coefficient $r_s=0.672$ $P=0.001$). The three within-population diversity indices did not significantly differ across French regions and other European countries (Global Kruskal–Wallis test $P=0.136$). More precisely, diversity in isolated populations such as Ireland and Landes (France) was not significantly lower than in other regions. Similar tests showed no significant differences for the three indices among groups of individuals associated with different hosts, between insects at single-host and mixed-host sites or between adults and larvae.

Low among-population differentiation

Nei's unbiased genetic distance ranged from 0.0039 between populations 11 and 12 to 0.0935 between populations 1 and 14 (Table 3). Pairwise exact tests of population differentiation indicate that there are no significant genetic differences among French sites. Finnish and Irish sites were differentiated from nearly all the other sites, whereas the Estonian site did not significantly differ from the Polish site or from several French sites.

The global *Theta* value among countries was 0.03 (95% confidence interval (CI) 0.023–0.049) and it was 0.07 (95% CI 0.057–0.085) among the 20 sites, indicating weak among-population differentiation. In France, the global *Theta* among sites and among regions (Ardèche, Landes, Limousin) was 0.05 (95% CI 0.042–0.070) and 0.03 (95% CI 0.016–0.053), respectively.

At the European scale, the major part of genetic variation was found within countries (94.46%), with only 5.54% of variation among countries (Table 4, a1). Exact tests showed a strong genetic differentiation in all

Table 2 Within-population genetic diversity indices of the 20 sites studied

Country	*Region*	*Site*	*Number of polymorphic loci*	*Percentage of polymorphic loci (P%)*	*Nei's diversity index (H_E)*	*Shannon index (I)*
Finland	—	Jalkala	62	74.70%	0.201 (0.170)	0.318 (0.240)
	—	Kalakukkokangas	81	97.59%	0.278 (0.147)	0.434 (0.186)
			T: 82	M: 86.14% (11.44%)	M: 0.239 (0.038)	M: 0.376 (0.058)
Estonia			T: 74	89.16%	0.289	0.439
Pologne			T: 83	100%	0.288 (0.133)	0.450 (0.166)
France	Ardèche	Les Quatre Vios	83	100%	0.285 (0.142)	0.445 (0.178)
		Lachamp Raphaël	77	92.77%	0.293 (0.157)	0.448 (0.206)
		Mézilhac	72	86.75%	0.266 (0.168)	0.409 (0.226)
		S^t Etienne de Lugdares	65	78.31%	0.288 (0.182)	0.429 (0.253)
			t: 83	m: 91.32% (8.0%)	m: 0.283 (0.010)	m: 0.432 (0.015)
	Landes	Le Sen	66	79.52%	0.268 (0.177)	0.406 (0.246)
		Pontenx	72	86.75%	0.274 (0.171)	0.418 (0.231)
			t: 79	m: 83.13% (3.61%)	m: 0.271 (0.003)	m: 0.412 (0.006)
	Limousin	Annouillards	80	96.39%	0.280 (0.145)	0.436 (0.184)
		Basville	69	83.13%	0.261 (0.170)	0.401 (0.233)
		Bellechassagne	70	84.34%	0.261 (0.159)	0.404 (0.220)
		Ebraly	82	98.80%	0.268 (0.140)	0.423 (0.179)
		Maussac	80	96.39%	0.290 (0.148)	0.448 (0.190)
		Pontgibaud	65	78.31%	0.272 (0.190)	0.407 (0.262)
		Puits de la Blanche	67	80.72%	0.256 (0.177)	0.392 (0.243)
		Royère	63	75.90%	0.260 (0.182)	0.393 (0.254)
			t: 83	m: 86.75% (8.48%)	m: 0.268 (0.011)	m: 0.413 (0.019)
			T: 83	M: 87.00% (8.06%)	M: 0.273 (0.012)	M: 0.418 (0.019)
Ireland	—	Callan	69	83.13%	0.254 (0.183)	0.388 (0.252)
	—	Galway	51	61.45%	0.196 (0.194)	0.298 (0.275)
			T: 76	M: 72.29% (10.84%)	M: 0.225 (0.029)	0.343 (0.045)

Total (T) or mean (M) values for the different scales (uppercase for countries; lowercase for regions). Standard deviations in parentheses.

pairwise comparisons of the five countries ($P<0.0001$), except for Estonia and Poland, which were not significantly different ($P=0.1030$). At the regional scale in France, the genetic variation was principally found within sites in regions 93.12 *versus* 2.93% among regions (Table 4, a2). When Ardèche and Limousin regions were analysed separately, the greatest part of genetic variation was found within sites rather than among sites and the differentiation across the sites was much lower in Ardèche (Table 4, a2.1 and a2.2). Exact test indicated a clear genetic differentiation among the three French regions ($P<0.0001$).

