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Host-plant diversity of the European corn borer *Ostrinia nubilalis*: what value for sustainable transgenic insecticidal *Bt* maize?

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The strategies proposed for delaying the development of resistance to the *Bacillus thuringiens*is toxins produced by transgenic maize require high levels of gene flow between individuals feeding on transgenic and refuge plants. The European corn borer *Ostrinia nubilalis* (Hübner) may be found on several host plants, which may act as natural refuges. The genetic variability of samples collected on sagebrush (*Artemisia* sp.), hop (*Humulus lupulus* L.) and maize (*Zea mays* L.) was studied by comparing the allozyme frequencies for six polymorphic loci. We found a high level of gene flow within and between samples collected on the same host plant. The level of gene flow between the sagebrush and hop insect samples appeared to be sufficiently high for these populations to be considered a single genetic panmictic unit. Conversely, the samples collected on maize were genetically different from those collected on sagebrush and hop. Three of the six loci considered displayed greater between-host-plant than within-host-plant differentiation in comparisons of the group of samples collected on sagebrush or hop with the group of samples collected on maize. This indicates that either there is genetic isolation of the insects feeding on maize or that there is host-plant divergent selection at these three loci or at linked loci. These results have important implications for the potential sustainability of transgenic insecticidal maize.

Keywords: *Ostrinia nubilalis*; European corn borer; pheromone; transgenic insecticidal maize; pest management; genetically modified organism

1. INTRODUCTION

Genetic modifications in response to man-made changes provide the best-known examples of adaptation to a new environment (e.g. Macnair 1991; McKenzie & Batterham 1994; Lenormand *et al*. 1999). For example, intensive pesticide treatment has resulted in the selection of insecticide resistance alleles within a very short space of time (Raymond *et al*. 1991). Therefore, it is generally accepted that one of the most important elements in the husbandry of transgenic crops producing *Bacillus thuringiensis* toxins (*Bt* crops) is the development and implementation of effective resistance management plans in order to delay the appearance of resistance to *Bt* in target pests (Gould 1998). The most widely accepted resistance management strategy is the high-dose–refuge model, which has been implemented in North America (Alstad & Andow 1995). Refuges are defined as non-*Bt* plants that can be used by the target pest and planted and maintained in close proximity to *Bt* crops (Gould 1998). The principle underlying this system of resistance management is that any resistant insects emerging from *Bt* crops are more likely to mate with one of the much larger number of susceptible adult pest insects emerging from the refuges than with each other, thereby decreasing the selection of *Bt* resistance alleles.

Bt crops, including transgenic varieties of cotton and maize, are toxic to many Lepidoptera, including Noctuidae and Pyralidae. *Ostrinia nubilalis* Hübner (Pyralidae), the European corn borer (ECB), is one of the most damaging pests of maize in North America and Europe. We have previously assessed the extent of gene flow in this pest species within and between 29 samples sites located in maize fields from all over France (Bourguet *et al*. 2000). We found that random mating and high levels of gene flow occurred within and between ECB populations over large geographical distances (several hundred kilometres). This suggests that non-*Bt* maize planted in the vicinity of *Bt* maize crops may act as an effective ECB refuge. However, the size and location of such refuges are still a matter of considerable debate (e.g. Caprio 1998; Onstad & Gould 1998; Roush 1998; Peck *et al*. 1999). Gould (1998) suggested that, for some generalist pest species such as *Heliothis virescens*, wild hosts and other crops could serve as part of a larger refuge. *Ostrinia nubilalis* is known to be remarkably polyphagous and will attack almost any robust herbaceous wild or cultivated plant with stems large enough for the larvae to enter (Hudon *et al*. 1989). Lewis (1975) reported 223 species of plant on which it can become established. Nevertheless, to be considered as complementary or alternative refuges, these plants must host ECB populations that will randomly mate with those emerging from maize. Although generalist herbivores are able to use a wide range of host plants, they usually have a preference for one or a few host species because of the differences in resources and toxic compounds even between closely related plants (Tikkanen *et al*. 1999). Depending on the relative importance of phenotypic

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Table 1. *Characteristics of the samples of* O. nubilalis*: location, date of sampling, host plant and number* (*n*) *of ECBs analysed* (S, sagebrush; H, hop; M, maize.)

