Biological Journal of the Linnean Society, 2007, **90**, 239–246. With 2 figures

Phylogeography of a host-specific insect: genetic structure of *Ips typographus* in Europe does not reflect past fragmentation of its host

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Received 2 June 2005; accepted for publication 1 March 2006

The phylogeography of the bark beetle *Ips typographus* was assessed using five microsatellite markers. Twenty-eight populations were sampled throughout Europe on the host tree *Picea abies*. *I. typographus* showed very low levels of genetic diversity, and the study revealed a lack of genetic structure across Europe. No significant barrier to gene flow was found, even though *P. abies* has a fragmented distribution. A weak but significant effect of isolation by distance was found. These results suggest a high dispersal capacity of *I. typographus*, which leads to low genetic differentiation between populations. Its high dispersal capacity is likely to have prevented *I. typographus* from developing important local adaptations to its host, which would have influenced its genetic structure. The nuclear data was compared to previously published mitochondrial data that showed strong differentiation between Central–Northern European populations and Russian–Baltic populations, and a founder effect in Scandinavia, probably reflecting the postglacial history of *I. typographus*. Discrepancies between nuclear and mitochondrial markers could be due to the maternal inheritance of mitochondrial DNA, and to sex-biased dispersal in *I. typographus*. The overall low genetic diversity observed on both markers on a large geographical scale is discussed. © 2007 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2007, **90**, 239–246.

ADDITIONAL KEYWORDS: bottleneck – gene flow – glacial periods – host specialization – microsatellites – phylogeography – Scolytinae.

INTRODUCTION

Ips typographus (L.) (Coleoptera: Scolytinae) attacks *Picea* spp. stands throughout Eurasia. These bark beetles generally establish in decaying trees, where their brood completes larval development within the phloem. In Europe, the wide distribution range of *I. typographus* is closely related to that of *P. abies* (L.) Karsten, which includes Alpine, Hercynian-Carpathian and Baltic-Northern domains (Arbez, 1987). During the last ice ages, however, *P. abies* was restricted to the Dinaric Alps, the Carpathian Alps, the Apennines and the Northern area of Moscow (Lagercrantz & Ryman, 1990), from which Central and Northern Europe were recolonized after amelioration of temperature. *P. abies* still reveals high levels of population differentiation on a European scale (Vendramin *et al*., 2000; Gugerli *et al*., 2001) and the authors suggested three to four postglacial recolonization routes.

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During glaciations, *I. typographus* was necessarily restricted to some or all of the refugial areas of its host. Logically, it could therefore be hypothesized that *I. typographus* and *P. abies* have followed the same postglacial colonization routes, and that the geographical and genetic structures of *P. abies* favour the subdivision of *I. typographus* populations. However, as *I. typographus* and *P. abies* have drastically different life-history traits (generation time, dispersal capacities), the postglacial colonization routes and the genetic structure of *I. typographus* could well differ from those of *P. abies*. Previous analyses of mitochondrial DNA (mtDNA) suggested a different evolution for *I. typographus* (Stauffer, Lakatos & Hewitt, 1999). The insect may have migrated from the southern refuge to Central and Northern Europe and no influence from the Russian refuge was detected in the rest of Europe. Mitochondrial data, however, represent only partial information on migration routes because of their maternal mode of inheritance. Analysis of three polymorphic isozymes did not reveal any clear distinction of the populations (Stauffer, Lakatos & Hewitt, 1999).

We therefore used five microsatellite loci to address the following objectives: (i) to provide a phylogeographical framework for the recent evolution of *I. typographus* populations in Europe compared with the history of *P. abies*, and (ii) to assess the relative genetic isolation of present-day populations, in order to understand the historical and ecological factors that play a key role in *I. typographus* population biology. Comparing the postglacial histories and the genetic structures of the herbivore and its host will improve our current understanding of the evolution and maintenance of the plant–insect interaction.

MATERIAL AND METHODS

Sampling

I. typographus were sampled from 28 localities throughout Europe and two localities from Asia (Table 1, Fig. 1). The Asian populations are hereafter referred to as *I. t. japonicus*. Other than populations SK and SY, which were collected from pheromone traps, all populations were adults from fallen or standing trees. The beetles were stored in absolute or 70% ethanol at –20 °C.