Performing a hierarchical AMOVA allowed to simultaneously test for the effect of the geography and host plant on genetic differentiation. We defined three geographical groups containing nonsignificantly differentiated sites according to the exact test (Table 3). We retained all sites in Ardèche, but excluded site 14 in Limousin and site 1 in Finland. Each group was partitioned into two subgroups corresponding to pools of larvae collected on pine and spruce. Sample sizes were 28, 75 and 38 on pine, and 50, 30 and 35 on spruce, respectively. Most of the genetic variation (corrected for sample size) was found within host-plant groups (94.34%); the genetic variation between geographic groups was weak (4.53%), whereas that between host plants within the geographical groups was significant but low (1.13%) (Table 4b). When each geographic region was considered separately, the AMOVA revealed low but significant genetic variation between larvae collected on pine and those collected on spruce: 1.16 and 1.75%, respectively, for Ardèche and Limousin ($P<0.01$), but no significant variation between host-plants for the mixed site in Finland (not shown). Testing the reciprocal hierarchical grouping (ie two groups based on the host-plant type, each containing three geographical subgroups) revealed a comparable amount of genetic variation explained by the geographic structure (6.1% $P<0.0000$). However, in this case, the host-plant grouping did not significantly structure the observed genetic variation (−1.49% $P=0.68$). Significant divergence between spruce and pine pools of larvae was found for five *loci* (among 83) in Ardèche and nine *loci* in Limousin. Averaged F_{st} for those outlier *loci* across host-plant groups was comparable for the two regions: 0.15 and 0.13, respectively. Ardèche and Limousin shared one outlier *locus* responsible for 13 and 12.5% of variation, respectively, in each region.

Genetic *vs* geographical structure

The overall Mantel test based on the 20 sites showed a marginally significant positive correlation between geographic and genetic distances ($r=0.3909$; $P=0.016$). In France, there was no correlation when considering the 14 localities ($r=0.251$; $P=0.125$), but a positive significant correlation was found at the regional scale in Limousin ($r=0.642$; $P=0.013$).

Assignment tests

Individuals were grouped by geographic level of assignment (Figure 1). For Estonia, Finland and Poland, the dotted pattern indicates the proportion of individuals that were not assigned to their country of origin but to one of the two others (they were grouped in a north-eastern 'supra-region' in the analysis). In France, the mean percentage of correct assignment (in white) to localities was low (21%), but the mean proportion of individuals assigned outside France (in black) was also weak (16%). Most individuals were correctly assigned

Table 3 Pairwise Nei's unbiased genetic distance among populations of European pine weevils and exact tests of population differentiation