location	sample	host plant	latitude	longitude	date	*n*
Steenvorde (Pennequin)	Moi	S	50° 49′ N	2° 37′ E	September 1998	40
	Pen	H	50° 49′ N	2° 37′ E	September 1998	40
Steenvorde (Daneels)	Dan	S	50° 49′ N	2° 37′ E	September 1998	40
	Vor	H	50° 49′ N	2° 37′ E	September 1998	22
Steenvorde (Degrick)	Sten	S	50° 49′ N	2° 37′ E	September 1998	40
Bailleul	Isa	S	50° 45′ N	2° 45′ E	September 1998	21
	Bek	H	50° 45′ N	2° 45′ E	September 1998	39
Solesmes	Lef	S	50° 11′ N	3° 28′ E	September 1998	22
	Sol	M	50° 11′ N	3° 28′ E	September 1998	40
Avesnes les Aubert	Dro	S	50° 12′ N	3° 23′ E	September 1998	22
	Ave	M	50° 12′ N	3° 23′ E	September 1998	21
Cap Gris nez	Gri	S	50° 52′ N	1° 35′ E	November 1998	32
Steenwerck	Wer	S	50° 52′ N	1° 35′ E	September 1998	16
Benifontaine	Chti	H	50° 29′ N	2° 49′ E	August 1998	22
Laventie	Hou	H	50° 38′ N	2° 47′ E	April 1997	68
Les Rues des Vignes	Mar	M	50° 06′ N	3° 14′ E	September 1998	40

plasticity, genetic variability, demographic dynamics and migration patterns, this heterogeneity in habitat results in one of two different situations. One is a source–sink system (Pulliam 1988) in which high-quality host plants (sources) produce an excess of insects, whereas lower-quality host plants (sinks) do not produce enough insects, such that populations may not persist without immigration from sources (Dias 1996). The second involves the adaptation of insects to their host plants, resulting in partial genetic isolation and potential sympatric speciation (Rice 1984, 1987). Comparisons of population structure and gene flow within and between habitats may provide insight into the occurrence of source and sink populations (e.g. Dias *et al*. 1996) and of partial genetic isolation (e.g. Feder *et al*. 1988). Therefore, unlike our previous study (Bourguet *et al*. 2000) in which we assessed the extent of gene flow within and between populations sampled on a single given host (maize) over large geographical distances**,** this study was devoted to assessing genetic differentiation within and between samples of *O. nubilalis* collected over a restricted area on three different host plants: maize (*Zea mays* L.), sagebrush (*Artemisia* sp.) and hop (*Humulus lupulus* L.).

2. MATERIAL AND METHODS

(a) *Sampling sites*

Samples were taken from 16 sites spread over a restricted area in northern France. All samples except Gri were located along a transect of 90 km (figure 1); Gri was located 80–150 km away from the other sampled sites (table 1 and figure 1). Individuals were collected as larvae diapausing in plants, their sex was determined and they were directly frozen at −80 °C. Three different host plants were considered: sagebrush (*Artemisia* sp.), hop (*H. lupulus* L.) and maize (*Z. mays* L.). We collected seven samples from sagebrush, six from hop and three from maize (table 1 and figure 1). Some samples were sympatric as they were collected from overlapping host-plant populations: (i) Moi, Dan and Isa were sampled from sagebrushes occurring within the hop fields from which Pen, Vor and Bek, respectively, were collected, and (ii) Lef and Dro were collected from sagebrushes located at the edges of the maize fields from which Sol and Ave, respectively, were sampled.

(b) *Electrophoresis*

Each larva was homogenized in 150 µl of Tris–EDTA, pH 6.8, after removal of the head for further analysis. Horizontal starch gel electrophoreses of the homogenates were carried out using Tris–borate–EDTA (pH 8.6) buffer systems (Pasteur *et al*. 1987). Six polymorphic enzymes allowing unequivocal genetic interpretation were revealed, as described by Bourguet *et al*. (2000). These enzyme systems were phosphoglucomutase (PGM, EC 8.4.2.2), mannose-6-phosphate isomerase (MPI, EC 5.3.1.8), hydroxybutyrate dehydrogenase (HBDH, EC 1.1.1.30), glucose-phosphate isomerase (GPI, EC 5.3.1.9), aspartate-aminotransferases (AAT EC 2.6.1.1) and triose-phosphate isomerase (TPI, EC 5.3.1.1). *Tpi* is located on sexual chromosome Z (Glover *et al*. 1990). Females are heterogametic (ZW) whereas males are homogametic (ZZ). Thus, at this locus females are hemiploid whereas males are diploid.