DNA preparation and microsatellite amplification

Genomic DNA was extracted from the head and pronota of each individual, using either the DNeasy Tissue Kit (Qiagen, Hilden, Germany) or the Sigma GenElute extraction kit (Sigma-Aldrich, St Louis, MO, USA). Samples were genotyped for the five microsatellite loci developed by Sallé *et al*. (2003), namely GAA3F10, GAA5D8, GT1B6, GT434 and GAA4C3. Polymerase chain reaction (PCR) amplifications were carried out in reaction volumes of 10 μL following the protocol of Sallé *et al*. (2003). Fluorescent dyes used for primer labelling were either HEX (Sigma), 6-FAM (Sigma) or NED (Applied Biosystems, Foster City, CA, USA). The amplified products were detected on an ABI 3100 automatic sequencer and their sizes were analysed using Genescan (Applied Biosystems).

Data analysis

Observed and unbiased expected heterozygosities (Nei, 1978) were calculated using GENETIX version 4.04 (Belkhir *et al*., 1996–2004). For each locus and population, deviations from Hardy–Weinberg equilibrium were tested with FSTAT version 2.9.3.2 (Goudet, 1995), with 3000 permutations. As sample sizes were different, allelic richness was calculated for each locus and population with reference to the smallest sample size ($N = 11$) (Leberg, 2002), after 3000 permutations, using FSTAT.

Population structure was analysed using Fst (Weir & Cockerham, 1984). Fst estimates (θ_{ST}) were calculated using Genepop version 3.3 (Raymond & Rousset, 1995). The population pairwise θ_{ST} were calculated using Arlequin version 2.001 (Schneider, Roessli & Excoffier, 2000). Their significance was estimated with 3000 permutations. As the sample sizes were different, a multilocus *G*-test of population differentiation (Goudet *et al*., 1996) was also carried out, with 8700 permutations, using FSTAT. A Bayesian analysis was also performed on the dataset including or excluding the Asian samples, using BAPS 2.0 (Corander, Waldmann & Sillanpää, 2003). Based on the posterior distributions of the population structure and allele frequencies, posterior probabilities for all the different combinations of populations were estimated using a Markov Chain Monte Carlo simulation.

To test the influence of geographical distance on population differentiation, a Mantel test for isolation by distance was performed using Genepop. A regression was done between pairwise distance $[\theta_{ST}/(1 - \theta_{ST})]$ (Rousset, 1997) and the logarithm of the geographical distance among populations. Significance was assessed with 10 000 permutations. The significance level was set at $\alpha = 0.05$.

RESULTS

Observed heterozygosities differed considerably among loci, from 0.27 for locus GAA3F10 to 0.78 for locus GAA5D8, but not among populations (0.41 in Fs to 0.62 in CN). Only two significant deviations from

