Molecular Ecology (2005) **14**, 4207–4219 doi: 10.1111/j.1365-294X.2005.02740.x

The voyage of an invasive species across continents: genetic diversity of North American and European Colorado potato beetle populations

ALESSANDRO GRAPPUTO, SANNA BOMAN, LEENA LINDSTRÖM, ANNE LYYTINEN and JOHANNA MAPPES
Department of Biological and Environmental Science, PO Box 35, FIN-40014, University of Jyväskylä, Finland

Abstract

The paradox of successful invading species is that they are likely to be genetically depauperate compared to their source population. This study on Colorado potato beetles is one of the few studies of the genetic consequences of continent-scale invasion in an insect pest. Understanding gene flow, population structure and the potential for rapid evolution in native and invasive populations offers insights both into the dynamics of small populations that become successful invaders and for their management as pests. We used this approach to investigate the invasion of the Colorado potato beetle (*Leptinotarsa decemlineata*) from North America to Europe. The beetles invaded Europe at the beginning of the 20th century and expanded almost throughout the continent in about 30 years. From the analysis of mitochondrial DNA (mtDNA) and amplified fragment length polymorphism (AFLP) markers, we found the highest genetic diversity in beetle populations from the central United States. The European populations clearly contained only a fraction of the genetic variability observed in North American populations. European populations show a significant reduction at nuclear markers (AFLPs) and are fixed for one mitochondrial haplotype, suggesting a single successful founder event. Despite the high vagility of the species and the reduction of genetic diversity in Europe, we found a similar, high level of population structure and low gene flow among populations on both continents. Founder events during range expansion, agricultural management with crop rotation, and selection due to insecticide applications are most likely the causes partitioning genetic diversity in this species.

Keywords: AFLP, cytochrome oxidase II, invasion, *Leptinotarsa decemlineata*, pest species

Received 22 May 2005; revision accepted 10 August 2005

Introduction

The term 'invasive species' usually refers to introduced species that have a negative impact on biodiversity, human economic activities, and health. The rapid spread of exotic species has mostly been investigated from an ecological point of view, while the evolutionary aspects of invasions have been substantially unexplored (Lee 2002). Invasions are rapid evolutionary events in which populations are usually subjected to founder effect during the colonization event followed by a rapid expansion (reviewed in Sakai *et al*. 2001). Thus, newly arrived populations are likely to be less variable than the original population from which they derived (Barrett & Kohn 1991). This leads to a paradox: while small population size is considered harmful when this causes inbreeding depression (Frankham *et al*. 2002), many successful invasions are undertaken by small introduced populations. Indeed, reduction in genetic variability has been shown to be advantageous in the invasiveness of Argentine ants (Tsutsui *et al*. 2000) at least in the short term (Queller 2000). Furthermore, some invasive species are not genetically depauperate. Newly introduced populations can stem from different source populations, thus transforming the among-population variation in the native range to within-population variation in the newly established population (Baker 1992; Kolbe *et al*. 2004). In turn, these new variable populations could act as the source for

Correspondence: Alessandro Grapputo, Fax: +358 14 2602321; E-mail: grapputo@cc.jyu.fi

© 2005 Blackwell Publishing Ltd

secondary invasions elsewhere, increasing the costs of management of the invasive species (Kolbe *et al*. 2004).

Molecular markers with different modes of inheritance can be very useful for studying the invasion history and population structure of invasive species. Genetic markers can be used to measure the amount of genetic diversity in invasive populations and also provide an indication of the amount of genetic variation lost during the colonization bottleneck or provide evidence of multiple sources of introduction (Sakai *et al*. 2001). Mitochondrial DNA is subject to strong genetic drift because of its maternal and haploid mode of inheritance (Avise 1994) and most of its variation can be lost during an introduction bottleneck (Villablanca *et al*. 1998). However, it can be very informative in the case of multiple sources of invasion (Kolbe *et al*. 2004). Nuclear markers retain variation for a longer period of time (Neigel & Avise 1986; Villablanca *et al*. 1998) and techniques based on polymerase chain reaction (PCR), such as amplified fragment length polymorphism (AFLP) (Vos *et al*. 1995), allow us to obtain a large number of markers without previous knowledge of the species under study and avoid the identification of variable nuclear sequences or the long phase needed to isolate microsatellites.

The Colorado potato beetle (*Leptinotarsa decemlineata*, Say) is a serious insect pest of potatoes (Hare 1980). Both adult and larvae feed on potato leaves and the damage they inflict can greatly reduce potato yields (Ferro *et al*. 1985). Beetles can also be a pest of other solanaceous plants such as tomato, aubergine, tobacco and peppers. The spread of this insect pest is historically well documented (Johnson 1967 and references therein; EPPO — European and Mediterranean Plant Protection Organization 1998 and also available at www.eppo.org/QUARANTINE/insects/Leptinotarsa_decemlineata/LPTNDE_map.pdf) although the number of invasion events in Europe is not known. The Colorado potato beetle originated in Mexico, where beetles are still present, and fed on wild relatives of potato such as buffalo bur (*Solanum rostratum*). The transition to potato is estimated to have taken place in the 1860s in the American Midwest and by 1880 the beetles reached the US East Coast. In the early 1920s beetles were accidentally introduced to western France (Bordeaux) and from there they spread throughout most of Europe in about 30 years. Currently, the beetle is spreading towards Siberia (S. Fasulati, personal communication) and Finland (where several unsuccessful introductions have been documented: in 1948, 1983, 1998 and most recently in 2002). Several observations indicate that Colorado potato beetles are weak fliers, but they can engage in long-distance migration in the presence of strong winds (Wiktelius 1981; Ferro *et al*. 1985; Voss & Ferro 1990). These observations are in line with data that suggest substantial gene flow among populations (Jacobson & Hsiao 1983; Zehnder *et al*. 1992). However, other data suggest substantial genetic subdivision among populations (Azeredo-Espin *et al*. 1996) and the presence of three chromosomal races within the species (Hsiao & Hsiao 1983).

Once an invasive species is established and starts to spread in a new area, eradication and control become the key priorities (Sakai *et al*. 2001). Knowledge of the dispersal mode and population structure of invasive species can improve management strategies. The rate and extent of invasive fronts may depend on the amount and pattern of gene flow among populations of an invasive species. While a large amount of gene flow is expected to bring high genetic variation at the periphery, which is required for the evolution of local adaptation, gene flow from the centre of a species' distribution could also prevent local adaptation at the periphery and limit the range expansion (Kirkpatrick & Barton 1997). Our study investigates the population structure and genetic variability of North American and European populations of Colorado potato beetles using mitochondrial DNA (mtDNA) sequences and AFLP markers. We also assessed the level of long-distance gene flow and whether a single or multiple introductions occurred in Europe. Understanding gene flow is particularly important for beetle management given that insecticide resistance is widespread in this species.

Materials and methods

Colorado potato beetles were collected from 13 populations (Fig. 1), five in North America [Colorado (36 beetles), Idaho (37), Kentucky (30), Minnesota (19) and New Brunswick (27)] and eight in Europe [Spain (37), France (38), North and South Italy (55 and 18, respectively), Poland (51), Estonia (38), Russia (25) and Finland (32)]. Specimens were collected in potato fields during the summers of 2001 and 2002, and stored in 90% ethanol.