(c) *Data analysis*

We estimated the allelic frequencies (available on request from the first author), mean number of alleles (n_{all}), observed and expected heterozygosities (H_o and H_e) and $\hat{f}$-values (i.e. F_{is} estimates according to Weir & Cockerham (1984)) for each sample using Fstat 2.3 software (J. Goudet, Institute of Ecology, Lausanne, Switzerland). We tested for deviation from Hardy–Weinberg expectations at each locus and calculated genotypic linkage disequilibria between loci within each sample with GENEPOP 3.1d software (Raymond & Rousset 1995). We also carried out Ohta's (1982) variance analysis using the LINKDOS program (Garnier-Gere & Dillmann 1992) in order to determine whether epistatic natural selection or genetic drift within a population was responsible for the observed linkage disequilibria. As *Tpi* is a sex-linked locus, the H_o and $\hat{f}$-values estimated and tests for deviations from Hardy–Weinberg expectations were carried out for males only. If the tests involved replicated independent tests, Fisher's method for combining independent results (Sokal & Rohlf 1981) was used. The genetic structure between samples

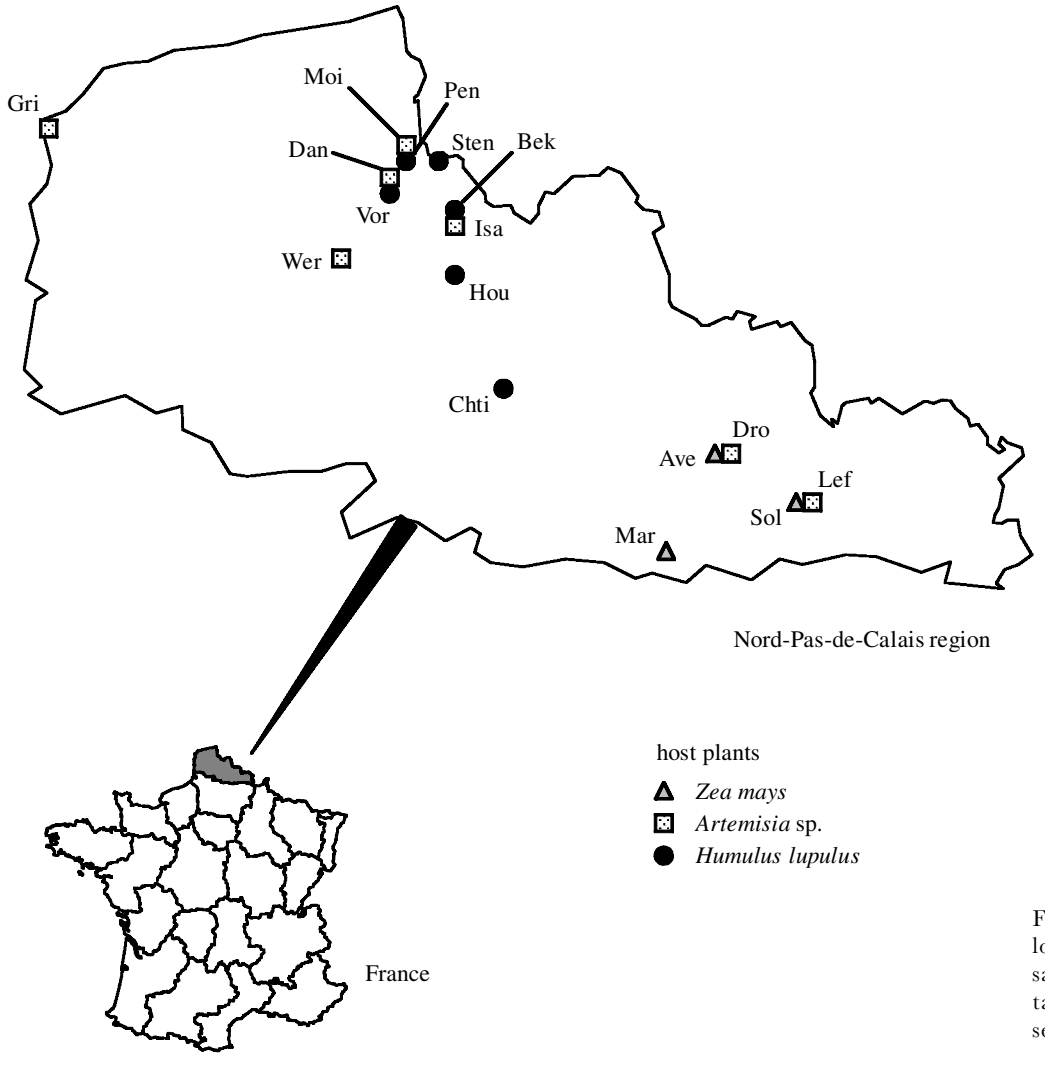

Figure 1. Geographical locations of the 16 sampling sites reported in table 1 (for definitions, see table 1).

and between host groups (a host group includes all samples collected on a given host plant over the whole area) was analysed by testing for allelic differentiation using exact tests and calculating the $\hat{\theta}$ estimator of F_{ST} as described by Weir & Cockerham (1984) using GENEPOP 3.1d (Raymond & Rousset 1995). Isolation by distance patterns (Slatkin 1993) was also tested by assessing the independence of the geographic and genetic distances for various geographical levels. The null hypothesis that the geographic and genetic distances were independent was tested against an alternative hypothesis of a positive correlation expected under isolation by distance, which was estimated as Spearman's rank correlation coefficient. The calculated correlation coefficient was compared with the distribution of the correlation coefficients obtained from Mantel-like permutations of the genetic $(\hat{\theta}/(1-\hat{\theta}))$ and geographic (ln(geographical distance)) matrices