	1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*	*13*	*14*	*15*	*16*	*17*	*18*	*19*	*20*
2	*0.0239* ***	—																		
3	0.0465 ***	0.0083 *	—																	
4	0.0324 ***	0.0063 ***	0.0113 NS	—																
5	0.0299 ***	0.0104 ***	0.0143 ***	0.0057 ***	—															
6	0.0609 **	0.0238 NS	0.0171 NS	0.0088 NS	*0.008* NS	—														
7	0.0424 *	0.015 **	0.0108 NS	0.008 NS	*0.0078* NS	*0.0139* NS	—													
8	0.0514 ***	0.0195 ***	0.0145 NS	0.0077 NS	*0.0094* NS	*0.0084* NS	*0.0103* NS	—												
9	0.0377 ***	0.0202 ***	0.0192 ***	0.0136 ***	0.0076 ***	0.0113 NS	0.0100 NS	0.0118 NS	—											
10	0.0538 **	0.0301 ***	0.0246 **	0.024 ***	0.0241 ***	0.0258 NS	0.0226 NS	0.0262 NS	*0.0173* NS	—										
11	0.0216 NS	0.0092 NS	0.0226 NS	0.005 NS	0.007 NS	0.0218 NS	0.0139 NS	0.0204 NS	*0.0107* NS	*0.0222* NS	—									
12	0.0257 ***	0.0155 ***	0.02 ***	0.0101 ***	0.0085 ***	0.0148 NS	0.0124 NS	0.0214 ***	*0.0058* **	*0.0154* NS	*0.0039* NS	—								
13	0.0378 ***	0.016 ***	0.0159 **	0.0116 ***	0.0089 ***	0.0104 NS	0.0078 NS	0.0152 NS	*0.005* NS	*0.0137* NS	*0.0061* NS	*0.0067* NS	—							
14	0.0935 ***	0.0574 ***	0.0426 ***	0.0504 ***	0.0475 ***	0.0434 NS	0.0365 NS	0.0410 **	*0.0344* ***	*0.0499* NS	*0.0417* *	*0.0413* ***	*0.0363* ***	—						
15	0.0496 **	0.0267 **	0.0263 NS	0.0199 NS	0.013 NS	0.0182 NS	0.0132 NS	0.0308 NS	*0.0134* NS	*0.0208* NS	*0.0219* NS	*0.0119* NS	*0.0119* NS	*0.0485* *	—					
16	0.0501 NS	0.0212 NS	0.0153 NS	0.022 NS	0.0198 NS	0.0192 NS	0.0141 NS	0.0275 NS	*0.0156* NS	*0.0194* NS	*0.0139* NS	*0.0175* NS	*0.0161* NS	*0.0257* NS	*0.0286* NS	—				
17	0.0541 **	0.0361 ***	0.0277 NS	0.0342 NS	0.0273 NS	0.0348 NS	0.0322 NS	0.0258 NS	0.0292 NS	0.0402 NS	0.0342 NS	0.0351 NS	0.0303 NS	0.0542 NS	0.0406 NS	0.0267 NS	—			
18	0.0571 ***	0.0284 ***	0.0162 **	0.0276 ***	0.0205 ***	0.0328 NS	0.0189 NS	0.0244 **	0.0224 ***	0.0332 ***	0.0296 *	0.0257 ***	0.0206 ***	0.0368 ***	0.0239 NS	0.0213 NS	*0.0128* NS	—		
19	0.0297 NS	0.0231 ***	0.0381 ***	0.019 ***	0.018 ***	0.0457 NS	0.0376 ***	0.038 ***	0.0339 ***	0.0418 ***	0.0126 NS	0.0217 ***	0.0308 ***	0.0857 ***	0.0357 ***	0.0466 **	0.0555 ***	0.0441 ***	—	
20	0.053 ***	0.0195 ***	0.0178 ***	0.0136 ***	0.0192 ***	0.0265 NS	0.019 ***	0.0175 ***	0.0244 ***	0.0437 ***	0.021 ***	0.0251 ***	0.0243 ***	0.0426 ***	0.0348 ***	0.0224 *	0.0371 ***	0.0259 ***	*0.0319* ***	—

Populations numbered as in Figure 1. Pairwise comparisons within regions are in italic.
Significance levels of exact tests of differentiation are shown as follows: NS: nonsignificant; *$0.01 < P < 0.05$; **$0.001 < P < 0.01$; ***$P < 0.001$.

Table 4 Analysis of molecular variance (AMOVA) of *Hylobius abietis* populations using 83 AFLP *loci*

Source of variation	*d.f.*	*Sum of squares*	*Variance*	*% total*	*Φ statistics*	P
(a) Geographical grouping						
(a1) European Scale						
Among countries	4	261.747	0.909	5.54		
Within countries	362	5615.038	15.511	94.46		
Total	366	5876.785	16.420		$\Phi_{ST} = 0.0554$	<0.0001
(a2) Regional scale in France						
Among regions	2	117.805	0.477	2.93	$\Phi_{CT} = 0.0293$	0.0009
Among sites within regions	11	255.791	0.641	3.94	$\Phi_{SC} = 0.0406$	<0.0001
Within site	199	3015.681	15.154	93.13		
Total	212	3389.277	16.298		$\Phi_{ST} = 0.0687$	<0.0001
(a2.1) Ardèche						
Among sites	3	57.003	0.301	1.91		
Within site	74	1142.343	15.437	98.09		
Total	77	1199.346	15.738		$\Phi_{ST} = 0.0191$	0.0117
(a2.2) Limousin						
Among sites	7	185.431	0.843	5.30		
Within site	107	1611.195	15.057	94.70		
Total	114	1796.626	15.900		$\Phi_{ST} = 0.0530$	<0.0001
(b) Hierarchical grouping: geographical – host plant						
Among geographical groups[a]	2	171.028	0.734	4.53	$\Phi_{CT} = 0.0453$	<0.0001
Among host-plant groups within regions	3	67.029	0.183	1.13	$\Phi_{SC} = 0.0118$	<0.0001
Within host-plant group	270	3824.416	15.298	94.34		
Total	275	4062.473	16.215		$\Phi_{ST} = 0.0566$	<0.0001