Table 1. Sampling sites of *Ips typographus* for the microsatellite survey

Country	Location	Abb.	Longitude	Latitude	Altitude (m)	Collected by	Year	Sample size (n)
France	Charleville-Mézières	Fcm	49°50′-N	4°41′-E	250	A. Sallé	2002	30
France	Vouziers	Fvz	49°23′-N	4°51′-E	150	A. Sallé	2002	30
France	Planchez	Fmo	47°10′-N	4°01′-E	650	A. Sallé	2002	30
France	Servières	Fs	45°38′-N	2°50′-E	1200	A. Sallé	2002	30
France	Muhlbach	Fmu	48°02′-N	7°03′-E	600	A. Sallé	2002	30
France	Climbach	Fcl	49°01′-N	7°51′-E	450	A. Sallé	2001	30
France	Labergement	Fl	46°46′-N	6°18′-E	1000	A. Sallé	2001	30
France	Annecy	Fru	45°25′-N	6°09′-E	1100	A. Sallé	2002	30
France	La Pradelle	Fpy	42°30′-N	2°10′-E	1045	G. Vouland	1994	14
Slovenia	Kamnik	SLO	46°13′-N	14°37′-E	430	D. Jurc	1994	17
Slovakia	Pol'ana	SK	48°37′-N	19°28′-E	900	R. Jakus	2002	30
Estonia	Jaaniveski	ESTj	58°52′-N	24°33′-E	120	K. Voolma	2002	15
Estonia	Maëltse	ESTm	58°51′-N	22°45′-E	10	K. Voolma	1995	15
Russia	Losiniy Ostrov	RU	56°30′-N	38°40′-E	123	E. Mozolevskaya	1995	20
Austria	Kindberg	Ak	47°30′-N	15°26′-E	1000	P. Baier	1994	30
Austria	Gosau	Ag	47°34′-N	13°31′-E	1100	P. Kitzberger	1994	15
Germany	Altenberg	D	50°46′-N	13°45′-E	413	S. Prien	1994	19
Norway	As	N	59°40′-N	10°48′-E	110	E. Christiansen	1994	20
Sweden	Torsby	So	60°08′-N	13°00′-E	120	J. Byers	1994	20
Sweden	Tylasberget	Sy	60°22′-N	14°43′-E	270	A. Lindelöw	2003	30
Bulgaria	Plovdiv	BG	42°09′-N	24°45′-E	890	M. Subchev	1994	20
Lithuania	Kaunas	LT	55°02′-N	24°12′-E	78	P. Zolubas	1994	20
Luxembourg	Luxembourg	L	49°36′-N	6°07′-E	270	J.-C. Grégoire	1994	20
Switzerland	Richterwil	CH	47°13′-N	8°42′-E	630	B. Wermelinger	1994	20
Poland	Bialowieza	PL	52°40′-N	23°50′-E	409	G. Gutowski	1994	20
Italy	Asiago	I	45°52′-N	11°30′-E	1150	A. Battisti	1994	20
Romania	Cluj Racatan	RO	46°35′-N	23°07′-E	1300	V. Mihalciuc	1994	20
Finland	Ruovesi	FIN	61°50′-N	24°15′-E	80	K. Heliövaara	1994	20
China	Shanghai	CN	41°30′-N	128°00′-E	1150	E. Führer	1994	12
Japan	Rubeshibe	JP	43°47′-N	143°37′-E	400	A. Ueda	2003	11

Abb, abbreviation.

Hardy–Weinberg equilibrium were observed out of the 150 tests performed, using a Bonferroni correction. These disequilibria were dispatched throughout the populations, and no single population ever had more than one disequilibrium. All populations were thus considered to be at equilibrium.

The allelic richness was low, with a mean of 3.9 per locality, and did not differ significantly among populations, ranging from 3.19 in LT to 4.1 in CN. All population structure parameters were low (Table 2). Without the Asian populations, the global θ_{ST} was as low as 0.01. The pairwise θ_{ST} values indicated that the Chinese and Japanese populations were significantly different from all other populations (θ_{ST} values ranged from 0.224 to 0.331). Considering each locus separately, these populations differed from all the other ones for all loci except GAA4C3 (two differences out of 28 comparisons) for the Chinese population, and

Table 2. Global F-statistics estimates for each microsatellite locus and for all loci in *Ips typographus*

	All populations			Europe only		
Locus	θ_{ST}	θ_{IT}	θ_{IS}	θ_{ST}	θ_{IT}	θ_{IS}
GAA3F10	0.041	0.109	0.071	0.013	0.097	0.085
GAA4C3	0.008	0.044	0.036	0.006	0.043	0.037
GAA5D8	0.024	0.059	0.037	0.016	0.05	0.034
GT434	0.028	0.003	–0.026	0.009	–0.005	–0.014
GT1B6	0.073	0.138	0.070	0.001	0.076	0.076
All loci	0.035	0.065	0.031	0.010	0.045	0.036

The estimates for 'Europe only' exclude the two Asian populations.