as described by Rousset (1997) and included in GENEPOP 3.1d. We also carried out hierarchical analyses of the population structure by partitioning $\hat{\theta}$ into $\hat{\theta}_{wg}$ and $\hat{\theta}_{bg}$ which indicates the genetic differentiation of samples within-groups and between-groups, respectively (Weir & Cockerham 1984), using TFPGA 1.3 software (Miller 1997). An unrooted tree was produced using the neighbour-joining method based on Reynolds's distance (Reynolds *et al.* 1983). Calculations were made and the tree drawn with PHYLIP 3.5c (Felsenstein 1993) and TREEVIEW (Page 1996) software, respectively.

3. RESULTS

Two alleles were observed for the *Tpi* locus, four for the *Gpi* locus, two for the *Hbdh* locus, four for the *Aat* locus, four for the *Mpi* locus and six for the *Pgm* locus for the samples from the 16 sampled sites analysed. However, the populations at only two of the 16 sites sampled showed polymorphism at the *Hbdh* locus (table 2).

Analysis of the genotypic associations for each pair of loci in each sample detected seven non-random associations out of the 15 tested (*Gpi* and *Hbdh*, *Gpi* and *Aat*, *Gpi* and *Mpi*, *Hbdh* and *Aat*, *Aat* and *Mpi*, *Aat* and *Pgm* and *Mpi* and *Pgm*). None occurred in all the samples (one to four samples out of 16). Analysis of the variance components of disequilibrium (Ohta 1982) indicated that genetic drift rather than selection was responsible for the non-random associations. Within samples (table 2), the mean number of alleles was 2.33–3.17 for the six loci tested. The observed and expected heterozygosities were almost identical and were 0.21–0.31 (observed) and 0.20–0.36 (expected). The $\hat{f}$-value estimates did not show a large excess or deficit of heterozygotes and deviations from Hardy–Weinberg expectations for the six loci were significant for only one of the 16 sampled sites (i.e. Sol) ($p < 10^{-3}$). However, this deviation was not significant

Table 2. *Within-sample polymorphism*

(n, P, n_{all}, H_o, H_e and $\hat{f}$ are the sample size, number of polymorphic loci, mean number of alleles, observed and expected heterozygosities and estimator of the F_{is} index, respectively. For each of these parameters, the mean number and standard error across loci are given.)