[a]Ardèche, Limousin and Finland.
The source of variation was tested using three scales of geographic partitioning (European, regional and local) and according to the larvae's host plant.
df, degrees of freedom; *P*, probability of obtaining a more extreme component estimate by chance alone (1000 permutations).

to one of the sites in the same forest region (in grey, 43%). In general, Irish individuals were correctly allocated to their own site and none of them was allocated to the other Irish site.

Discussion

Within-population diversity

Whatever the geographic level considered, most of the genetic diversity was found within populations of the large pine weevil. A similar pattern (with 82–86% of variation within populations) was found using mitochondrial sequences in the Scolytid *Tomicus piniperda* L. (Kerdelhué *et al*, 2002). Similarly, RAPD analysis of the common pine sawfly *Diprion pini* L. also indicated that on average 84% of the genetic diversity comprised intrapopulation variation (Baumann *et al*, 2003). Comparable large proportions of genetic variation within populations have been found in insect species with recent range expansion, and may be due to the increased availability of a cultivated host: for example, the boll-weevil (*Anthonomus grandis* Boheman), which took advantage of the extensive culture of cotton in North America (Kim and Sappington, 2004).

H. abietis was first described as a forest pest at the early beginning of the 19th century (Leather *et al*, 1999). Extensive conifer plantations in Western Europe go back to the two last centuries (Agnoletti and Anderson, 2000). The host plant's expansion of geographic range together with the modern harvesting techniques used in forests boosted the population dynamics of *H. abietis*. Indeed, recent estimates of *H. abietis* larval population size ranged from 46 400 to 170 825 individuals per hectare in clearcut sites (Day *et al*, 2004). Such large populations would appear to be unlikely to loose alleles through genetic drift and our results support the supposition that they do not undergo drastic size reduction. They do not appear to be driven by a severe outbreak regime. Even apparently isolated sites (two sites from Ireland and two sites from the Landes forest in France) did not exhibit the reduced diversity that would be expected if substantial genetic drift was occurring.

Among-population dispersal

The overall F_{st} estimate ($\theta = 0.03$) was weak, and may reflect high migration rates across populations. However, we did not attempt to estimate migration rates from observed F_{st} because *Hylobius* populations are unlikely to be at demographic equilibrium, especially in managed forests. Such weak spatial genetic structuring has been reported for highly migratory insect species like the grain aphid *Sitobion avenae* (Llewellyn *et al*, 2003).

The assignment tests failed to accurately allocate individuals to their sampling site, but they were correctly allocated to their forest region. Accordingly, the proportion of genetic diversity revealed by the AMOVA was higher across regions than among the sites and most of pairwise comparisons among sites were nonsignificant within regions. The regional scale therefore seems to be a more coherent geographic level for genetically homogeneous unit definition in *H. abietis*. The Estonia–Finland–Poland group demonstrates that there is a

geographic coherence in assignments as the majority of the outside-allocated individuals were allocated to one of those three eastern countries.

Comparative studies of population structure in phytophagous insects have shown that genetic structure is mostly determined by the vagility of the species (Peterson and Denno, 1998). The very low genetic variation observed among *H. abietis* populations is likely to reflect its high dispersal ability, which has been observed in nongenetic studies (Solbreck, 1980; Nilssen, 1984). Using an indirect method (ie Mills experiments), Solbreck (1980) estimated that female weevils might disperse as far as 80 km during the entire flight season. This interpretation was supported by the results of Nilssen (1984), who found many *H. abietis* larvae developing on trap-logs set out at more than 80 km from the nearest coniferous forest in Finland. However, our study provides the first indication of wider dispersal behaviour in *H. abietis*, given the wide geographic range surveyed. In the Limousin region, the isolation-by-distance pattern indicated that adults tend to move preferentially between neighbouring sites. However, the low F_{st} observed between Landes, Limousin and Ardèche, and the lack of isolation-by-distance at the scale of France suggests that adults may be able to disperse up to 300 km.