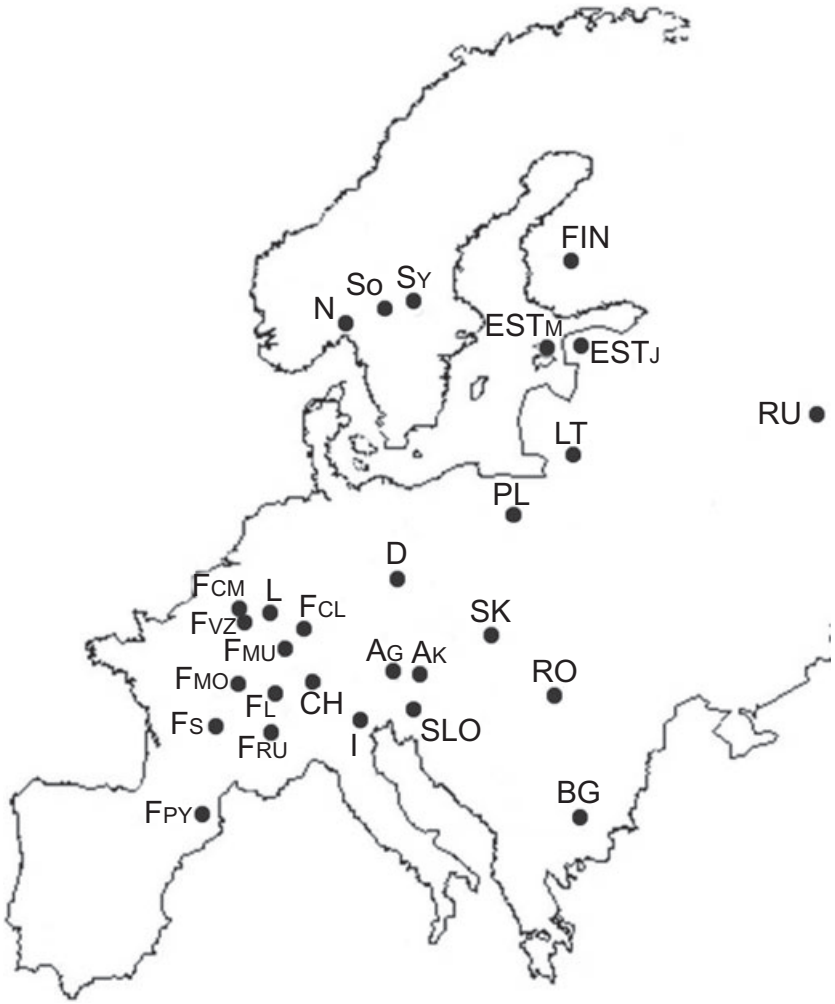

Figure 1. Sampling localities for microsatellite survey of *Ips typographus* populations in Europe. Coding for the localities is as in Table 1.

GAA4C3 (3/28) and GAA3F10 (8/28) for the Japanese population. Within European populations, pairwise θ_{ST} never exceeded 0.073 (and even 0.05 if French population Fs was excluded); the overwhelming majority of pairwise θ_{ST} were below 0.04. Except for populations Fs, SK, Ak and PL, all sampled populations diverged significantly from fewer than eight localities. The differences were observed mostly with: loci GAA3F10 (18 differences out of 26 comparisons), GT1B6 (9/26), and GAA5D8 (12/26) for population Fs; loci GT434 (15/26) and GAA5D8 (10/26) for SK; loci GAA5D8 (18/26) and GAA4C3 (9/26) for Ak; locus GAA5D8 (20/26) for PL. However, no coherent geographical group could be identified based on analysis of pairwise θ_{ST}. On the other hand, the multilocus *G*-test of population differentiation only showed significant differences between the Asian and the European populations. Within Europe, significant structure was found only between Fs and So, and between Fs and Ak. Similarly, the Bayesian analysis of population structure exhibited the highest posterior probability ($P = 0.655$) for a partition in two groups, namely Asian vs. European populations. The analysis without the Asian *I. t. japonicus* populations did not show any clustering within Europe. A significant effect of isolation by distance was found within European populations, although the dispersion was great ($P < 0.001$, $r = 0.27$, Fig. 2).

