

Differentiation and gene flow among island and mainland populations of the true armyworm, *Pseudaletia unipuncta* (Haworth) (Lepidoptera: Noctuidae)

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Abstract: The genetic structure of populations of the true armyworm, *Pseudaletia unipuncta* (Haworth) (Lepidoptera: Noctuidae), in the Azores archipelago was studied using polyacrylamide-gel electrophoresis. Four enzyme systems (aldehyde oxidase, esterase, phosphoglucomutase, and phosphoglucose isomerase) were examined in six populations from islands in the Azores (Santa Maria, São Miguel, Terceira, Pico, Faial, Flores) and compared with those from populations from mainland Portugal and Canada. The North American and European populations are not clearly separated from the Azorean ones. Similarly, studies of different enzyme systems (aldehyde oxidase, esterase, malic enzyme, sorbitol dehydrogenase, manose-6-phosphate isomerase, and phosphoglucomutase) over 2 years (1997 and 1998) at different times of the year (spring, summer, and autumn) and at three different altitudes (0, 250, and 500 m above sea level) on three different islands (Santa Maria, São Miguel, and Faial) uncovered no distinct differences. These results, obtained from classically used loci, suggest that there is still some gene flow between sites or that island populations have not been isolated for sufficient time to have diverged from founder populations.

Résumé : La structure génétique des populations açoriennes de la légionnaire uniponctuée *Pseudaletia unipuncta* (Haworth) (Lepidoptera : Noctuidae), a été étudiée à l'aide d'enzymes séparées par électrophorèse sur gel de polyacrylamide. Quatre systèmes enzymatiques (aldéhyde oxydase, estérase, phosphoglucomutase et phosphoglucose isomérase) ont été analysés chez six populations des Açores (îles de Santa Maria, São Miguel, Terceira, Pico, Faial et Flores) et chez des populations portugaise et canadienne. Les populations européenne et américaine ne sont pas nettement séparées des populations açoriennes. De la même manière, l'étude de six systèmes enzymatiques (aldéhyde oxydases, estérase, enzymes maliques, sorbitol déshydrogénases, manose-6-phosphate isomérases et phosphoglucomutases) durant deux années (1997 et 1998), à différentes périodes de l'année (printemps, été et automne) et à trois altitudes différentes (0, 250 et 500 m) sur trois îles (Santa Maria, São Miguel et Faial) n'a pas dévoilé de divergences évidentes. Ces résultats obtenus à partir de locus classiquement utilisés et en nombre suffisant, suggèrent qu'il subsiste un certain flux génétique entre les populations étudiées, et (ou) que les populations insulaires sont isolées depuis trop peu de temps pour avoir pu diverger des populations fondatrices.

Introduction

Data on the movement of organisms, often essential for ecological and evolutionary studies, may be obtained by direct means (e.g., mark-recapture; Anglade 1969; King et al. 1990) or indirect means (e.g., genetic markers; Caprio and

Tabashnik 1992; Neigel 1997; Megléc et al. 1998; Beerli and Felsenstein 1999). Direct estimates, especially when movement is over considerable distances, have a number of limitations that include cost (in both time and money) and the significance for the gene flow (Caprio and Tabashnik 1992; Crochet 1996). The use of indirect techniques to study the genetic structure of populations may offer some insight into dispersal (Caprio and Tabashnik 1992; Thorpe and Solé-Cava 1994; Harry et al. 1998) when direct methods are either not possible or not cost effective.

The true armyworm, *Pseudaletia* (= *Mythimna*) *unipuncta* (Haworth) (Lepidoptera: Noctuidae), is an important pest of graminaceous crops, including pasture, in Europe, North America, and the Azores. It is considered a seasonal migrant in North America and Europe, establishing temporary summer populations in northern areas, where over-winter survival is not possible (Cayrol et al. 1974; Fields and McNeil 1984; Buès et al. 1986). A similar pattern has also been inferred or demonstrated for a number of other noctuids, such as *Helicoverpa zea* (Boddie) (Westbrook et al. 1997), *Agrotis ipsilon* (Hufnagel) (Buès et al. 1996; Showers 1997), *Peridroma saucia* (Hübner) (Cayrol et al. 1974), *Mythimna*