			n_{all}		H_o		H_e		
sample	n	P	mean	s.e.	mean	s.e.	mean	s.e.	$\hat{f}$
sagebrush									
Moi	40	5 (HBDH)	2.50	1.05	0.25	0.20	0.26	0.21	0.05
Dan	22	5 (HBDH)	2.83	1.17	0.30	0.22	0.36	0.18	0.00
Isa	21	5 (HBDH)	2.33	1.03	0.21	0.22	0.23	0.17	0.09
Wer	16	5 (HBDH)	2.50	0.84	0.23	0.14	0.27	0.15	0.13
Lef	22	5 (HBDH)	2.50	0.84	0.27	0.21	0.27	0.18	− 0.06
Dro	22	5 (HBDH)	2.33	0.82	0.24	0.23	0.24	0.20	− 0.08
Gri	32	5 (HBDH)	2.67	1.21	0.21	0.21	0.20	0.20	− 0.05
hop									
Chti	22	4 (HBDH, TPI)	2.67	1.37	0.25	0.20	0.27	0.21	0.09
Sten	40	6	3.17	0.98	0.31	0.22	0.29	0.20	− 0.06
Pen	40	5 (HBDH)	2.83	1.17	0.25	0.19	0.29	0.19	0.07
Vor	40	5 (HBDH)	2.83	1.33	0.27	0.22	0.27	0.17	− 0.07
Bek	39	6	3.00	0.89	0.27	0.21	0.26	0.16	− 0.08
Hou	68	5 (HBDH)	3.17	1.47	0.24	0.21	0.23	0.20	− 0.03
maize									
Mar	40	5 (HBDH)	2.83	1.17	0.28	0.27	0.29	0.25	− 0.05
Sol	40	5 (HBDH)	2.67	1.37	0.23	0.25	0.29	0.24	0.19**
Ave	21	5 (HBDH)	2.17	0.75	0.28	0.27	0.30	0.20	− 0.03

**$p < 10^{-3}$.

Table 3. *Between-sample (within-group) differentiation*

(Differentiation is measured by the Téta. p corresponds to the probability value for the exact test of allelic differentiation over the whole study and within each group of samples according to the host.)

	Tpi	*Gpi*	*Hbdh*	*Aat*	*Mpi*	*Pgm*	all
whole study							
$\hat{\theta}$	0.012	0.019	0.016	0.032	0.048	0.015	0.030
p	$< 10^{-3}$	$< 10^{-5}$	0.055	$< 10^{-5}$	$< 10^{-5}$	$< 10^{-5}$	$< 10^{-5}$
sagebrush							
$\hat{\theta}$	0.008	0.016	—	0.039	0.017	0.020	0.021
p	0.010	0.005	—	$< 10^{-3}$	0.026	0.003	$< 10^{-5}$
hop							
$\hat{\theta}$	0.000	0.000	0.017	0.016	0.000	0.003	0.002
p	0.056	0.883	0.032	0.019	0.513	0.015	0.002
maize							
$\hat{\theta}$	0.000	0.000	—	0.044	0.026	0.030	0.012
p	0.952	0.808	—	0.007	0.057	0.055	0.016

($p > 0.05$) when we took the multiple tests into account (Bonferroni test) (Holm 1979). These results were consistent with those reported by Bourguet *et al.* (2000).

The overall differentiation between samples was significant ($p < 10^{-5}$), but the mean $\hat{\theta}$-value was low (0.030) (table 3). Such low $\hat{\theta}$-values were also observed for the analysis of the samples of each host group separately (table 3). The lowest value was observed for the samples collected on the hop plants ($\hat{\theta} = 0.002$ and $p = 0.002$), whereas the highest was observed in the sagebrush group ($\hat{\theta} = 0.021$ and $p < 10^{-5}$).

We carried out a hierarchical analysis of the distribution of the genetic variability in order to determine the components of variance due to differentiation within and between host groups. $\hat{\theta}_{wg}$ was 0.038 and $\hat{\theta}_{bg}$ was 0.027; both were significantly different from zero. This indicated that significant but low-level genetic differentiation occurred not only within host groups but also between host groups. In addition, the genetic differentiation between each pair of host groups indicated that the maize group was differentiated from the sagebrush and hop groups whereas the sagebrush and hop groups were genetically very similar (table 4). This result is illustrated in figure 2 with an unrooted tree based on Reynolds's distance (F_{ST} based) in which the three samples collected on maize (Ave, Mar and Sol) are clustered separately from all the samples collected on sagebrush and hop. The position of the three maize samples relative to the others is supported by a bootstrap value of 74% (1000 resamplings) whereas the relative positions of the samples collected on