Total genomic DNA was extracted from three legs of each beetle using the DNeasy Tissue Kit (QIAGEN) following the manufacturer's protocol for animal tissues. For a subsample of beetles from each population (Table 1), a 577-bp fragment of mtDNA spanning from the 3′ end of the COI gene to the 5′ end of the COII gene was amplified using the primers S2792 (Brower 1994) and C2-N-3389 (Simon *et al*. 1994). The amplifications were carried out in a total volume of 25 μL, with 10 mM of Tris-HCl, 1.5 mM of $MgCl_2$, 5 pmoles of each primer, 200 μM of each dNTP, 1 U of *Taq* polymerase (Biotools) and 20–50 ng of DNA. PCR amplifications were performed in MBS satellite thermocyclers using the following temperature cycling profile: a 2-min denaturation step at 94 °C followed by 30 cycles of denaturation for 30 s at 94 °C, annealing for 30 s at 45 °C and extension for 1 min at 72 °C. A final step at 72 °C for 5 min followed the 30 cycles. The forward and reverse primers, labelled with a fluorescent dye (IDR-800 and IDR-700, respectively; LI-COR), were used with the Thermo Sequenase DYEnamic Direct Cycle Sequencing Kit (Amersham)

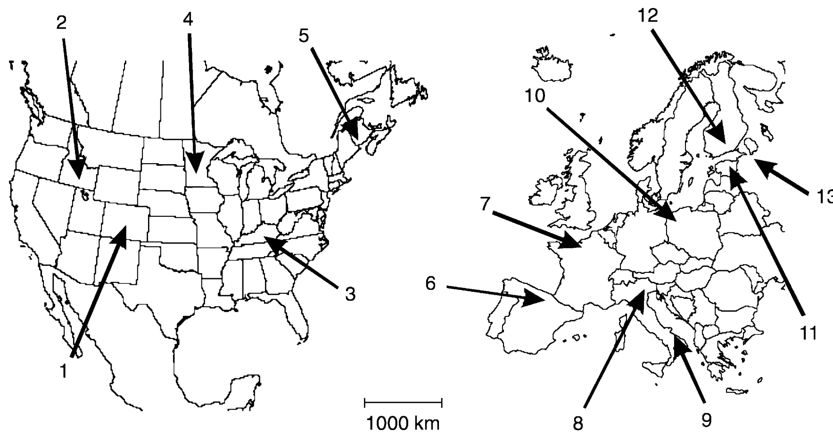

Fig. 1 Location of the North American and European populations of Colorado potato beetles. (1) Colorado, (2) Idaho, (3) Kentucky, (4) Minnesota, (5) New Brunswick, (6) Spain, (7) France, (8) North Italy, (9) South Italy, (10) Poland, (11) Estonia, (12) Finland, (13) Russia.

as described in the technical bulletin #71 (LI-COR). DNA sequences were obtained from sequencing reactions using a LI-COR DNA bidirectional sequencer 4200.

The AFLP protocol of Vos *et al*. (1995) was used with two-primer combinations (*Eco*RI-AGA/*Mse*I-CGA and *Eco*RI-ACT/*Mse*I-CGT). DNA solution (approximately 250 ng) was digested with 10 units of *Mse*I and 10 units of *Eco*RI restriction enzymes (Fermentas) and ligated to specific adapters using 5 units of T4 DNA ligase (Fermentas) in a total volume of 25 µL. After 3 h incubation at 37 °C, each sample was diluted 10-fold and 3 µL were used as template in the preselective PCR amplification. PCR amplifications were performed in MBS satellite thermocyclers using the following temperature cycling profile: a 2-min step at 72 °C, a 2-min denaturation step at 94 °C followed by 30 cycles of denaturation for 30 s at 94 °C, annealing for 30 s at 56 °C and extension for 2 min at 72 °C. A final step at 60 °C for 30 min followed the 30 cycles. The product of the preselective PCR was diluted sevenfold and 2.5 µL were used in the selective PCR amplification. In the selective amplification, the *Eco*RI primers were labelled with either IRDye-700 or IRDye-800. The selective PCR profile was as follows: a denaturation step of 2 min at 94 °C was followed by 13 cycles of denaturation at 94 °C for 30 s, annealing (begun at 65 °C and decreased by 0.7 °C per cycle) for 30 s and an extension at 72 °C for 2 min. These 13 cycles were followed by another 24 cycles with annealing temperature fixed at 56 °C. A final step at 72 °C for 10 min ended the PCR. The two selective PCRs of each sample were mixed together and loaded in a LI-COR DNA bidirectional sequencer 4200 and run overnight. Molecular standards labelled with IRDye-700 and IRDye-800 (LI-COR) were loaded every five samples to calculate the bands' molecular weight and compare fragment mobility in different runs. Fingerprinting patterns were visualized with GENEPROFILER version 3.56 (Scanalytic) and bands were scored manually. AFLP profiles (columns) were recorded for each sample (rows) with the presence (1) or absence (0) of bands. Both polymorphic and monomorphic bands were scored.

Mitochondrial DNA analyses

Sequences were aligned using CLUSTAL_X (Thompson *et al*. 1997). The degree of polymorphism of each population was estimated as haplotype diversity (*h*) (Nei 1978) using ARLEQUIN version 2.001 (Schneider *et al*. 2000). Relationships among haplotypes were represented as a haplotype network obtained with the statistical parsimony method using the TCS software (Clement *et al*. 2000). Population structure was analysed by AMOVA using Φ_{ST} statistics (Excoffier *et al*. 1992) with the Kimura 2-parameter distance model. The statistical significance of each Φ_{ST} value was assessed under the null hypothesis of genetic homogeneity, performing 1000 permutations of the original data set in ARLEQUIN version 2.001 (Schneider *et al*. 2000).

Analyses of AFLP markers

Descriptive statistics providing information on the percentage of polymorphic loci (*p*), the average expected heterozygosity (Nei 1978) for each population and the overall genetic differentiation among populations (F_{ST}) were obtained using the AFLP-SURV program (Vekemans *et al*. 2002). Allele frequency estimates were obtained by a Bayesian method with nonuniform prior distribution of allele frequencies and assuming Hardy–Weinberg equilibrium (Zhivotovsky 1999). Statistics of genetic diversity and population genetic structure were computed following the approach of Lynch & Milligan (1994). The confidence interval for F_{ST} was obtained by bootstrapping over loci.