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separata (Walker) (Mills et al. 1996), and *Spodoptera exempta* (Walker) (Dingle 1991; Gatehouse 1997). The conclusion that *P. unipuncta* is a seasonal migrant has been based on indirect evidence from field studies on overwintering individuals (Fields and McNeil 1984) and seasonal patterns of individuals caught in light and pheromone traps (McNeil 1987), together with parallel laboratory studies examining the effects of temperature and photoperiod on the reproductive biology of males and females (Turgeon and McNeil 1983; Delisle and McNeil 1987; Dumont and McNeil 1992). Adults subjected to short-day, low-temperature conditions reduce the production of juvenile hormone (Cusson et al. 1990), leading to a delay in the onset of reproduction to facilitate migration from a deteriorating habitat (McNeil et al. 1994, 1996, 1997).

In the Azores, *P. unipuncta* is present year-round (Tavares 1989; Tavares et al. 1992; Vieira et al. 1994), and when compared with North American populations in terms of behaviour and physiology associated with sexual maturation (McNeil et al. 1996, 2000), these island populations appear to be nonmigratory. Given the volcanic origin of the Azores archipelago and its relatively young geological age (Santa Maria is considered the oldest island, dating from about 8 million years ago), it is clear that these *P. unipuncta* populations are of continental origin, but it is not known whether they came from North America, Europe, or both. Moreover, the rates of gene flow between islands and continents, or within the archipelago, are unknown. Furthermore, it is not known whether the seasonal fluctuations in population density that occur at different altitudes (Anunciada 1983; Tavares 1989; Tavares et al. 1992; Vieira et al. 1994) are associated with local climatic conditions or result from the movement of adults in search of the most appropriate habitat at different times of the year.

As electrophoresis has been used to study dispersal in other Lepidoptera (Pashley et al. 1985; Daly 1989; Buès et al. 1994), we used this approach to compare (i) different island populations within the Azores archipelago with those from mainland Portugal and Canada, and (ii) genetic variability between islands as a function of season and altitude. These comparisons will allow some conclusions to be drawn about the rate of gene flow.

Materials and methods

To compare *P. unipuncta* populations from islands and continents, material was obtained from the islands of Santa Maria, São Miguel, Terceira, Pico, Faial, and Flores in the Azores, as well as from Aveiro and Coimbra on the mainland of Portugal and from Normandin in Quebec, Canada. Cultures were established using eggs obtained from mated females collected during the summer of 1994 in black-light traps (a minimum of 50 females assumed to be representative of each population). Eggs from all females captured at a given site were pooled and reared at $22 \pm 1^\circ\text{C}$ and $70 \pm 5\%$ RH under a 16 h light : 8 h dark photoperiodic regime on an artificial diet (Poitout and Buès 1970). Pupae were sexed (Breeland 1958) and after emergence, adult pairs were randomly formed in individual cylindrical cages (157 cm^3) with a food source of 8% sucrose. Eggs from each pair were reared through one additional generation before eggs and

3rd- and 6th-instar larvae were randomly collected from each family and then stored at -25°C (≤ 2 months) until analysis.

Analyses were carried out on individual eggs, individual 3rd-instar larvae, and the haemolymph of 6th-instar larvae that were homogenized, using the methodology described by Pintureau (1987), Vieira and Pintureau (1993), and Vieira (2000). Vertical polyacrylamide gel electrophoresis (PAGE) was performed on 10 enzymatic systems: esterase (EST, EC 3.1.1.1), aldehyde oxidase (AO, EC 1.2.3.1), phosphoglucosyltransferase (PGM, EC 5.4.2.2), phosphoglucose isomerase (PGI, EC 5.3.1.9), alcohol dehydrogenase (EC 1.1.1.1), glutamate-oxaloacetate transferase (EC 2.6.1.1), glucose-6-phosphate dehydrogenase (EC 1.1.1.49), lactate dehydrogenase (EC 1.1.1.27), malate dehydrogenase (EC 1.1.1.37), and leucine-aminopeptidase (EC 3.4.1.1). However, only four systems produced genetically interpretable results: EST (loci from eggs and hemolymph of the 6th larval instar), AO, PGM, and PGI (loci from 3rd-instar larvae).