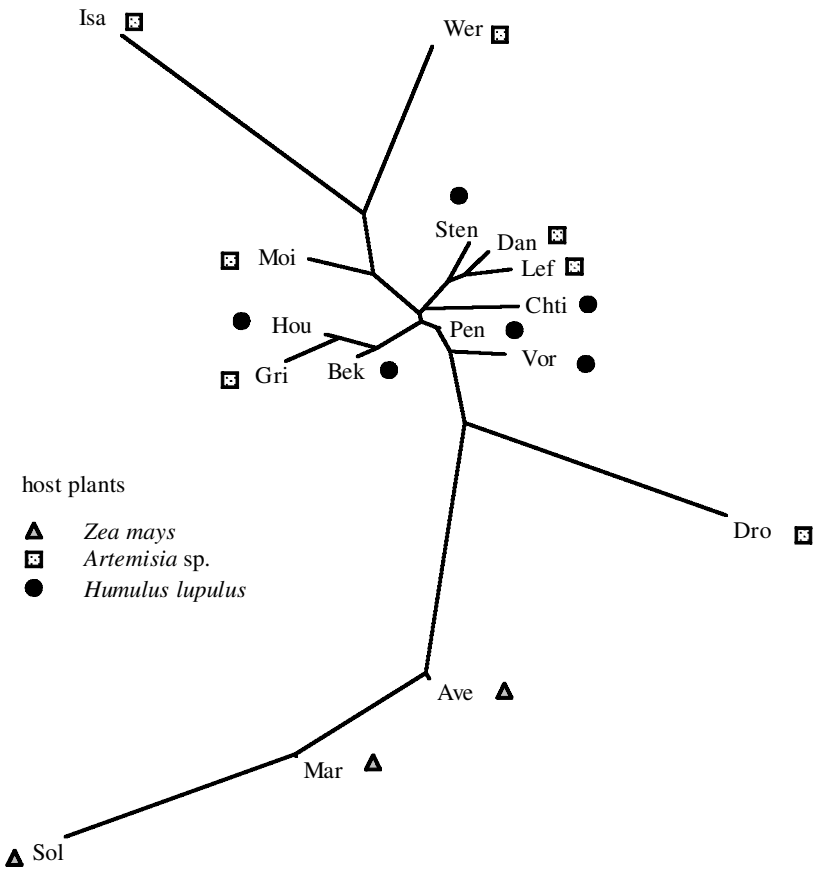

Figure 2. Unrooted dendrogram inferred from Reynolds's genetic distance (neighbour-joining method). Genetic distances were calculated and the tree drawn using PHYLIP 3.5c (Felsenstein 1993) and TREEVIEW software (Page 1996), respectively.

Table 4. *Between-group differentiation with each group defined as the samples collected from a given host*

($\hat{\theta}$-values were calculated as described by Weir & Cockerham (1984) and exact tests were carried out using GENEPOP 3.1d software.)

	Tpi	*Gpi*	*Hbdh*	*Aat*	*Mpi*	*Pgm*	all
sagebrush versus hop							
$\hat{\theta}$	0.006	0.007	0.004	0.002	0.000	0.000	0.002
p	0.190	$< 10^{-3}$	0.080	0.330	0.950	0.170	0.020
sagebrush versus maize							
$\hat{\theta}$	0.010	0.056	—	0.030	0.114	0.012	0.057
p	0.040	$< 10^{-5}$	—	$< 10^{-3}$	$< 10^{-5}$	$< 10^{-5}$	$< 10^{-5}$
hop versus maize							
$\hat{\theta}$	0.060	0.011	0.002	0.005	0.050	0.007	0.024
p	$< 10^{-3}$	$< 10^{-4}$	0.330	0.070	$< 10^{-5}$	$< 10^{-3}$	$< 10^{-5}$

sagebrush and hop have low bootstrap values (below 70%). We analysed the genetic structure of each pair of samples more precisely. Most of the significant exact tests for allelic differentiation between samples involved one of the three samples collected on maize (figure 3).

Finally, the Mantel-like tests of the independence of the geographic and genetic distances were significant for the whole data set ($p = 0.012$). This may have resulted from the differentiation of the maize group from the two other groups. Indeed, in separate analyses of the data sets for each host group, none exhibited a significant isolation by distance pattern ($p = 0.35$, 0.38 and 0.50 for the sagebrush, hop and maize groups, respectively), consistent with the high level of gene flow which is estimated to occur within each host group.