The assumption of Hardy–Weinberg equilibrium may not be valid for estimating genetic diversity using dominant markers (Keiper & McConchie 2000; Salvato *et al*. 2002), and therefore, the phenetic distances between AFLP profiles also were calculated using the simple matching method (Apostol *et al*. 1993). The resulting matrix was used to investigate population structure by AMOVA using ARLEQUIN version 2.001 (Schneider *et al*. 2000). The significance of pairwise Φ_{ST} values was assessed under the null hypothesis

© 2005 Blackwell Publishing Ltd, *Molecular Ecology*, 14, 4207–4219

Table 1 Mitochondrial variability: number of analysed beetles (N), number of each haplotype and haplotype diversity (± SE) for each population. European populations are pooled because we found only one haplotype

Populations	N	Haplotypes																				Haplotype diversity (h)
		H1	H2	H3	H4	H5	H6	H7	H8	H9	H10	H11	H12	H13	H14	H15	H16	H17	H18	H19	H20	
1. Colorado	10	1	–	–	–	–	–	–	–	–	–	–	–	–	3	1	1	1	1	1	1	0.933 ± 0.077
2. Idaho	10	10	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0
3. Kentucky	10	–	3	1	2	1	2	1	–	–	–	–	–	–	–	–	–	–	–	–	–	0.889 ± 0.075
4. Minnesota	10	1	–	–	5	–	–	–	1	2	1	–	–	–	–	–	–	–	–	–	–	0.756 ± 0.130
5. New Brunswick	18	1	–	–	12	–	–	–	–	–	–	2	1	1	1	–	–	–	–	–	–	0.562 ± 0.134
Europe*	51	51	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0

*Europe consists of 4 to 10 specimens from each European population.

of genetic homogeneity, performing 10 000 permutations of the original data set in ARLEQUIN version 2.001 (Schneider *et al*. 2000). PHYLIP version 3.6 (Felsenstein 1989) was used in neighbour-joining (NJ) analyses first to search for population groupings based on Nei's D distances (Lynch & Milligan 1994) and then to elaborate groupings of individuals using the simple matching distance (Apostol *et al*. 1993). An assignment test (Paetkau *et al*. 1995) was performed using the AFLPPOP version 1.1 program (Duchesne & Bernatchez 2002).

Results

mtDNA variability

Sequencing of the amplified mtDNA from 109 beetles from 13 populations resulted in 20 different haplotypes (GenBank Accession nos: AJ884950–AJ884969). The percentage of variable sites was 3.81%. The mitochondrial haplotypes were differentially distributed among the North American populations (Table 1). Three haplotypes (H1, H4 and H14) were shared among different populations and all others were restricted to a single population. Only one haplotype (H1) was recovered from the 51 European beetles collected from eight populations (Table 1). This haplotype was also fixed in the Idaho population.

The relationships among mitochondrial haplotypes were reconstructed by a haplotype network (Fig. 2). The network showed three haplotypes groups for which the net divergence between groups ranged from 0.6% to 1.4% (calculated as described in Kumar *et al*. 1993). One haplotype group contained only haplotypes from Kentucky (H2, H3, H5, H6 and H7). A second group of haplotypes was formed by H14 and other haplotypes diverging from it by one or two mutational steps, and a third group was formed by H1 and H4, the most common haplotypes found in our sample.

AMOVA performed on the mtDNA data of the North American populations confirmed significant population differentiation, with 44% of the variation explained by the subdivision among populations ($\Phi_{ST} = 0.444$, $P < 0.001$). However, this significant subdivision was due to the Idaho and Kentucky populations. Pairwise Φ_{ST} among Colorado, Minnesota and New Brunswick populations were not significant after Bonferroni correction (Table 2).

Nuclear variability

The AFLP analyses, using two primer combinations, resulted in a total of 297 bands (in the interval from 50 to 220 bp) of which 295 (99.3%) were polymorphic. The highest level of polymorphism was recorded in Colorado ($P \approx 75\%$) while the lowest was observed in France ($P \approx 38\%$) (Table 3). The estimated heterozygosity ranged from 0.25 in New

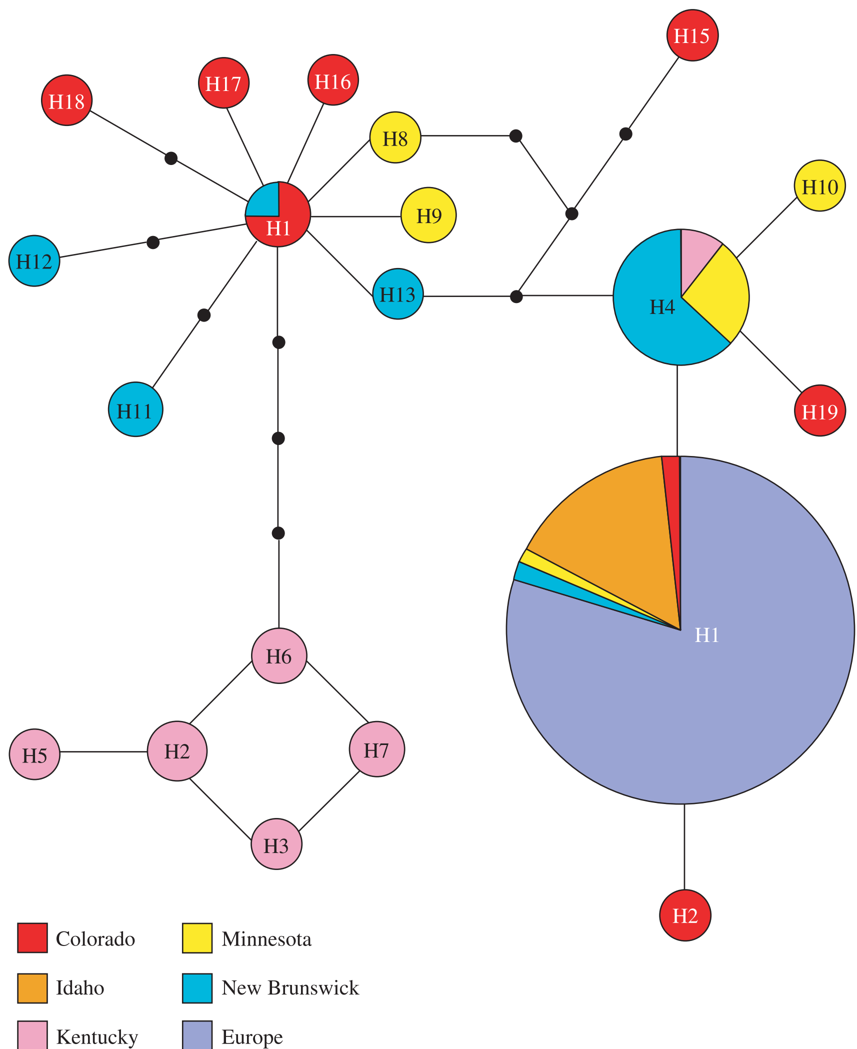

Fig. 2 Mitochondrial haplotype network. The areas of the circles are proportional to the number of samples sharing each haplotype. Lines represent single nucleotide mutations and black circles represent haplotypes not observed in the sample. Different colours represent different populations (see legend).

Table 2 Pairwise Φ_{ST} between populations based on Kimura 2-parameter distance between mtDNA haplotypes

Populations	Colorado	Idaho	Kentucky	Minnesota	New Brunswick	Europe
1. Colorado	—					
2. Idaho	0.488*	—				
3. Kentucky	0.415†	0.757†	—			
4. Minnesota	0.055	0.465†	0.529†	—		
5. New Brunswick	0.139	0.461†	0.598†	−0.027	—	
Europe	0.772†	0.000	0.913†	0.757†	0.703†	—

*indicates a significant value at the 5% global level after sequential Bonferroni correction (Rice 1989) and †indicates a significant value at 1% global level.