Island populations were compared using males that were trapped alive in pheromone traps baited with 300 μg of Z11-16:Ac containing 0.2–0.5% Z11-16OH (Turgeon et al. 1983). Traps were installed 1 m above ground at the edge of permanent pastures at low (100 m), medium (250 m), and high (500 m) altitudes on the islands of Santa Maria, São Miguel, and Faial in 1997 and 1998. The sites (from low to high) were located at Paúl, Almagreira, and Fontinhas on Santa Maria, Relva, Remédios da Lagoa, and Lagoa do Congro on São Miguel, and Courelas, Flamengos, and Caldeira on Faial. Material was collected during flights in spring (May–June), summer (July–August), and autumn (October–November). For any given trapping period, all adults at every site were captured within a 1-week interval. All males were held at -25°C until analysis. The thoracic muscle of each male individual was homogenized in 250 μL of Trudgill solution (Pintureau 1987) and then centrifuged (27 000g at 5°C for 5 min) to obtain a clear supernatant for electrophoresis. Only 40 μL of this supernatant was used for each enzymatic system. PAGE was performed using the techniques described by Pintureau (1987), Vieira and Pintureau (1993), and Vieira (2000) on six polymorphic enzyme systems: EST, PGM, AO, malic enzyme (ME, EC 1.1.1.40), sorbitol dehydrogenase (SDH, EC 1.1.1.14), and manose phosphate isomerase (MPI, EC 5.3.1.8). To compare males and females, the same analyses were carried out on individuals captured in black-light traps at all sites on São Miguel and Faial during the 1998 season.

Individuals were identified according to island (Santa Maria, São Miguel, or Faial), year (1997 or 1998), season (spring, summer, or autumn), and altitude (high, medium, or low). Loci were designated according to criteria defined by Vieira and Pintureau (1993): enzyme (AO, EST, ME, PGI, MPI, PGM, SDH), insect stage (egg (E), 6th-instar haemolymph (H), whole body of 3rd-instar larva (L), adult thorax (T)) and arabic numerals (1, 2, 3, or 4) indicating the anodal position of encoded bands.

Allele frequencies (genotypes were inferred from electromorph phenotypes), mean number of alleles per locus (MNA), percentage of polymorphic loci (P), observed heterozygosity (H_o , direct count), and Nei's (1978) unbiased expected heterozygosity (H_e) were estimated using the

BIOSYS-1 version 1.7 package (Swofford and Selander 1989). H_e was calculated using Levene's correction for small samples (Swofford and Selander 1989). The genotype frequencies at each polymorphic locus were compared with those expected from Hardy-Weinberg equilibrium with the probability test implemented by the GENEPOP version 3 (Raymond and Rousset 1995). According to Pashley et al. (1985), population heterogeneity can be assessed in two ways: Wright's F statistics (Wright 1978) and contingency χ^2 . We opted for F statistics computed with FSTAT (Goudet 1996) for populations (eight including the continental ones, or 52 subpopulations from three Azores islands) and for individuals and loci using Weir and Cockerham's (1984) method, which accounts for sampling variation. We used weighted averaging over alleles and loci and jackknifed over loci to provide an error estimate for each index as suggested by Weir and Cockerham (1984). Thus, F_{IS} values estimated deviations from random mating within populations for each locus, while F_{IT} values measured the global deficit of heterozygotes (i.e., the inbreeding coefficient of the individuals relative to the total population). Positive values of F_{IS} and F_{IT} indicate a deficiency of heterozygotes, while negative values indicate an excess. F_{ST} values measuring the genetic variance among populations (see Wright 1951, 1978) were also calculated in order to examine the genetic structure of *P. unipuncta*. Whenever needed, the sequential Bonferroni procedure was employed to control for the probability of incorrectly rejecting one or more true null hypotheses at the 0.05 level of significance (Rice 1989).

Gene flow among populations (Nm , where N is the effective local population size and m is the average migration rate) was estimated by the method of private alleles (Slatkin 1985; Barton and Slatkin 1986) using the GENEPOP program (Raymond and Rousset 1995). This estimator is the most appropriate when levels of gene flow are low (Barton and Slatkin 1986). Nei's (1978) unbiased genetic distance between populations was calculated with BIOSYS (Swofford and Selander 1989).