DISCUSSION

For the first time, microsatellite markers have been used to infer the population genetic structure of a scolytid beetle. As for Lepidoptera (Zhang, 2004), isolation of microsatellite markers in the coleopteran subfamily Scolytinae is quite difficult, mostly because of the low efficiency of enrichment protocols in this

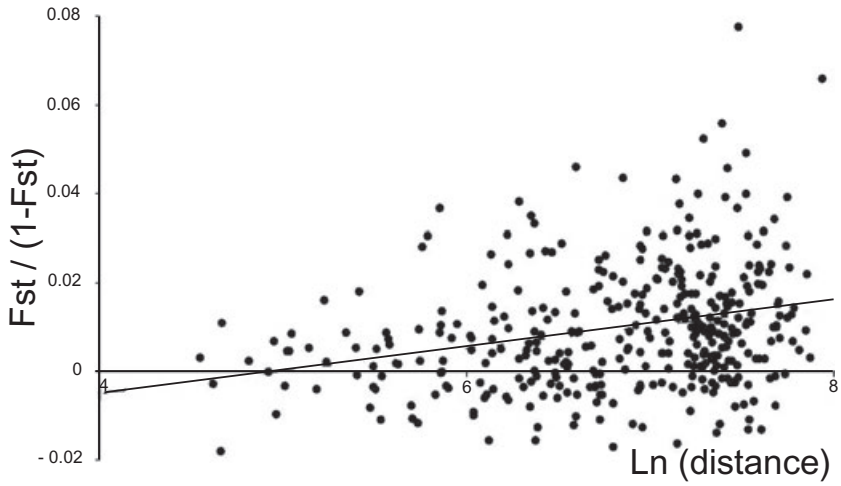

Figure 2. Isolation by distance pattern in Europe. Regression of genetic differentiation [estimated as $\theta_{ST}/(1 - \theta_{ST})$] against the logarithm of geographical distance (in km) for all pairs of sampled populations of *Ips typographus* (except for Asian populations).

group, high level of redundancy and multiple banding patterns (A. Sallé, C. Kerdelhué, W. Arthofer and C. Stauffer, unpubl. data). As a result of this technical difficulty, we were able to use only five microsatellite loci to infer the phylogeography of *I. typographus* in Europe. However, the results of all tests and data analyses were consistent and unambiguous, despite the relatively low number of available loci. Our study did not reveal any significant population differentiation on the European scale. The global θ_{ST} value (0.01) was clearly below the 5% threshold; a value above this threshold can be taken to indicate the populations to be fairly differentiated (Balloux & Lugon-Moulin, 2002). Similarly, the pairwise population comparisons did not indicate any spatially coherent group, or differences between populations located in natural or introduced areas of *P. abies*. Even the multilocus *G*-test – known to be particularly efficient when the actual population structure is low (Petit, Balloux & Goudet, 2001) – and the Bayesian analysis failed to detect any significant population structure. Since no differences occurred even among the border populations of our sampling, it is unlikely that the lack of populations within some areas had any impact on the analyses. Our results are thus similar to those previously obtained with isozymes (Stauffer *et al*., 1999), and show that no significant barrier to gene flow exists within Europe. The main hypothesis that could explain the genetic patterns found in our study is that *I. typographus* has a very high effective dispersal rate. After emergence from their host tree, males disperse to find a new suitable host; ecological studies have suggested that they are able to disperse up to 40 km (Nilssen, 1984), and that individuals might be transported over long distances by the wind (Forsse & Solbreck, 1985). Our data suggest that such long-range dispersal plays an effective role in genetically homogenizing *I. typographus* populations and is representative for the species rather than being a rare and exceptional event. Like *I. typographus*, most bark beetles develop on weakened or recently dead trees, which are usually scarce and dispatched in the environment. To cope with the fluctuating abundance and location of their hosts, these insects have developed relative developmental plasticity (Sallé, Baylac & Lieutier, 2005) and aggregation pheromones (Schlyter *et al*., 1987), and they have probably evolved towards good foraging capacities to maximize their chances of finding a suitable host. Host availability and distribution thus probably played a role in the evolution of highly dispersing insects. The pattern of weak but significant isolation by distance found in our study is consistent with the review of Peterson & Denno (1998) that shows that phytophagous insects dispersing more than 20 km typically show limited but significant isolation by distance, characterized by a lot of scatter.