Brunswick to 0.14 in France (Table 3). Both the level of polymorphism and the estimated heterozygosity were significantly higher in North American than in European populations (*p*: Mann–Whitney *U*-test $P = 0.002$; *H*: Mann–Whitney *U*-test $P = 0.006$). The overall F_{ST} was 0.2010 (95% CI, 0.1826–0.2208), indicating strong differentiation among populations.

Neighbour-joining analysis of populations revealed differentiation between the populations from the two continents, as well as two separate groups within the European

Table 3 Nuclear DNA: number of beetles analysed (*N*) and descriptive statistics for the AFLP markers

Populations	*N*	% polymorphic loci (*p*)	Expected heterozygosity (*H*) ± SE
North America			
1. Colorado	36	74.75	0.244 ± 0.010
2. Idaho	37	58.25	0.192 ± 0.011
3. Kentucky	30	66.00	0.217 ± 0.011
4. Minnesota	19	70.37	0.218 ± 0.010
5. New Brunswick	27	68.01	0.246 ± 0.011
Europe			
6. Spain	37	38.38	0.164 ± 0.011
7. France	38	35.35	0.137 ± 0.010
8. Italy North	55	38.05	0.160 ± 0.011
9. Italy South	12	51.52	0.154 ± 0.011
10. Poland	51	57.91	0.207 ± 0.011
11. Estonia	38	50.17	0.190 ± 0.012
12. Finland	25	54.88	0.184 ± 0.011
13. Russia	32	57.58	0.201 ± 0.011

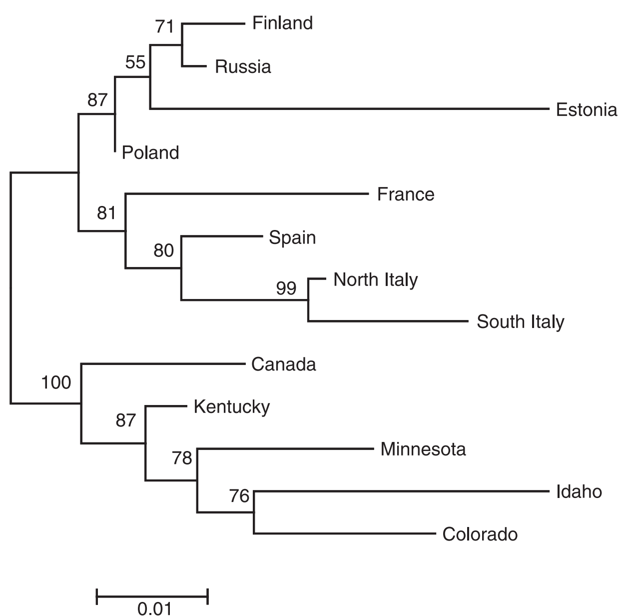

Fig. 3 Neighbour-joining tree obtained with the Nei's *D* distance among populations from the AFLP data set. Bootstrap values are presented at the nodes.

sample formed by the western (Spain, France and Italy) and the eastern populations (Poland, Estonia, Russia and Finland) (Fig. 3).

AMOVA clearly indicates a strong genetic differentiation between the populations of the two continents: 13% of the total variation was attributed to variation among the two groups ($P = 0.001$) and 17% of the variance was attributed to population variation within groups ($P < 0.001$). When the populations from the two continents were considered separately, the level of population differentiation was similar among North American populations and among European populations ($\Phi_{ST} = 0.201$, $P < 0.001$ and $\Phi_{ST} = 0.196$, $P < 0.001$, respectively). The amount of genetic variance between the eastern and western European populations amounted to 3.4% ($P = 0.028$) confirming the geographical differentiation observed on the NJ tree. Pairwise Φ_{ST} values among North American populations ranged from 0.279 between Idaho and Minnesota to 0.122 between Kentucky and Minnesota. Among European populations, Φ_{ST} values ranged from 0.342 between South Italy and Estonia to 0.057 between Russia and Finland (Table 4).

The NJ tree of individual AFLP profiles (Fig. 4) confirms the presence of geographical structure in the AFLP data set. North American and European beetles formed two separate clusters. Most of the beetles from the same population tended to cluster together. This was particularly true for the North American populations with the exception of the beetles from New Brunswick, which formed two groups and several beetles from Kentucky which did not cluster with other beetles from the same population. Among the European populations, the phenetic relationships among beetles were more complex. Estonian and Spanish beetles both clustered as single populations. French beetles formed two separate groups. Italian beetles also formed two clusters, one of which included all the southern Italian beetles. Russian and Finnish beetles were mostly mixed and closely related to the Estonian beetles. In general, however, individual European beetles tended to cluster in a western and an eastern group, as seen in the NJ tree of populations (Fig. 4), with the exception of the Polish beetles. Polish beetles were widely spread on the tree with some of them close to Russian and Finnish beetles and others closer to French and Italian beetles. The variable placement of beetles in the NJ tree could be due either to the lack of power in the cluster analysis or to different geographical origin of these beetles and thus represent gene flow.