This high dispersal ability in *H. abietis* might result from an adaptation to the patchy and ephemeral breeding resource in unmanaged forests. Although *H. abietis* exploits woody plants for breeding, the conifer stumps are only suitable for egg laying during a given time-window which varies with the tree species (von Sydow and Birgersson, 1997). It has been shown that evolution may increase dispersal ability in insects for which availability of suitable breeding habitat is unpredictable in space or time (Roff, 1994; Gandon *et al*, 1998). These theoretical studies are supported by several observations in forest insects (Eidmann, 1985). For example, longhorn beetles developing on decaying host plants exhibit higher dispersal ability than taxonomically close species developing on living host plants (see the review on Cerambycid beetles by Hanks, 1999). High dispersal ability was also shown by genetic analysis in *Ips typographus*, a bark beetle attacking weakened trees (Stauffer *et al*, 1999). Thus, habitat persistence may influence gene flow through its effect on the evolution of dispersal behaviour (Roderick, 1996). Our study supports this hypothesis in *H. abietis*.

Furthermore, *H. abietis* adults can live up to 4 years (Day *et al*, 2004), which increases the potential distance that a weevil can cover during its life. Moreover, as compared to larvae, adults are relatively polyphagous: they were shown to be able to feed in the crown of mature trees in the absence of young conifer seedlings (Örlander and Karlsson, 2000). This feeding plasticity reduces the potential barriers to weevil dispersal. For the adults, the forest landscape certainly appears 'fine-grained' for feeding, although it can appear 'coarse-grained' for egg laying.

The role of the host plant

This study suggests that the host plant does not constitute a strong barrier to gene flow in *H. abietis*. Although a host-plant effect was found in two of the three tested forest regions, the reciprocal hierarchical AMOVA showed that geography plays a stronger role in differentiating populations than the host plant over this geographic range. The amount of genetic variation due to host plant is very low and does not suggest host-plant-associated selection.

The larvae sampled on the two alternate host plants are genetically differentiated at 5–9 *loci*. The few genetic differences observed across host-plant larval pools may be due to within-generation selection, rather than stable genetic differences between host-associated populations. High admixture among adults might be the re-homogenise diverging pools. Alternatively, these differences may reflect the early stages of host-specialisation process occurring in some geographical locations (France) but not in others (Finland). Such geographical variation in the pattern of adaptation to the host plant is predicted by the geographic mosaic theory of coevolution, which states that the form of selection between interacting species varies across a landscape with coevolution being important and active in some locations (ie, coevolutionary hotspots) but not in others (ie, coevolutionary coldspots, Thompson, 2005). Very few AFLP studies to detect the *loci* involved in adaptation in wild populations have been conducted so far. In their study on 306 AFLP markers in intertidal snails, Wilding *et al* (2001) identified 15 outliers (4.9 %) linked to different morphological types, and Campbell and Bernatchez (2004) found 14 outliers out of 440 AFLP markers (3.2%) in the whitefish linked to trophic specialization. In the present study, we found 5–9 atypical *loci* out of 83 markers (6.0–10.8 %) in only two out of three geographical regions. Although our study suggests that few genes may be underlying processes of local adaptation, it is possible that increasing the number of markers could reveal more outlier *loci* linked to host-plant specialisation.

Given the limited evidence for genetic differentiation across host plants, is this weevil likely to evolve host races? Factors favouring host-race formation include host fidelity and correlation between host choice and mate choice (Drès and Mallet, 2002). Although *H. abietis* has been extensively studied to assess whether it is attracted to different synthetic chemicals (reviewed by Schlyter, 2004), its precise mating behaviour in the field is largely unknown. To date, no study has directly tested for host fidelity or assortative mating. In other phytophagous insects, a phenological shift in host-plant availability has been shown to promote divergence of host-associated pools of insects, notably the apple maggot fly, *Rhagoletis pomonella* (Feder *et al*, 1993) and the European corn borer, *Ostrinia nubilalis* (Thomas *et al*, 2003). The monitoring of chemical and physiological properties of Norway spruce and Scots pine stumps showed that the pine becomes suitable as a breeding substrate earlier than the spruce (von Sydow and Birgersson, 1997). By contrast, the length of the pre-oviposition period was shown to be shorter when females are fed on Norway spruce rather than on Scot pine (Wainhouse *et al*, 2001). Therefore, the phenological delay in host availability (pine first, then spruce) is counterbalanced by the opposite phenological delay in female maturity (spruce first, then pine), precluding a divergence based on phenological delay. Mating in *H. abietis* does not take place immediately after emergence on the host-plant stump, as adult feeding on conifer transplants or on twigs of mature trees is