The variances of allele frequency of seven loci in the three island populations studied were compared using a four-way ANOVA (island, year, season, and altitude as principal factors) (program SAS®; SAS Institute Inc. 1985; for a description and interpretation of the method see also Excoffier et al. 1992; Excoffier 2001). Data were arcsine-transformed prior to the ANOVA. Multivariate analyses applied to the allele frequencies may improve the description of the population structure (Lessa 1990), so a factorial correspondence analysis (program ANAMUL™ of Febvay and Bonnot 1991) was carried out on the allele frequencies of the 52 subpopulations, by altitude or by season, to examine the geographical differentiation of *P. unipuncta*. On the other hand, Fisher's exact tests were used to compare males and females captured in light traps on São Miguel and Faial.

As with other species (Allegrucci et al. 1997; Peterson and Denno 1998), we investigated whether a correlation exists between genetic distances and geographical distances separating continental and island populations, using Mantel tests (Mantel 1967) after log transformation of the values (Slatkin 1993; Peterson and Denno 1998). Finally, to confirm their membership of the same population, we compared individuals from Santa Maria showing null alleles (AO) and

alleles only readable under rather specific conditions (ME) with individuals from the same sites that did not show such alleles, on the basis of seven common loci, using Fisher's exact tests (Sokal and Rohlf 1997).

Results

Comparison of island and continental populations

Of the enzyme systems used, seven loci were interpretable. Comparisons of individuals from Santa Maria with and without specific alleles yielded no evidence of genetic divergence (Fisher's exact test, $p > 0.05$). Loci AO-L1 (L for larvae) and PGI-L1 were entirely monomorphic, while EST-E2 (E for egg), EST-H2 (H for haemolymph), EST-H3, AO-L2, and PGM-L1 were polymorphic. However, locus AO-L2 exhibited a single allele in populations from Santa Maria, São Miguel, Flores, mainland Portugal, and Canada (Table 1). With the exception of Faial and Pico, the mean percentage of polymorphic loci in the Azorean populations (71.43% for each island) is similar to that in both the Portuguese and the Canadian populations (57.14%). The deviation from Hardy-Weinberg equilibrium, tested at each polymorphic locus and corrected by the Bonferroni method, is only significant for EST-E2 (Portugal) and EST-H2 (Santa Maria, São Miguel, and Terceira) (Table 1).

The two negative F_{IS} values calculated for EST-E2 and AO-L2 indicate an excess of heterozygotes (Table 2), but the mean F_{IS} value is positive, indicating that homozygotes are more often in excess. The mean F_{ST} value (0.098) is relatively distant from zero, showing that matings are not panmictic among all the studied populations and, thus, that these populations are partially isolated (Table 2). The overall allelic heterogeneity (eight populations), as indicated by the F_{ST} values, is relatively high. Very similar values were obtained from the six Azorean populations ($F_{ST} = 0.105$, confidence interval (CI) = 0.045–0.188) and from a comparison of these populations with the Portuguese mainland ($F_{ST} = 0.101$, CI = 0.055–0.161) and Canadian populations ($F_{ST} = 0.105$, CI = 0.043–0.190). F_{ST} analyses show that only about 10.2% of the total variance in allelic frequency is due to genetic differences among the studied populations.

The Nm value of 5.7 obtained with the method of private alleles suggests moderate gene flow between populations each generation. Nei's genetic distances between pairs of Azorean populations (0.060 ± 0.004) and between the Azorean and continental populations (0.016–0.095 and 0.039–0.068 for mainland Portugal and Canada, respectively) are generally relatively low (Table 3).

Furthermore, the correlation between genetic distance and geographic distance (Mantel's test, $Z = -0.238$, $p = 0.111$) was not significant, suggesting that gene flow between the studied populations exists now or has existed in the recent past and that the geographical distances do not explain the genetic structure of *P. unipuncta*.

Inter-island comparisons as a function of season and altitude

Seven polymorphic loci were used in the analyses of the 52 subpopulations observed at different altitudes over three seasons on the islands of Santa Maria, São Miguel, and Faial. The number of alleles per locus ranged from 3 ± 0.4

Table 1. Allele frequencies at seven loci in eight populations (laboratory samples) of the true armyworm, *Pseudaletia unipuncta*, from six islands in the Azores (Santa Maria, São Miguel, Terceira, Faial, Pico, and Flores), mainland Portugal, and Quebec, Canada.