Contrary to the findings in *I. typographus*, the two Asian populations of *I. t. japonicus* were fairly differentiated from each other according to the pairwise θ_{ST}, although they were not more distant from each other than were the most separated European populations. Different hypotheses could be formulated to explain this higher population structure, including the occurrence of several host species throughout Asia and the isolation of the Japanese population. Nonetheless, further sampling in continental Asia would be necessary to infer the actual population structure of this subspecies.

The geographical structures of *I. typographus* and *P. abies* are incongruent, as the insect populations are not structured, while the host tree populations are. Two main gene pools (Sarmathic–Baltic and Alpine–Central) have been identified previously for *P. abies* using several markers, that resulted from postglacial colonization of Europe from the Russian, Balkan and Carpathian refuge areas (Lagercrantz & Ryman, 1990; Vendramin *et al*., 2000; Gugerli *et al*., 2001). A high genetic differentiation also occurs between western and eastern Alpine populations, probably as a consequence of the postglacial colonization of the western Alps from an additional refuge area located in the central plains of Italy (Vendramin *et al*., 2000; Gugerli *et al*., 2001). Given the incongruence found between the genetic structures of *I. typographus* and *P. abies*, we can hypothesize that local insect–tree relationships and coadaptations are unlikely to be the main forces that have shaped the evolutionary history of *I. typographus*. The host specificity of European populations of *I. typographus*, their endophagous habits, and the longevity of *P. abies* relative to the *I. typographus* generation time, are likely to have promoted the population subdivision of the insect (Mopper, 1996). Nonetheless, the high migration rate that we found is probably responsible for the lack of congruence between the *I. typographus* genetic structure and that of its specific host, as concluded on local or regional spatial scales for the weevil *Larinus cynarae* F. (Michalakis *et al*., 1993) and, although to a lesser extent, for *Pameridea* species (Hemiptera) (Anderson *et al*., 2004).

The previous phylogeography of *I. typographus* based on mitochondrial sequences showed a quite different pattern on a European scale. One haplotype was restricted to Russia and Lithuania, while a founder effect was detected in Scandinavia, where populations were fixed for one haplotype. None of these patterns was observed with microsatellite markers. In a variety of organisms, microsatellite and mitochondrial data have been found to yield incongruent results (e.g. Johnson, Toepfer & Dunn, 2003). One explanation could be that mitochondrial markers are more sensitive than are nuclear ones to factors restricting effective population sizes and shortening coalescence times (Moore, 1995). It should also be considered that this previous study used rather short sequences of mtDNA cytochrome c oxidase subunit I (COI) (567 bp), and was thus unlikely to exhibit a high level of diversity; this could have skewed the interpretation of the results. Sex-biased dispersal might also explain these differences, and would confirm previous observations by Pavlíček, Zurovcova & Stary (1997). Females are smaller than males and could consequently have a reduced flight capacity (Gries, 1985). Moreover, because they stop dispersing as soon as they detect aggregation pheromones, their dispersal range might be shorter compared with that of pioneering males. In this case, the mitochondrial markers would still show historical isolation and drift (as in Russia and Lithuania), or a founder effect (as in Scandinavia), while the genetic composition of the populations at microsatellite markers would have been homogenized by the long-range dispersal of the males.

The low level of genetic diversity exhibited by *I. typographus* is congruent with previous mitochondrial data (eight haplotypes for 136 individuals; see Stauffer *et al*., 1999). As stated earlier in our Discussion, this previous study could have underestimated the genetic diversity of *I. typographus*. However other members of the genus *Ips* investigated with even shorter sequences of mtDNA COI showed higher levels of intraspecific variability (*I. pini* Say (34 haplotypes for 217 individuals) (Cognato, Seybold & Sperling, 1999) and *I. confusus* LeConte (15 haplotypes for 95 individuals) (Cognato, Harlin & Fisher, 2003)). Two ecological factors could explain the reduced genetic diversity in *I. typographus*. One hypothesis involves the quite narrow host specificity of *I. typographus* in Europe, because host specialization has been found to be associated with extreme reduction in genetic diversity in scolytids (Kelley, Farrell & Mitton, 2000). The low genetic variability might also be a consequence of demographic fluctuations. The relatively frequent and extensive *I. typographus* outbreaks are qualified as 'pulse eruptive' because both gradation and retrogradation are short and intense (Berryman, 1987). The rapid population crashes following outbreaks could be responsible for the extinction of rare alleles, thus contributing to the overall low genetic diversity observed in *I. typographus*.