required for sexual maturation of weevils (Wainhouse, 2001). As males and females are often found around the stumps during the reproductive period, mating may also take place while the adults feed on conifer seedlings of different tree species. Indeed, adults' feeding choice is less specific than that of larvae; hence a strong correlation between host choice and mate choice is unlikely.

Our study has shown that the current pattern of AFLP genetic diversity in *H. abietis* populations from managed European forests was strongly influenced, both by sylvicultural practices (expansion of coniferous forests outside their natural range, clear felling and planting) and by the biological characteristics of this insect. It was originally adapted to a scarce and relatively ephemeral breeding resource. Plasticity in life-history traits, longevity and polyphagy of *H. abietis* adults enhance their 'gap-crossing ability' (*sensu* Tscharntke and Brandl, 2004) and their harmfulness for the European forestry. Large-scale adult migration is likely to prevent local adaptation, preventing the formation of two host-races. The weak genetic structure observed throughout Europe suggests that an efficient biological control agent could be used widely.

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References

Agnoletti M, Anderson S (2000). *Forest history: international studies on socioeconomic and forest ecosystem change CAB International*. CAB International: Wallingford.

Batley J, Edwards KJ, Barker JHA, Dawson KJ, Wiltshire CW, Glen DM *et al* (2004). Population structure of the beetle pests *Phyllodecta vulgatissima* and *P. vitellinae* on UK willow plantations. *Insect Mol Biol* **13**: 413–421.

Baumann R, Schubert R, Heitland W, Auger-Rozenberg M, Faivre-Rimpant P, Müller-Starck G (2003). Genetic diversity within and among populations of *Diprion pini* (Hym., Diprionidae) determined by random amplified polymorphic DNA-polymerase chain reaction of haploid males. *J App Ent* **127**: 258–264.

Beaumont MA, Nichols RA (1996). Evaluating loci for use in the genetic analysis of population structure. *Proc R Soc Lond B Biol Sci* **263**: 1619–1626.

Bonin A, Bellemain E, Bronken Eidesen P, Pompanon F, Brochmann C, Taberlet P (2004). How to track and assess genotyping errors in population genetics studies. *Mol Ecol* **13**: 3261–3273.

Bourguet D, Bethenod MT, Trouve C, Viard F (2000). Host-plant diversity of the European corn borer *Ostrinia nubilialis*: what value for sustainable transgenic insecticidal Bt maize? *Proc R Soc Lond B Biol Sci* **267**: 1177–1184.

Campbell D, Bernatchez L (2004). Generic scan using AFLP markers as a means to assess the role of directional selection in the divergence of sympatric Whitefish ecotypes. *Mol Biol and Evol* **21**: 945–956.

Day KR, Nordlander G, Kenis M, Halldorson G (2004). General biology and life cycles of bark weevils. In: Lieutier F, Day KR, Battisti A, Grégoire JC, Evans HF (eds) *Bark and Wood Boring Insects in Living Trees in Europe, a Synthesis*. Kluwer Academic: Dordrecht. pp 331–349.

Drès M, Mallet J (2002). Host races in plant-feeding insects and their importance in sympatric speciation. *Phil Trans Royal Soc of London B* **357**: 471–492.

Duchesne P, Bernatchez L (2002). AFLPOP: a computer program for simulated and real population allocation, basedon AFLP data. *Mol Ecol Notes* **2**: 380–383.

Eidmann HH (1985). Silviculture and insect problems. *Z Ang Entomol* **99**: 105–112.

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 Biol Sci* **271**: 97–105.

Excoffier L, Smouse PE, Quattro JM (1992). Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. *Genetics* **131**: 479–491.