	Santa Maria	São Miguel	Terceira	Faial	Pico	Flores	Mainland Portugal	Canada
EST-E2								
69	—	0.058	—	—	—	0.063	0.013	—
76	—	0.096	0.167	—	—	0.250	0.020	0.012
83	0.258	0.375	0.150	0.116	0.081	0.400	0.033	0.045
90	0.452	0.471	0.567	0.744	0.757	0.287	0.820	0.670
100	0.290	—	0.116	0.140	0.162	—	0.114	0.273
H_o	0.839	0.500	0.600	0.442	0.486	0.825	0.213	0.636
H_e	0.656	0.631	0.625	0.418	0.400	0.700	0.315	0.479
F_{IS}	-0.300	0.200	0.024	-0.069	-0.233	-0.194	0.319	-0.343
P_{ex}	0.152	0.013	0.132	0.262	0.081	0.744	0.000*	0.016
EST-H2								
98	0.093	0.530	0.190	0.094	0.268	0.196	0.349	0.470
100	0.703	0.318	0.476	0.500	0.125	0.304	0.443	0.309
105	0.185	0.136	0.334	0.344	0.482	0.500	0.142	0.221
107	0.019	0.016	—	0.062	0.125	—	0.066	—
H_o	0.185	0.152	0.286	0.500	0.571	0.536	0.528	0.500
H_e	0.466	0.608	0.633	0.639	0.677	0.631	0.663	0.644
F_{IS}	0.599	0.747	0.543	0.192	0.140	0.135	0.196	0.212
P_{ex}	0.000*	0.000*	0.001*	0.026	0.715	0.464	0.012	0.317
EST-H3								
98	0.045	0.031	0.013	0.038	0.067	0.083	0.011	0.039
99	0.303	0.313	0.162	0.423	0.433	0.292	0.222	0.145
100	0.500	0.469	0.500	0.385	0.433	0.396	0.445	0.329
102	0.091	0.187	0.287	0.154	0.050	0.187	0.289	0.355
104	0.061	—	0.038	—	0.017	0.042	0.033	0.132
H_o	0.667	0.563	0.550	0.538	0.433	0.542	0.400	0.711
H_e	0.654	0.667	0.647	0.674	0.628	0.730	0.676	0.735
F_{IS}	-0.035	0.130	0.140	0.169	0.298	0.242	0.402	0.021
P_{ex}	0.498	1.000	1.000	0.329	0.022	0.008	0.071	0.031
AO-L1								
100	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
AO-L2								
71	—	—	0.038	—	—	—	—	—
81	—	—	0.138	—	0.048	—	—	—
90	—	—	0.112	—	0.029	—	—	—
100	1.000	1.000	0.712	0.975	0.923	1.000	1.000	1.000
110	—	—	—	0.025	—	—	—	—
H_o	—	—	0.575	0.050	0.077	—	—	—
H_e	—	—	0.465	0.049	0.146	—	—	—
F_{IS}	—	—	-0.252	-0.026	0.469	—	—	—
P_{ex}	—	—	0.017	0.873	0.019	—	—	—
PGI-L1								
100	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
PGM-L1								
100	0.955	0.543	1.000	0.485	0.871	0.824	0.500	0.700
107	0.045	0.457	—	0.515	0.129	0.176	0.500	0.287
114	—	—	—	—	—	—	—	0.013
H_o	0.091	0.571	—	0.441	0.086	0.135	0.415	0.300
H_e	0.088	0.504	—	0.507	0.227	0.294	0.506	0.433
F_{IS}	-0.048	-0.151	—	0.117	0.617	0.533	0.171	0.298
P_{ex}	1.000	0.503	—	0.442	0.004	0.005	0.347	0.125

Table 1 (concluded).