A further possibility for the low mitochondrial polymorphism could be *Wolbachia*, a maternally inherited endosymbiont that can spread through populations rapidly (Turelli & Hoffmann, 1991) by inducing cytoplasmic incompatibility. By this process, the mitochondrial haplotype infected with *Wolbachia* hitchhikes through the populations, thereby replacing the original mitochondrial haplotypes and reducing mitochondrial polymorphism in its host species (reviewed by Hurst & Jiggins, 2005). Stauffer, Van Meer & Riegler (1997) detected *Wolbachia* in *I. typographus*, and it was recently detected in several Ipinae species (K. Koivista and H.R. Braig, pers. comm.).

ACKNOWLEDGEMENTS

We wish to thank E. Magnoux, J. Garcia and A. Stradner for technical assistance. We are grateful to Messrs de Falvard, Oriol, Adam, Delannoye, Chermette, Durrmann, Ducret and Pagnier for facilitating access to forest stands in France. The colleagues cited in Table 1 are gratefully acknowledged

for sending us *I. typographus* samples. This study was supported by a grant of the Direction Générale de la Forêt et des Affaires Rurales (DERF 61.45.01/01), the WTZ Austria–France, Contrat Egide 'Amadeus' 2003–04 and the Austrian Science Foundation FWF P16235-B06. The PhD thesis of A. Sallé was funded by the Région Centre (France).

REFERENCES

Anderson B, Olivieri I, Lourmas M, Stewart BA. 2004. Comparative population genetic structures and local adaptation of two mutualists. *Evolution* **58:** 1730–1747.

Arbez M. 1987. *Les ressources génétiques forestières en France. Tome 1: conifères*. Paris: INRA – BRG.

Balloux F, Lugon-Moulin N. 2002. The estimation of population differentiation with microsatellite markers. *Molecular Ecology* **11:** 155–165.

Belkhir K, Borsa P, Chikhi L, Raufaste N, Bonhomme F. 1996–2004. *GENETIX 4.05, logiciel sous Windows TM pour la génétique des populations.* Montpellier: Laboratoire Génome, Populations, Interactions: CNRS UMR. 5000, Université de Montpellier II.

Berryman AA. 1987. The theory and classification of outbreaks. In: Barbosa P, Schultz JC, eds. *Insect outbreaks*. London: Academic Press Inc., 3–30.

Cognato AI, Harlin AD, Fisher ML. 2003. Genetic structure among pinyon pine beetle populations (Scolytinae: *Ips confusus*). *Environmental Entomology* **30:** 1262–1270.

Cognato AI, Seybold SJ, Sperling FAH. 1999. Incomplete barriers to mitochondrial gene flow between pheromone races of the North American pine engraver *Ips pini* (Say) (Coleoptera: Scolytidae). *Proceedings of the Royal Society of London B* **266:** 1843–1850.

Corander J, Waldmann P, Sillanpää MJ. 2003. Bayesian analysis of genetic differentiation between populations. *Genetics* **163:** 367–374.

Forsse E, Solbreck C. 1985. Migration in the bark beetle *Ips typographus* L.: duration, timing and height of flight. *Zeitschrift für Angewandte Entomologie* **100:** 47–57.

Goudet J. 1995. FSTAT Version 1.2.: a computer program to calculate F-statistics. *Journal of Heredity* **86:** 485–486.

Goudet J, Raymond M, de Meeus T, Rousset F. 1996. Testing differentiation in diploid populations. *Genetics* **144:** 1933–1940.

Gries G. 1985. Zur frage der dispersion des buchdruckers (*Ips typographus* L.). *Zeitschrift für Angewandte Entomologie* **99:** 12–20.

Gugerli F, Sperisen C, Büchler U, Magni F, Geburek T, Jeandroz S, Senn J. 2001. Haplotype variation in a mitochondrial tandem repeat of Norway spruce (*Picea abies*) populations suggests a serious founder effect during postglacial re-colonization of the western Alps. *Molecular Ecology* **10:** 1255–1263.