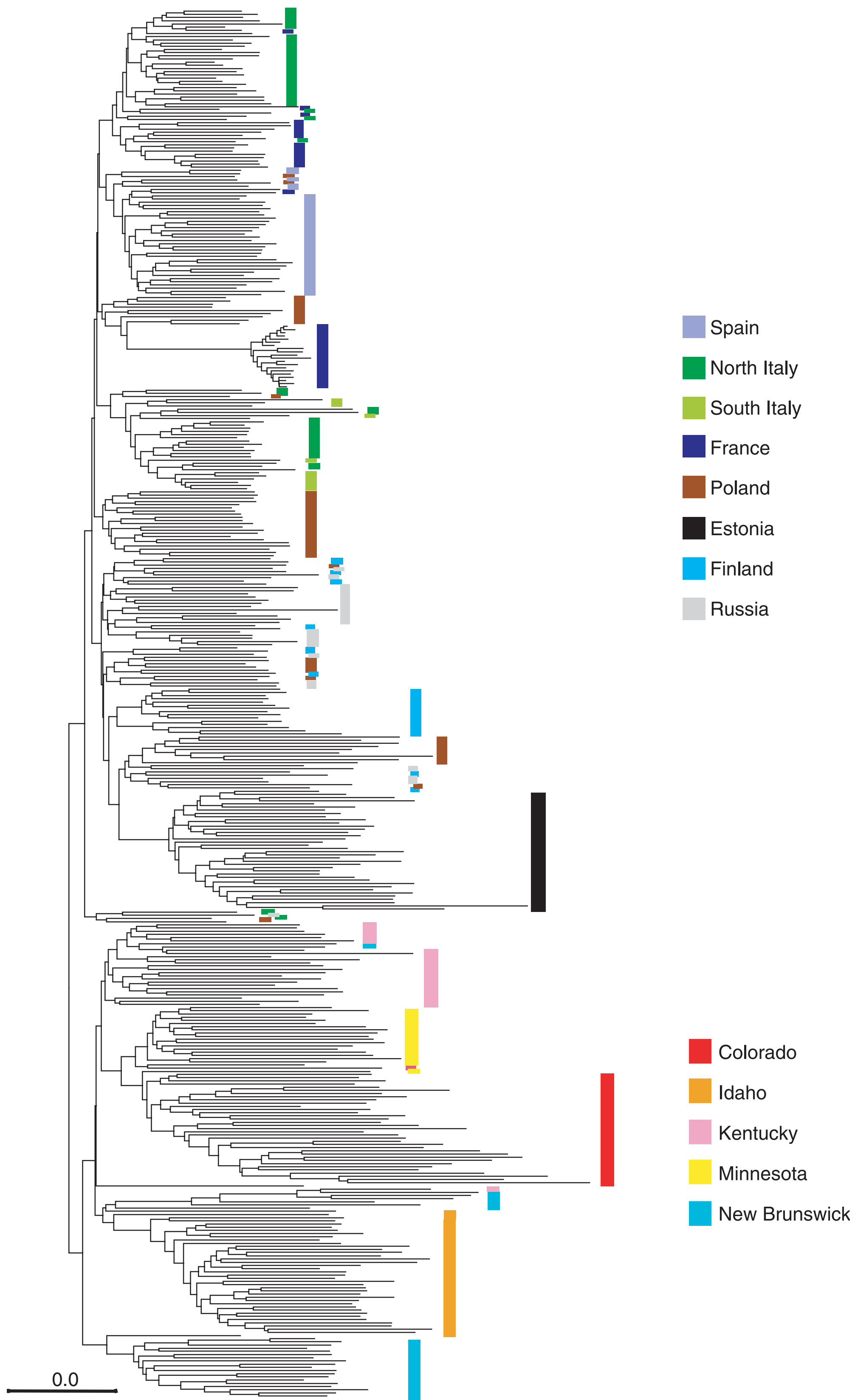

Fig. 4 Neighbour-joining tree representing the relationships among individual beetles obtained with the simple matching distance of the AFLP profiles. Different colours represent different populations (see legend).

Table 4 Phenetic pairwise Φ_{ST} between populations based on the AFLP data set. All comparisons are statistically significant at the 1% global level

Populations	Colorado	Idaho	Kentucky	Minnesota	New Brunswick	Spain	France	Italy N	Italy S	Poland	Estonia	Finland	Russia
1. Colorado	—												
2. Idaho	0.217	—											
3. Kentucky	0.194	0.227	—										
4. Minnesota	0.165	0.279	0.122	—									
5. New Brunswick	0.194	0.245	0.136	0.186	—								
6. Spain	0.293	0.358	0.244	0.305	0.261	—							
7. France	0.354	0.399	0.291	0.368	0.311	0.240	—						
8. Italy North	0.335	0.394	0.252	0.333	0.238	0.170	0.243	—					
9. Italy South	0.294	0.388	0.263	0.324	0.244	0.226	0.366	0.131	—				
10. Poland	0.261	0.308	0.155	0.217	0.200	0.114	0.153	0.102	0.159	—			
11. Estonia	0.369	0.413	0.304	0.343	0.322	0.304	0.312	0.301	0.342	0.190	—		
12. Finland	0.302	0.357	0.217	0.297	0.229	0.198	0.254	0.184	0.247	0.082	0.223	—	
13. Russia	0.285	0.335	0.190	0.267	0.192	0.185	0.249	0.142	0.203	0.063	0.216	0.057	—

Gene flow

Contemporary patterns of migration can be investigated by genetic tagging (Paetkau *et al*. 1998) and AFLP have proved to be very efficient in determining the source of an individual among putative populations (Campbell *et al*. 2003). The result of the assignment test is shown in Table 5. No beetles were allocated across the two continents. All North American beetles were allocated to their respective populations except for one beetle collected in Minnesota and allocated to Kentucky. All beetles from Estonia and Spain were assigned to their respective populations. About 50% of the southern Italian beetles were assigned to North Italy and a total of 13 European beetles could not be confidently assigned to one European population. Most of the Finnish beetles were assigned to either Finland or Russia or ambiguously to both countries. One Finnish beetle was assigned to both Finland and Poland.

Discussion

This study on Colorado potato beetles is one of the few studies of the genetic consequences of continent-scale invasion in an insect pest. Our results show high levels of mitochondrial and nuclear variability in North American beetle populations, with the highest genetic variability in populations from the central United States. Rapid expansion in North America could have resulted in a series of founder events and bottlenecks that partitioned the genetic variability of the species. Different studies found a fixed mitochondrial haplotype in the northwest of the United States, in Idaho (our results) and in Washington (Zehnder *et al*. 1992; Azeredo-Espin *et al*. 1996), which could suggest a unique founder event in this area. A more detailed investigation, however, is needed because the fixed haplotypes found by the three studies are not directly comparable. Thus, we cannot rule out different founder events by different maternal lineages in the different populations. A reduction in genetic variability was also observed in the Idaho population with the AFLP markers, although it was not as evident as in the mtDNA. European populations, instead, show a significant reduction in genetic diversity both in AFLP markers and mtDNA.

Beetle populations in Europe are fixed for a single mitochondrial haplotype (H1) which suggests a unique founder event (or a single successful one). However, European populations could also have arisen as a result of multiple introductions of the same haplotype. H1 haplotype is also fixed in the Idaho population, and it was common across the North American range.

Given the lack of mitochondrial DNA variation in Europe, mtDNA is not informative for understanding the dispersal history of Colorado potato beetles on this continent (Villablanca *et al*. 1998). Nuclear data also support our

© 2005 Blackwell Publishing Ltd, *Molecular Ecology*, 14, 4207–4219

Table 5 Assignment test obtained with AFLP-POP version 1.1 for North American and European populations

Beetle collected in:	Beetle assigned to:													
	Colorado	Idaho	Kentucky	Minnesota	New Brunswick	Spain	France	Italy N	Italy S	Poland	Estonia	Finland	Russia	None
1. Colorado	36	—	—	—	—	—	—	—	—	—	—	—	—	—
2. Idaho	—	37	—	—	—	—	—	—	—	—	—	—	—	—
3. Kentucky	—	—	30	—	—	—	—	—	—	—	—	—	—	—
4. Minnesota	—	—	1	18	—	—	—	—	—	—	—	—	—	—
5. New Brunswick	—	—	—	—	27	—	—	—	—	—	—	—	—	—
6. Spain	—	—	—	—	—	37	—	—	—		—	—	—	—
7. France	—	—	—	—	—	—	30	1	—	4	—	—	—	3
8. Italy N	—	—	—	—	—	—	—	53	1	—	—	—	—	1
9. Italy S	—	—	—	—	—	—	—	5	7	—	—	—	—	—
10. Poland	—	—	—	—	—	—	—	—	—	47	1	—	—	3
11. Estonia	—	—	—	—	—	—	—	—	—	—	38	—	—	—
12. Finland	—	—	—	—	—	—	—	—	—	—	—	17	4	4
13. Russia	—	—	—	—	—	—	—	—	—	2	—	—	28	2