	Santa Maria	São Miguel	Terceira	Faial	Pico	Flores	Mainland Portugal	Canada
Global								
H_o	0.254 (0.132)	0.255 (0.105)	0.287 (0.109)	0.282 (0.251)	0.236 (0.094)	0.291 (0.128)	0.222 (0.086)	0.307 (0.119)
H_e	0.266 (0.118)	0.344 (0.123)	0.339 (0.122)	0.327 (0.303)	0.297 (0.106)	0.336 (0.130)	0.309 (0.118)	0.327 (0.122)
N	45.3 (6.6)	35.1 (4.2)	36.3 (2.0)	27.9 (5.1)	37.7 (3.9)	34.1 (2.5)	52.0 (5.6)	38.0 (1.7)
MNA	2.43 (0.61)	2.43 (0.57)	2.71 (0.64)	2.43 (0.48)	2.71 (0.57)	2.43 (0.61)	2.71 (0.71)	2.57 (0.61)
P	57.14	57.14	57.14	71.43	71.43	57.14	57.14	57.14

Note: Values in parentheses show the standard error. H_o , observed heterozygosity; H_e , expected heterozygosity; P_{χ^2} , conformity with Hardy-Weinberg equilibrium; F_{IS} , Wright's fixation index; N , mean number of individuals studied for each locus; MNA, mean number of alleles per locus; P , mean percentage of polymorphic loci.

* $p < 0.001$, indicating rejection of Hardy-Weinberg equilibrium after correction of probability values by the Bonferroni method.

Table 2. Estimated F statistics by polymorphic locus for eight *P. unipuncta* populations (laboratory samples) from Azores islands, mainland Portugal, and Canada.

Locus	F_{IS}	F_{IT}	F_{ST}
EST-E2	-0.038	0.094	0.128
EST-H2	0.371	0.435	0.101
EST-H3	0.192	0.212	0.024
AO-L2	-0.031	0.111	0.138
PGM-L1	0.210	0.361	0.191
Mean	0.190	0.269	0.098
SD	0.091	0.082	0.034
95% CI ^a	0.018-0.320	0.128-0.402	0.048-0.167

^aInferior and superior limits of CI at 95%.

Table 3. Matrix of Nei's genetic distances for eight populations (laboratory samples) of *P. unipuncta*, calculated from allele frequencies at five polymorphic loci.

Population	Santa Maria	São Miguel	Terceira	Faial	Pico	Flores	Mainland Portugal
São Miguel	0.081	—					
Terceira	0.032	0.090	—				
Faial	0.065	0.046	0.085	—			
Pico	0.064	0.072	0.046	0.050	—		
Flores	0.050	0.047	0.045	0.072	0.052	—	
Mainland Portugal	0.083	0.032	0.084	0.016	0.063	0.095	—
Canada	0.064	0.039	0.054	0.045	0.044	0.068	0.020

(mean \pm SE) (Santa Maria, 1998, autumn, medium altitude) to 4 ± 0.6 (São Miguel, 1998, spring, low altitude), with 3.6 ± 0.1 overall. The observed heterozygosity varied between 0.233 ± 0.037 (São Miguel, 1998, autumn, high altitude) and 0.519 ± 0.081 (Santa Maria, 1998, spring, medium altitude), with an overall mean of 0.333 ± 0.059 . Table 4 provides the data for each island and year. Deviation from Hardy-Weinberg proportions was observed at each locus in some of the 52 subpopulations studied: 12 subpopulations for AO-T2 (T for thorax), 1 for ME-T2, 20 for SDH-T2, 10 for MPI-T1, 6 for PGM-T1, 10 for EST-T2, and 21 for EST-T3. These deviations could be caused by null alleles not detected, alleles subjected to low selection, or imperfect sampling.

The positive yearly and seasonal F_{IS} values (Table 5) indicate a heterozygotic deficiency. However, with the exception of loci MPI-T1 and EST-T2, an excess of heterozygotes was

detected sporadically in certain subpopulations. The global F_{ST} value (0.053; Table 6) was lower than that obtained in the island-continent comparisons (Table 2) and indicates that there is little between-year or seasonal inter-island differentiation. The F_{ST} values are similar for 1997 ($F_{ST} = 0.052$, CI = 0.023-0.102) and 1998 ($F_{ST} = 0.052$, CI = 0.006-0.120).

No private allele was detected with respect to year, but some exist with respect to altitude within a given season. When private alleles were recorded, Nm values suggest that moderate gene flow exists between subpopulations, at a level similar to that found in the island-continent comparisons. The genetic distance between subpopulations varied little: 0.084 ± 0.004 in 1997 and 0.088 ± 0.004 in 1998, with 0.091 ± 0.002 overall. Table 7 shows these distances after subpopulations are grouped according to island and year.

Table 4. Allele frequencies at seven loci in six populations (field samples) of *P. unipuncta* from Azores islands (Santa Maria, São Miguel, and Faial).