Feder JL, Hunt TA, Bush L (1993). The effects of climate, host plant phenology and host fidelity on the genetics of apple and hawthorn infesting races of *Rhagoletis pomonella*. *Entomol Exp Appl* **69**: 117–135.

Gandon S, Ebert D, Olivieri I, Michalakis Y (1998). Differential adaptation in spatially heterogeneous environments and host-parasite coevolution. In: Mopper S, Strauss S (eds) *Genetic Structure and Local Adaptation in Natural Insect Populations*. Chapman & Hall: New York. pp 325–343.

Grégoire JC, Evans HF (2004). Damage and control of BAWBILT organisms – an overview. In: Lieutier F, Day KR, Battisti A, Grégoire JC, Evans HF (eds) *Bark and Wood Boring Insects in Living Trees in Europe, a Synthesis*. Kluwer Academic: Dordrecht. pp 19–37.

Hanks LM (1999). Influence of the larval host plant on reproductive strategies of Cerambycid Beetles. *Ann Rev Entomol* **44**: 483–505.

Kerdelhué C, Roux-Morabito G, Forichon J, Chambon J, Robert A, Lieutier F (2002). Population genetic structure of *Tomicus piniperda* L. (Curculionidae: Scolytinae) on different pine species and validation of *T. destruens* (Woll.). *Mol Ecol* **11**: 483–494.

Kim KS, Sappington TW (2004). Boll Weevil (*Anthonomus grandis* Boheman) (Coleoptera: Curculionidae) dispersal in the Southern United States: evidence from mitochondrial DNA variation. *Environ Entomol* **33**: 457–470.

Leather S, Day KR, Salisbury AN (1999). The biology and ecology of the large pine weevil, *Hylobius abietis* (Coleoptera: Curculionidae): a problem of dispersal? *Bull Entomol Res* **89**: 3–16.

Lempérière G, Julien J (2003). Protection contre l'hylobe du pin: efficacité d'un insecticide systémique à base de carbosulfan. *Rev Forest Franç* **LV**: 129–140.

Lewontin RC (1972). The apportionment of human diversity. *Evolution* **6**: 381–394.

Lieutier F, Day KR, Evans HF, Langström B (2004). General conclusion and research priorities for BAWBILT organisms in Europe. In: Lieutier F, Day KR, Battisti A, Grégoire JC, Evans HF (eds) *Bark and Wood Boring Insects in Living Trees in Europe, a Synthesis*. Kluwer Academic: Dordrecht. pp 541–552.

Llewellyn KS, Loxdale HD, Harrington R, Brookes CP, Clark SJ, Sunnucks P (2003). Migration and genetic structure of the grain aphid (*Sitobion avenae*) in Britain related to climate and clonal fluctuation as revealed using microsatellites. *Mol Ecol* **12**: 21–34.

Luikart G, England PR, Tallmon D, Jordan S, Taberlet P (2003). The power and promise of population genomics: from genotyping to genome typing. *Nat Rev* **4**: 981–994.

Miller MP (1997). *Tools for Population Genetic Analyses (TFPGA) 1.3: A Windows Program for the Analysis of Allozyme and Molecular Population Genetic Data*. Department of Biological Sciences, Northern Arizona University: AZ, USA.

Mopper S (1996). Adaptative genetic structure in phytophagous insect populations. *TREE* **11**: 235–238.

Nei M (1978). Estimation of heterozygosity and genetic distance from a small number of individuals. *Genetics* **89**: 583–590.

Nilssen AC (1984). Long range aerial dispersal of bark beetles and bark weevils (Coleoptera, Scolytidae and Curculiondae) in northern Finland. *Ann Entomol Fenn* **50**: 37–42.

Örlander G, Karlsson C (2000). Influence of shelterwood density on survival and height increment of *Picea abies* advance growth. *Scand J Forest Res* **15**: 20–29.

Örlander G, Nilsson U, Nordlander G (1997). Pine weevil abundance on clearcuts of different ages: a 6-year study using pitfall traps. *Scand J Forest Res* **15**: 194–201.

Peakall R, Smouse P (2001). *GenAlEx V5: Genetic Analysis in Excel. Population Genetic Software for Teaching and Research*. Australian National University: Canberra, Australia.