conclusion of a single invasion, as beetles from each continent cluster together according to the AFLP profiles. Despite their significant reduction in genetic variability compared to North American populations, European populations have maintained genetic variability at the nuclear level. This could indicate that the founder population was relatively large or that multiple invasions stemming from the same or similar source populations occurred. Genetic variability at neutral markers is also associated with a high genetic variance in the life history traits of European beetles (S. Boman *et al*. unpublished). Loss of genetic diversity during colonization and spread through different continents has been observed in several invading insect pests such as the medfly (*Ceratitis capitata*) (Malacrida *et al*. 1998; Gasperi *et al*. 2002; Kourti 2002; Bonizzoni *et al*. 2004) which, in the Mediterranean area, was consistent with a sequential rather than a parallel colonization (Gomulski *et al*. 1998; Gasperi *et al*. 2002). Reduction in mitochondrial diversity has also been previously reported in *Aedes albopictus* (Kambhampati & Rai 1991) as well as in *Culex quinquefasciatus* (Guillemaud 1997) and attributed to their recent human-aided expansion through sequential bottlenecks that caused stochastic lineage survival and reduced haplotype diversity. Nevertheless, invading insects generally show the signature of multiple invasions. The colonization of South America by the medfly consisted of independent invasions from the native range in Africa and from the Mediterranean regions (Gomulski *et al*. 1998). Multiple invasions have also characterized the coffee berry borer *Hypothenemus hampei*'s colonization of South America, the *A. albopictus* mosquito's colonization of Italy and North America and *Aedes japonicus*' colonization of North America, neither of which showed any reduction of microsatellite diversity compared to native populations in Japan (Urbanelli *et al*. 2000; Fonseca *et al*. 2001; Benavides *et al*. 2005). These examples show that it is not simple to generalize about the dynamics of introduction and spread of invasive species and therefore comparison of the genetic diversity and population structure of native and introduced populations is a fundamental requirement to obtain a full picture.

Beetle population structure, observed with the nuclear markers, was very similar in North America and Europe. This high and similar population structure on the two continents suggests that the range expansion of Colorado potato beetles proceeded by a series of long-distance dispersal events and the establishment of outlying populations with local bottlenecks, instead of a simple advancing wave front with extensive gene flow (Sakai *et al*. 2001). Establishment of large outlying populations can maintain the required genetic variation for the evolution of local adaptation, while extensive gene flow can prevent local adaptation and further range expansion (Kirkpatrick & Barton 1997). In contrast to our results, Zehnder *et al*.'s (1992)

mtDNA data found no evidence of population structure within North American populations, which they attribute to the rapid range expansion of this species across the continent. Indeed, the high vagility of the species (Ferro *et al*. 1985; Voss & Ferro 1990), associated with the production of large populations that sometimes migrate and sometimes do not (Johnson 1967), should have favoured high gene flow between the old and newly established populations (Barrett & Husband 1990). However, another study on North American beetles using mtDNA markers confirms a strong population structure (Azeredo-Espin *et al*. 1996) which is consistent with differences in host plant affinity, photoperiodic response (Jacobson & Hsiao 1983) and insecticide resistance (Hare 1990). Furthermore, chromosomal studies suggested there are three different races within the species (Hsiao & Hsiao 1983). Similarly, local genetic drift has been proposed to explain the population genetic structure observed in *A. albopictus* in Italy (Urbanelli *et al*. 2000). Colorado potato beetles' and *Aedes* mosquitoes' genetic structure, instead, contrast with the spread of other invasive insects such as the medfly *C. capitata* (Gasperi *et al*. 2002), in which isolation by distance has been observed between populations in Africa (the native area) and in the Mediterranean basin (Gomulski *et al*. 1998; Malacrida *et al*. 1998; Gasperi *et al*. 2002; Kourti 2002), and in other potentially invasive fruit flies in Africa (Baliraine *et al*. 2004). However, beetle populations are thought to be demographically unstable (Hare 1990). The rotation of field cultivations, the management policies to control this pest and the strong selection imposed by the use of insecticides (Argentine *et al*. 1989) can cause oscillations in population densities which can increase the effect of drift and the partition of the genetic variability among populations (Sidorenko & Berezovska 2002) and partially explains differences in population structure reported by different studies. Loss of genetic diversity could also result from selective pressures as the beetles adapted to local environments in the northward range expansion toward colder regions than the original range in Mexico–southern United States. However, we did not observe a pattern in the reduction of genetic diversity across North America or across Europe. On the contrary, we observed higher nuclear variability in populations from the cooler northeastern countries than in populations from warmer countries, such as Spain and Italy (Table 3), making the hypothesis of selective pressure for adaptation to novel environments less probable. A temporal analysis of the population structure of Colorado potato beetles is also needed to fully understand the expansion dynamics of this invasive species.

Although the population structure observed with the nuclear markers was very similar on the two continents, the level of current migration as assessed by the assignment test indicates higher migration among European populations than among North America populations. In all but one case, North American beetles were assigned to the population from which they had been collected. In contrast, in Europe (excluding Finland which has been invaded very recently), 14 beetles were assigned to a population other than their own and nine other samples could not be confidently assigned between two populations. Assignments to populations other than those of collection were mostly between 'neighbour populations' as many southern Italian beetles were assigned to North Italy, Russian to Poland and Finnish to Russia, thus representing migrants among these populations. Alternatively, it is possible that greater, long-distance dispersal is more common in Europe than in North America, most likely due to commercial trade. Several beetles could not be assigned between their original population of collection and Poland. Poland and the East European countries in general are the greatest potato-producing countries in Europe (Huaccho & Hijmans 1999); thus, it is not surprising that many migrants could have come from these populations. These 'failed' assignments can represent migrants or the spreading history of Colorado potato beetles from their source populations. Accidentally introduced beetles have been found in Finland in supermarket and restaurant lettuce imported from other European countries [personal observation; Plant Production Inspection Centre (KTTK), personal communication]. It has been shown that AFLPs are very efficient in discriminating the source of an individual among putative populations even in the presence of low population structure (Campbell *et al*. 2003); thus, it seems unlikely that this lack of assignment for the European beetles could be due to the lower level of polymorphism at AFLP markers and thus, to the lower genetic variability compared to the North American beetles.

Three main steps are common to all invasions: an initial colonization event, a lag phase and a rapid population growth and range expansion (Sakai *et al*. 2001). Colorado potato beetles in Finland offer an opportunity to study all these steps of a biological invasion and the basic evolutionary processes involved in the adaptation to novel abiotic and biotic conditions. Previous introductions of beetles to Finland in 1948 (EPPO 1981), 1983 and 1998 (KTTK, personal communication) were not successful in establishing viable populations. The last and, currently, largest migration to Finland occurred during summer 2002 (KTTK, personal communication). Whether or not this migration has been successful in establishing viable populations is still uncertain but a few beetles were found in potato fields in 2003 and 2004 (personal observation; KTTK, personal communication). The source populations of the Finnish introduction were most likely in Russia (the St Petersburg region). Several observations point to this: (i) the abundance of beetles was highest in the southeastern part of Finland, along the border with Russia (KTTK, personal communication) and (ii) the meteorological conditions in the St

Petersburg region and southern Finland in summer 2002, with storms and strong westward winds (Finnish Meteorological Institute 2003) could have favoured the migration of the beetles westward. However, beetles are also widespread in Estonia which could also have served as source for beetles arriving in Finland. Under favourable meteorological conditions, beetles can fly over 100 km across the Baltic Sea to Scandinavia (Wiktelius 1981). Genetic analysis and the assignment test, however, exclude Estonia as the source of migrants. Finnish beetles were phenetically closer to Russian beetles than to Estonian beetles. Four samples were assigned to Russia and a further three, out of four specimens, could not be assigned with confidence between Russia and Finland. Interestingly, beetles of Russian populations show faster developmental times and have higher survival rates at low temperatures than beetles from other European populations (S. Boman *et al*., unpublished). These adaptations to cold climate conditions may help the Colorado potato beetles to invade further north, and the successful colonization of Scandinavia is probably only a matter of time.