	1997			1998		
	Santa Maria	São Miguel	Faial	Santa Maria	São Miguel	Faial
AO-T2						
81	0.056	0.104	0.067	0.069	0.070	0.072
90	0.254	0.297	0.242	0.245	0.220	0.282
100	0.543	0.454	0.467	0.479	0.521	0.509
110	0.147	0.145	0.225	0.207	0.189	0.137
H_o	0.492	0.432	0.389	0.564	0.403	0.424
H_e	0.617	0.675	0.671	0.665	0.640	0.638
F_{IS}	0.175	0.357	0.421	0.152	0.371	0.336
ME-T2						
100	0.851	0.873	0.794	0.786	0.860	0.831
113	0.149	0.127	0.206	0.214	0.140	0.169
H_o	0.259	0.208	0.240	0.294	0.211	0.189
H_e	0.255	0.222	0.328	0.337	0.241	0.281
F_{IS}	-0.079	0.097	0.268	0.127	0.123	0.330
SDH-T2						
100	0.379	0.446	0.333	0.466	0.575	0.375
109	0.550	0.511	0.624	0.445	0.394	0.574
118	0.071	0.043	0.043	0.089	0.030	0.050
H_o	0.213	0.126	0.128	0.178	0.122	0.100
H_e	0.550	0.539	0.499	0.579	0.513	0.528
F_{IS}	0.576	0.776	0.745	0.694	0.763	0.810
MPI-T1						
87	0.104	0.116	0.080	0.062	0.056	0.059
93	0.322	0.312	0.231	0.241	0.232	0.243
100	0.470	0.481	0.439	0.553	0.574	0.599
107	0.096	0.085	0.240	0.126	0.120	0.099
113	0.008	0.005	0.010	0.018	0.017	0.000
H_o	0.330	0.418	0.321	0.382	0.398	0.263
H_e	0.657	0.651	0.692	0.618	0.599	0.570
F_{IS}	0.390	0.411	0.538	0.382	0.335	0.540
PGM-T1						
89	0.116	0.221	0.223	0.237	0.228	0.333
100	0.451	0.418	0.487	0.411	0.461	0.402
107	0.338	0.297	0.210	0.220	0.237	0.207
114	0.095	0.064	0.080	0.131	0.074	0.057
H_o	0.605	0.421	0.520	0.491	0.433	0.316
H_e	0.661	0.685	0.665	0.711	0.675	0.682
F_{IS}	0.041	0.369	0.219	0.309	0.359	0.538
EST-T2						
98	0.257	0.361	0.388	0.285	0.299	0.359
100	0.631	0.545	0.497	0.633	0.652	0.545
105	0.111	0.094	0.115	0.082	0.049	0.096
H_o	0.267	0.268	0.341	0.266	0.277	0.308
H_e	0.524	0.564	0.591	0.513	0.484	0.566
F_{IS}	0.436	0.549	0.423	0.483	0.427	0.456
EST-T3						
97	0.027	0.030	0.065	0.009	0.041	0.019
98	0.275	0.130	0.127	0.198	0.197	0.189
99	0.307	0.283	0.201	0.256	0.262	0.285
100	0.275	0.288	0.272	0.302	0.282	0.208
102	0.096	0.214	0.228	0.192	0.179	0.243
104	0.019	0.046	0.059	0.034	0.036	0.042

Table 4 (concluded).

	1997			1998		
	Santa Maria	São Miguel	Faial	Santa Maria	São Miguel	Faial
106	0.000	0.010	0.049	0.009	0.003	0.015
H_o	0.374	0.353	0.525	0.390	0.256	0.453
H_e	0.745	0.772	0.810	0.768	0.779	0.780
F_{IS}	0.423	0.559	0.353	0.493	0.672	0.420
Global						
H_o	0.363 (0.053)	0.318 (0.045)	0.352 (0.054)	0.367 (0.050)	0.300 (0.044)	0.293 (0.047)
H_e	0.573 (0.060)	0.587 (0.068)	0.608 (0.059)	0.599 (0.054)	0.562 (0.065)	0.578 (0.059)
N	191.1 (4.4)	378.6 (5.4)	162.0 (5.3)	167.4 (8.7)	341.3 (6.1)	275.6 (2.4)
MNA	3.9 (0.5)	4.0 (0.6)	4.0 (0.6)	4.0 (0.6)	4.0 (0.6)	3.9 (0.6)

Note: Values in parentheses show the standard error. H_o , observed heterozygosity; H_e , expected heterozygosity; F_{IS} , Wright's fixation index; N , mean number of individuals studied for each locus; MNA, mean number of alleles per locus.