4. DISCUSSION

Both phenotypic plasticity and genetic adaptation may enable ECB populations to cope with the heterogeneity of their host plants. In theory, the balance between these two responses is determined by the extent of environmental variation relative to that of gene flow (Pulliam 1988). If individuals encounter frequently changing selection pressures, the evolutionary balance should tip towards greater phenotypic plasticity (Sibly 1995). Similarly, variations in host-plant quality may lead to genetic source–sink systems in which there is a net gene flow from favourable host plants to host plants of lower quality (Pulliam 1988; Dias 1996). In contrast, if populations encounter different but consistent selection pressures,

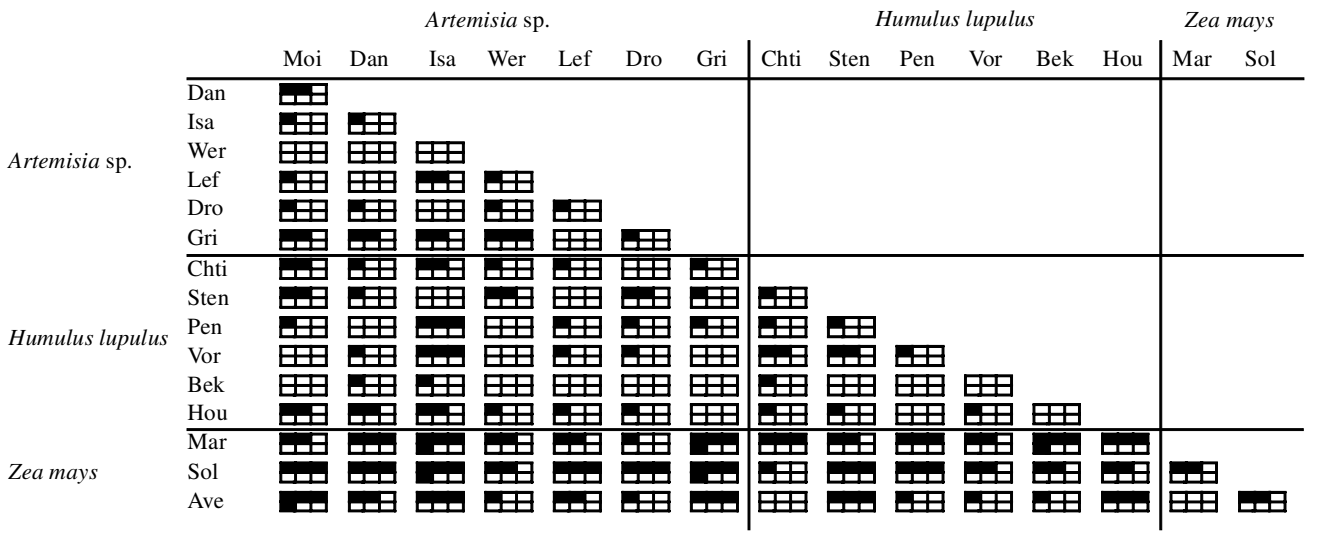

Figure 3. Pairwise exact test of allelic differentiation for each locus and pair of samples. For each pair of samples, the number of black squares indicates the number of loci for which exact tests were significant (e.g. two of six loci exhibited a significant *p*-value for the exact test of allelic differentiation between the Moi and Dan populations but none of the tests were significant in comparisons of Wer and Moi).

genetic differentiation is likely to occur as subpopulations adapt to local conditions (Endler 1979).

Our results show that the allele frequencies at six loci encoding presumably neutral polymorphic protein markers were statistically indistinguishable within and between the samples collected on *Artemisia* sp. and *H. lupulus* L. resulting in essentially no differentiation within and between host groups (tables 3 and 4). If the data from the samples collected from these two host plants were pooled, the probability values in the exact tests for deviations from Hardy–Weinberg expectations for the *Tpi*, *Gpi*, *Hbdh*, *Aat*, *Mpi* and *Pgm* loci were 0.57, 0.06, 1, 0.02, 0.28 and 0.07, respectively. Thus, the observed genotypic ratios for these protein markers are similar to the ratios expected under Hardy–Weinberg equilibrium, suggesting that interhost gene flow is sufficiently high for these populations to be considered a single genetic panmictic unit.