Genetics should play a larger role in the development of policy to manage and control invasive species because it may play an important part in the invasion process and in evolving defences against controlling agents (Allendorf & Lundquist 2003). The evolution of local adaptation requires genetic variation. Successful invasions are often the result of multiple introductions which increase the genetic variation of the introduced population and circumvent the harmful effect of small population size. This could imply that a planned introduction (i.e. biological control) which limits the amount of genetic variation of introduced populations may be safe (Sakai *et al*. 2001). However, the success of an invading species is not necessarily linked to multiple introductions as shown by Colorado potato beetles which seem to have successfully invaded Europe only once. This result is confirmed by both the mtDNA and nuclear markers analyses. Despite the reduction in genetic variability in European beetles, they managed to spread across the continent in a very short time and, in the face of efforts to control them, are continuing to spread towards northern latitudes. In the long run, however, this reduction in genetic diversity as well the high population structure should aid the management of this pest species.

Acknowledgements

We thank Ric Bessin, Gilles Boiteau, Whitney Cranshaw, Jeff Davis, Sergei Fasulati, Külli Hiessaar, Leonardo Melchionda, Thomas Mowry, Maria Pawinska, Andrea Pilastro, David Ragsdale, Julien Saguez, Gustavo Tomás and the Plant Production Inspection Centre (KTTK), for kindly providing us with the samples of Colorado potato beetles from the different populations. Sari Viniikainen provided technical help in the laboratory. We also thank E. Knott, M. Iversen, J. Dallas and three anonymous reviewers for their useful comments on a previous version of the manuscript. The study was financially supported by the Finnish Ministry of Agriculture and Forestry (MMM), the Academy of Finland (project number 102292) and by the Centre of Excellence in Evolutionary Ecology at the University of Jyväskylä.

References

Allendorf FW, Lundquist LL (2003) Population biology, evolution, and control of invasive species. *Conservation Biology*, **17**, 24–30.

Apostol BL, Black WCI, Miller BR, Reiter P, Beaty BJ (1993) Estimation of the number of full sibling families at an ovoposition site using RAPD-PCR markers: applications to the mosquito *Aedes aegypti*. *Theoretical and Applied Genetics*, **86**, 991–1000.

Argentine JA, Clark JM, Ferro DN (1989) Relative fitness of insecticide-resistant Colorado potato beetle strains (Coleoptera: Chrysomelidae). *Environmental Entomology*, **18**, 705–710.

Avise JC (1994) *Molecular Markers, Natural History and Evolution*. Chapman & Hall, New York.

Azeredo-Espin AML, Schroder RFW, Roderick GK, Sheppard WS (1996) Intraspecific mitochondrial DNA variation in the Colorado potato beetle, *Leptinotarsa decemlineata* (Coleoptera: Chrysomelidae). *Biochemical Genetics*, **34**, 253–268.

Baker AJ (1992) Genetic and morphometric divergence in ancestral European and descendant New-Zealand populations of chaffinches (*Fringilla coelebs*). *Evolution*, **46**, 1784–1800.

Baliraine FN, Bonizzoni M, Guglielmino CR *et al.* (2004) Population genetics of the potentially invasive African fruit fly species, *Ceratitis rosa* and *Ceratitis fasciventris* (Diptera: Tephritidae). *Molecular Ecology*, **13**, 683–695.

Barrett SCH, Husband BC (1990) Genetics of plant migration and colonization. In: *Plant Population Genetics, Breeding and Genetic Resources* (eds Brown AHD, Clegg MT, Kahler AL, Weir BS). Sinauer Associates, Sunderland, Massachusetts.

Barrett SCH, Kohn JR (1991) Genetic and evolutionary consequences of small population size in plants: implications for conservation. In: *Genetics and Conservation of Rare Plants* (eds Falk DA, Holsinger KE), pp. 3–30. Oxford University Press, New York.

Benavides P, Vega FE, Romero-Severson J, Bustillo AE, Stuart JJ (2005) Biodiversity and biogeography of an important inbred pest of coffee, coffee berry borer (Coleoptera: Curculionidae: Scolytinae). *Annals of the Entomological Society of America*, **98**, 359–366.

Bonizzoni M, Guglielmino CR, Smallridge CJ *et al.* (2004) On the origins of medfly invasion and expansion in Australia. *Molecular Ecology*, **13**, 3845–3855.

Brower AVZ (1994) Rapid morphological radiation and convergence among races of the butterfly *Heliconius erato* inferred from patterns of mitochondrial DNA evolution. *Proceedings of the National Academy of Sciences, USA*, **91**, 6491–6495.

Campbell D, Duchesne P, Bernatchez L (2003) AFLP utility for population assignment studies: analytical investigation and empirical comparison with microsatellites. *Molecular Ecology*, **12**, 1979–1991.

Clement M, Posada D, Crandall KA (2000) TCS: a computer program to estimate gene genealogies. *Molecular Ecology*, **9**, 1657–1659.

Duchesne P, Bernatchez L (2002) AFLPPOP: a computer program for simulated and real population allocation based on AFLP data. *Molecular Ecology Notes*, **2**, 380–383.

EPPO (1981) EPPO data sheet on quarantine organisms no. 113: *Leptinotarsa decemlineata. EPPO Bulletin*, **11**.

EPPO – European and Mediterranean Plant Protection Organization (1998) *Distribution Maps of Quarantine Pests for Europe*. CABI Publishing, Wallingford, UK.

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.

Felsenstein J (1989) PHYLIP — Phylogeny inference package (version 3.2). *Cladistics*, **5**, 164–166.

Ferro DN, Logan JA, Voss RH, Elkinton JS (1985) Colorado potato beetle (Coleoptera: Chrysomelidae) temperature-dependent growth and feeding rates. *Environmental Entomology*, **14**, 343–348.

Finnish Meteorological Institute (2003) *Annual Report 2002*. Painoprisma, Turku, Finland.

Fonseca DM, Campbell S, Crans WJ *et al.* (2001) *Aedes* (Finlaya) *japonicus* (Diptera: Culicidae), a newly recognized mosquito in the United States: analyses of genetic variation in the United States and putative source populations. *Journal of Medical Entomology*, **38**, 135–146.

Frankham R, Ballou JD, Briscoe DA (2002) *Introduction to Conservation Genetics*. Cambridge University Press, Cambridge, UK.

Gasperi G, Bonizzoni M, Gomulski LM *et al.* (2002) Genetic differentiation, gene flow and the origin of infestations of the medfly, *Ceratitis capitata*. *Genetica*, **116**, 125–135.

Gomulski LM, Bourtzis K, Brogna S *et al.* (1998) Intron size polymorphism of the Adh_1 gene parallels the worldwide colonization history of the Mediterranean fruit fly, *Ceratitis capitata*. *Molecular Ecology*, **7**, 1729–1741.