Hurst GDD, Jiggins FM. 2005. Problems with mitochondrial DNA as a marker in population, phylogeographic and phylogenetic studies: The effects of inherited symbionts. *Proceedings of the Royal Society of London B* **272:** 1525–1534.

Johnson JA, Toepfer JE, Dunn PO. 2003. Contrasting patterns of mitochondrial and microsatellite population structure in fragmented populations of greater prairie-chickens. *Molecular Ecology* **12:** 3335–3347.

Kelley ST, Farrell BD, Mitton JB. 2000. Effect of specialization on genetic differentiation in sister species of bark beetles. *Heredity* **84:** 218–227.

Lagercrantz U, Ryman N. 1990. Genetic structure of Norway spruce (*Picea abies*): concordance of morphological and allozyme variation. *Evolution* **44:** 38–53.

Leberg PL. 2002. Estimating allelic richness: effects of sample size and bottlenecks. *Molecular Ecology* **11:** 2445–2449.

Michalakis Y, Sheppard AW, Noël V, Olivieri I. 1993. Population structure of a herbivorous insect and its host plant on a microgeographic scale. *Evolution* **47:** 1611–1616.

Moore WS. 1995. Inferring phylogenies from mtDNA variation: mitochondrial-gene trees versus nuclear-gene trees. *Evolution* **49:** 718–726.

Mopper S. 1996. Adaptive genetic structure in phytophagous insect populations. *Trends in Ecology and Evolution* **11:** 235–238.

Nei M. 1978. Estimation of average 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 Curculionidae) in northern Finland. *Annales Entomologici Fennici* **50:** 37–42.

Pavlíček T, Zurovcova M, Stary P. 1997. Geographic population-genetic divergence of the Norway spruce bark beetle, *Ips typographus* in the Czech Republic. *Biologia Bratislava* **52:** 273–279.

Peterson MA, Denno RF. 1998. The influence of dispersal and diet breadth on patterns of genetic isolation by distance in phytophagous insects. *American Naturalist* **152:** 428–446.

Petit E, Balloux F, Goudet J. 2001. Sex-biased dispersal in a migratory bat: a characterization using sex-specific demographic parameters. *Evolution* **55:** 635–640.

Raymond M, Rousset F. 1995. *GENEPOP* Version 1.2.: a population genetics software for exact tests and eucumenicism. *Journal of Heredity* **86:** 248–249.

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

Sallé A, Baylac M, Lieutier F. 2005. Size and shape changes of *Ips typographus* L. (Coleoptera: Scolytinae) in relation to population level. *Agricultural and Forest Entomology* **7:** 297–306.

Sallé A, Kerdelhué C, Breton M, Lieutier F. 2003. Characterization of microsatellite loci in the spruce bark beetle *Ips typographus* (Coleoptera: Scolytinae). *Molecular Ecology Notes* **3:** 336–337.

Schlyter F, Birgersson G, Byers JA, Löfqvist J, Bergström G. 1987. Field response of the spruce bark beetle, *Ips typographus*, to aggregation pheromone candidates. *Journal of Chemical Ecology* **13:** 701–716.

Schneider S, Roessli D, Excoffier L. 2000. *Arlequin*, Version 2.001*: a software for population genetics data analysis.* Geneva: Genetics and Biometry Laboratory, University of Geneva.

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

Stauffer C, Van Meer MMM, Riegler M. 1997. The presence of *Wolbachia* in European *Ips typographus* populations and the consequences for genetic data. *Mitteilungen der Deutschen Gesselschaft für Allgemeine und Angewandte Entomologie* **11:** 709–711.

Turelli M, Hoffmann AA. 1991. Rapid spread of an inherited incompatiblity factor in Californian *Drosophila*. *Nature* **353:** 440–442.

Vendramin GG, Anzidei M, Madaghiele A, Sperisen C, Bucci G. 2000. Chloroplast microsatellite analysis reveals the presence of population subdivision in Norway spruce (*Picea abies* K.). *Genome* **43:** 68–78.

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

Zhang DX. 2004. Lepidopteran microsatellite DNA: redundant but promising. *Trends in Ecology and Evolution* **19:** 507–509.