There may be several reasons for this lack of differentiation.

(i) There is too little isolation for differentiation to be detected in analyses of population differentiation (Rousset 1999).
(ii) The sagebrush and hop populations may have become isolated very recently, such that current gene flow cannot be distinguished from the retention of ancestral polymorphisms at similar frequencies.
(iii) The sagebrush and hop phenologies are so similar that there is no selection pressure for promoting genetic adaptation in ECB populations.
(iv) These two host plants do differ in quality but the phenotypic plasticity of the ECB enables it to develop in both host plants, thereby preventing genetic adaptation.
(v) Populations of ECB are better adapted to one of the two host species, so one functions as a sink and the other acts as the source.

It is not possible to determine whether sagebrush and hop correspond to a source–sink system from the data reported here. There was almost no differentiation between populations on the two species, so the only possible genetic prediction is that the expected lifetime of any particular neutral gene is shorter in a sink than in a source host plant (Dias *et al*. 1996). Testing this prediction would require long-term studies. Moreover, Rousset (1999) recently showed that source and sink habitats cannot be distinguished by comparing their genetic diversities or by population structure analysis using *F*-statistics.

A different pattern emerges if the samples collected on *Z. mays* L. are compared with those collected on *Artemisia* sp. and *H. lupulus* L. Indeed, the genotypic differentiation between each pair of host groups indicated that the maize group has differentiated from the sagebrush and hop groups (table 4). The possible isolation of population insects collected on maize from those collected on the other two host plants is further illustrated in figure 3. The populations collected on the maize samples were genetically more similar to each other than to sympatric samples collected on sagebrush (Sol versus Lef and Ave versus Dro). Finally, three of the six loci (*Tpi*, *Gpi* and *Mpi*) showed greater between-group than within-group differentiation if the sagebrush or hop insect samples were compared with those collected on maize (tables 3 and 4). This suggests that either the populations feeding on maize are isolated or that there is host-divergent selection at these three loci or at linked loci (Rousset 1999). Other examples of two populations of the same species inhabiting the same geographical area and showing such patterns of differentiation include host races of the fruit fly *Rhagoletis pomonella* (Feder *et al*. 1988). In the fruit fly there is selective maintenance between sympatric host-plant races at six allozyme loci (Feder *et al*. 1997).

Several factors, alone or in combination, may be responsible for the observed differentiation between the ECB populations collected from maize and those

collected from hop and sagebrush. These factors include (i) pre- or post-mating reproductive isolation, (ii) a genetic bottleneck associated with the founding of the maize population, (iii) genetically based differences in host preference that might lead to a system of positive assortative mating based on host choice, (iv) differential host recognition by adult moths, and (v) temporal differences in the timing of adult emergence.

In North America, *O. nubilalis* consists of several morphologically indistinguishable races with different sex pheromone communication systems (Roelofs *et al*. 1985). Only a few studies have focused on the genetic relationships between these races. Harrison & Vawter (1977) and Cardé *et al*. (1978) found that two sympatric pheromonal races displayed slight differences in their allelic frequencies. Using the *Tpi* locus, Glover *et al*. (1991) found that gene flow between ECB pheromonal races was asymmetrical. Races with different pheromones have also been reported in France (Stengel & Schubert 1982; Anglade *et al*. 1984). Therefore, an investigation of the relationship between pheromones and host plant would probably provide further insight into the genetic differentiation of populations of ECB on maize and other host plants. More detailed analysis is also required in order to identify the ecological processes which may lead to differentiation. One possible line of research would be to compare the genetic structures of samples from the same population at different stages in their life cycle. Significant differences in the population structure between larvae and adults would provide evidence for population-wide selection (Stanton *et al*. 1997).

The results presented here have practical implications for preventing the evolution of resistance in pest species to *B. thuringiensis* toxins produced by transgenic crops. *Bt* resistance in *O. nubilalis* is currently managed by implementing a high-dose–refuge strategy (Alstad & Andow 1995). However, this study suggests that, although ECB populations are found on several different types of host plant, the ECB populations on non-maize plants may constitute separate subpopulations and, therefore, cannot necessarily be viewed as alternative refuges as proposed by Gould (1998) and Alstad & Andow (1995).

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