Guillemaud T (1997) Contrasting levels of variability between cytoplasmic genomes and incompatibility types in the mosquito *Culex pipiens*. *Proceedings of the Royal Society of London. Series B, Biological Sciences*, **264**, 245–251.

Hare JD (1980) Impact of defoliation by the Colorado potato beetle on potato yields. *Journal of Economic Entomology*, **73**, 369–373.

Hare JD (1990) Ecology and management of the Colorado potato beetle. *Annual Review of Entomology*, **35**, 81–100.

Hsiao TH, Hsiao C (1983) Chromosomal analysis of *Leptinotarsa* and *Labidomera* species (Coleoptera: Chrysomelidae). *Genetica*, **60**, 139–150.

Huaccho L, Hijmans RJ (1999) *A Global Geo-Referenced Database of Potato Distribution for 1995–1997 (GPOT97)*. International Potato Center, Lima, Peru.

Jacobson JW, Hsiao TH (1983) Isozyme variation between geographic populations of the Colorado potato beetle, *Leptinotarsa decemlineata* (Coleoptera: Chrysomelidae). *Annals of the Entomological Society of America*, **76**, 162–166.

Johnson CG (1967) International dispersal of insects and insect-borne viruses. *Netherlands Journal of Plant Pathology*, **73**, 21–43.

Kambhampati S, Rai KS (1991) Mitochondrial DNA variation within and among populations of the mosquito *Aedes albopictus*. *Genome*, **34**, 288–292.

Keiper FJ, McConchie R (2000) An analysis of genetic variation in natural populations of *Sticherus flabellatus* [R. Br. (St John)] using amplified fragment length polymorphism (AFLP) markers. *Molecular Ecology*, **9**, 571–581.

Kirkpatrick M, Barton NH (1997) Evolution of a species' range. *American Naturalist*, **150**, 1–23.

Kolbe JJ, Glor RE, Rodriguez Schettino L *et al.* (2004) Genetic variation increases during biological invasion by a Cuban lizard. *Nature*, **431**, 177–181.

Kourti A (2002) Estimates of heterozygosity and patterns of geographic differentiation in natural populations of the medfly (*Ceratis capitata*). *Hereditas*, **137**, 173–179.

Kumar S, Tamura K, Nei M (1993) *MEGA (Version 1.0): Molecular Evolutionary Genetics Analysis*. Pennsylvania State University, University Park, Pennsylvannia.

Lee CE (2002) Evolutionary genetics of invasive species. *Trends in Ecology & Evolution*, **17**, 386–391.

Lynch M, Milligan BG (1994) Analysis of population genetic structure with RAPD markers. *Molecular Ecology*, **3**, 91–99.

Malacrida AR, Marinoni F, Torti C *et al.* (1998) Genetic aspects of the worldwide colonization process of *Ceratitis capitata*. *Journal of Heredity*, **89**, 501–507.

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

Neigel JE, Avise JC (1986) Phylogenetic relationships of mitochondrial DNA under various demographic models of speciation. In: *Evolutionary Process and Theory* (eds Nevo E, Karlin S), pp. 515–534. Academic Press, New York.

Paetkau D, Calvert W, Stirling I, Strobeck C (1995) Microsatellite analysis of population structure in Canadian polar bears. *Molecular Ecology*, **4**, 347–354.

Paetkau D, Shields GF, Strobeck C (1998) Gene flow between insular, coastal and interior populations of brown bears in Alaska. *Molecular Ecology*, **7**, 1283–1292.

Queller DC (2000) Pax Argentinica. *Nature*, **405**, 519–520.

Rice WR (1989) Analysing tables of statistical tests. *Evolution*, **43**, 223–225.

Sakai AK, Allendorf FW, Holt JS *et al.* (2001) The population biology of invasive species. *Annual Review of Ecology and Systematics*, **32**, 305–332.

Salvato P, Battisti A, Concato S *et al.* (2002) Genetic differentiation in the winter pine processionary moth (*Thaumetopoea pityocampa–wilkinsoni* complex), inferred by AFLP and mitochondrial DNA markers. *Molecular Ecology*, **11**, 2435–2444.

Schneider S, Roessli D, Excoffier L (2000) *ARLEQUIN (Version 2.000): A software for population genetic data analysis*. Genetics and Biometry Laboratory, University of Geneva, Switzerland.

Sidorenko AP, Berezovska OP (2002) Genetic structure of populations of the Colorado potato beetle *Leptinotarsa decemlineata* (Coleoptera: Chrysomelidae). *Russian Journal of Genetics*, **38**, 1256–1261.

Simon C, Frati F, Beckenbach A, Crespi B, Liu H, Flook P (1994) Evolution, weighting, and phylogenetic utility of mitochondrial gene-sequences and a compilation of conserved polymerase chain-reaction primers. *Annals of the Entomological Society of America*, **87**, 651–701.

Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG (1997) The CLUSTAL_X Windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Research*, **24**, 4876–4882.

Tsutsui ND, Suarez AV, Holway DA, Case TJ (2000) Reduced genetic variation and the success of an invasive species. *Proceedings of the National Academy of Sciences, USA*, **97**, 5948–5953.

Urbanelli S, Bellini R, Carrieri M, Sallicandro P, Celli G (2000) Population structure of *Aedes albopictus* (Skuse): the mosquito which is colonizing Mediterranean countries. *Heredity*, **84**, 331–337.

Vekemans X, Beauwens T, Lemaire M, Roldan-Ruiz I (2002) Data from amplified fragment length polymorphism (AFLP) markers show indication of size homoplasy and of a relationship between

degree of homoplasy and fragment size. *Molecular Ecology*, **11**, 139–151.

Villablanca FX, Roderick GK, Palumbi SR (1998) Invasion genetics of the Mediterranean fruit fly: variation in multiple nuclear introns. *Molecular Ecology*, **7**, 547–560.

Vos P, Hogers R, Bleeker M *et al*. (1995) AFLP: a new technique for DNA fingerprinting. *Nucleic Acids Research*, **23**, 4407–4414.

Voss RH, Ferro DN (1990) Ecology of migrating Colorado potato beetles (Coleoptera: Chrysomelidae) in western Massachusetts. *Environmental Entomology*, **19**, 123–129.

Wiktelius S (1981) Wind dispersal of insects. *Grana*, **20**, 205–207.

Zehnder GW, Sandall L, Tisler AM, Powers TO (1992) Mitochondrial DNA diversity among 17 geographic populations of *Leptinotarsa decemlineata* (Coleoptera: Chrysomelidae). *Annals of the Entomological Society of America*, **85**, 234–240.

Zhivotovsky LA (1999) Estimating population structure in diploids with multilocus dominant DNA markers. *Molecular Ecology*, **8**, 907–913.

A. Grapputo is researcher at the University of Jyväskylä, with interests in genetic diversity and phylogeography. S. Boman is a PhD candidate interested in the evolution of adaptation to cold climate and the potential for spread of invasive insect species to northern latitudes. L. Lindström and A. Lyytinen are Post-Doctoral Researcher Fellows of the Academy of Finland interested in aposematism in insects and the evolutionary ecology of invasive species. J. Mappes is Professor of Evolutionary Ecology at the University of Jyväskylä with interests in predator–prey interactions and the evolutionary ecology of invasive species.