Peterson MA, Denno RF (1998). Life-history strategies and the genetic structure of phytophagous insect populations. In: Mopper S, Strauss SY (eds) *Genetic Structure and Local Adaptation in Natural Insect Populations*. Chapman & Hall: New York. pp 236–322.

Raymond M, Rousset F (1995). An exact test for population differentiation. *Evolution* **49**: 1280–1283.

Roderick GK (1996). Geographic structure of insect populations: gene flow, phylogeography, and their uses. *Ann Rev Entomol* **41**: 325–352.

Roff DA (1994). Habitat persistence and the evolution of wing dimorphism in insects. *Am Nat* **144**: 772–798.

Rousset F (1997). Genetic differentiation and estimation of gene flow from F statistics under isolation by distance. *Genetics* **145**: 1219–1228.

Salinas S (2002). Entomopathogenic nematodes in Norwegian forest soils. *Nematology* **4**: 197.

Savelkoul PH, Aarts HJM, de Haas J, Dijkshoorn L, Duim B, Osten M *et al* (1999). Amplified-fragment length polymorphism analysis: the state of an art. *J Clin Microbiol* **37**: 3083–3091.

Schlyter F (2004). Semiochemicals in the life of bark feeding weevils. In: Lieutier F, Day KR, Battisti A, Grégoire J-C, Evans HF (eds) *Bark and Wood Boring Insects in Living Trees in Europe, a Synthesis*. Kluwer: Dordrecht, The Netherlands. pp 351–364.

Schneider S, Küffer J, Rössli D, Excoffier L (2000). *Arlequin Version 2.0: a Software for Population Genetic Data Analysis*. Genetics and Biometry Laboratory, University of Geneva: Geneva.

Slatkin M (1973). Gene flow and selection in a cline. *Genetics* **75**: 733–756.

Solbreck C (1980). Dispersal distances of migrating pine weevils, *Hylobius abietis*, Coleoptera: Curculionidae. *Entomol Exp Appl* **28**: 123–131.

Speight MR, Wainhouse D (1989). *Ecology and Management of Forest Insects*. Oxford Science: Oxford.

Stauffer C, Lakatos F, Hewitt GM (1999). Phylogeography and postglacial colonization routes of *Ips typographus* L. (Coleoptera, Scolytidae). *Mol Ecol* **8**: 763–773.

Thomas Y, Bethenod M, Pelozuelo L, Frerot B, Bourguet D (2003). Genetic isolation between two sympatric host-plant races of the European corn borer, *Ostrinia nubilalis* Hübner. I. Sex pheromone, moth emergence timing, and parasitism. *Evolution* **57**: 261–273.

Thompson JN (2005). *The Geographic Mosaic of Coevolution*. University of Chicago Press: Chicago.

Thorpe KV, Day KR (2002). The impact of host plant species on the larval development of the large pine weevil *Hylobius abietis* L. *Agr Forest Entomol* **4**: 187–194.

Tscharntke T, Brandl R (2004). Plant-Insect interactions in fragmented landscapes. *Ann Rev Entomol* **49**: 405–430.

von Sydow F, Birgersson G (1997). Conifer stump condition and pine weevil (*Hylobius abietis*) reproduction. *Can J Forest Res* **22**: 1254–1262.

Vos P, Hogers R, Bleeker M, Reijans M, van de Lee T, Hornes M *et al* (1995). AFLP: a new technique for DNA fingerprinting. *Nuclear Acids Res* **23**: 4407–4414.

Wainhouse D, Ashburner R, Boswell R (2001). Reproductive development and maternal effects in the pine weevil *Hylobius abitetis*. *Ecol Entomol* **26**: 655–661.

Weir BS, Cockerham CC (1984). Estimating F-statistics for the analysis of populations structure. *Evolution* **38**: 1358–1370.

Wilding CS, Butlin RK, Grahame J (2001). Differential gene exchange between parapatric morphs of *Littorina saxatilis* detected using AFLP markers. *J Evol Biol* **14**: 611–619.

Yeh F, Yang R-C, Boyle T (1997). *POPGENE, the user-friendly shareware for population genetic analysis*. Molecular Biology and Biotechnology Centre, University of Alberta: Edmonton.

Zangerl AR, Berenbaum MR (2003). Phenotype matching in wild parsnip and parsnip webworms: causes and consequences. *Evolution* **57**: 806–815.