Table 5. F_{ST} estimates by polymorphic locus and F_{IS} estimates for different *P. unipuncta* populations (field samples) from islands in the Azores (Santa Maria, São Miguel, and Faial), during spring, summer, and autumn in 1997 and 1998.

		F_{ST}							
	F_{IS}	AO-T2	ME-T2	SDH-T2	MPI-T1	PGM-T1	EST-T2	EST-T3	Mean
1997									
Santa Maria									
Spring	0.237	0.010	0.013	0.011	0.017	0.046	0.035	0.046	0.027
Summer	0.325	0.004	0.003	0.007	0.001	0.006	0.005	0.009	0.005
Autumn	0.307	0.011	0.001	0.004	0.025	0.047	0.006	0.068	0.028
Intra-island	0.290	0.011	0.008	0.021	0.018	0.052	0.017	0.049	0.028
São Miguel									
Spring	0.283	0.006	0.013	0.083	0.048	0.050	0.009	0.021	0.035
Summer	0.391	0.051	0.057	0.146	0.048	0.145	0.026	0.032	0.072
Autumn	0.447	0.049	0.008	0.071	0.102	0.053	0.021	0.097	0.063
Intra-island	0.372	0.043	0.027	0.131	0.080	0.105	0.029	0.059	0.071
Faial									
Spring	0.414	0.021	0.009	0.384	0.055	0.043	0.030	0.006	0.075
Summer	0.382	0.060	0.015	0.229	0.041	0.213	0.079	0.037	0.096
Autumn	0.375	0.016	0.003	0.222	0.042	0.092	0.026	0.012	0.059
Intra-island	0.392	0.037	0.010	0.328	0.071	0.154	0.047	0.022	0.097
All islands	0.351	0.042	0.028	0.190	0.060	0.119	0.035	0.050	0.077
1998									
Santa Maria									
Spring	0.464	0.012	0.007	0.078	0.027	0.054	0.015	0.031	0.034
Summer	0.487	0.014	0.000	0.034	0.031	0.010	0.015	0.032	0.022
Autumn	0.333	0.013	0.005	0.015	0.012	0.072	0.006	0.003	0.021
Intra-island	0.434	0.026	0.008	0.243	0.029	0.089	0.015	0.032	0.064
São Miguel									
Spring	0.392	0.117	0.032	0.168	0.073	0.038	0.030	0.042	0.066
Summer	0.297	0.019	0.006	0.036	0.046	0.027	0.063	0.035	0.034
Autumn	0.326	0.020	0.021	0.243	0.036	0.050	0.026	0.058	0.065
Intra-island	0.336	0.070	0.027	0.228	0.113	0.049	0.115	0.067	0.095
Faial									
Spring	0.407	0.019	0.025	0.252	0.031	0.039	0.007	0.039	0.044
Summer	0.466	0.007	0.011	0.190	0.025	0.196	0.024	0.010	0.068
Autumn	0.482	0.022	0.014	0.289	0.010	0.130	0.020	0.004	0.072
Intra-island	0.453	0.018	0.034	0.324	0.042	0.128	0.020	0.023	0.082
All islands	0.405	0.044	0.030	0.309	0.082	0.106	0.065	0.049	0.098

However, some subpopulations (e.g., Faial in 1997, spring, high altitude; São Miguel in 1998, spring, medium altitude) deviated from the overall means. The differences, probably

due to small sample sizes, undoubtedly contributed considerably to the overall heterogeneity.

The observed differences did not indicate that the genetic

Table 6. Estimated F statistics by polymorphic locus for 52 *P. unipuncta* subpopulations (field samples) from islands in the Azores (Santa Maria, São Miguel, and Faial) collected during 1997 and 1998.

Locus	F_{IS}	F_{IT}	F_{ST}
AO-T2	0.318	0.326	0.011
ME-T2	0.158	0.164	0.007
SDH-T2	0.689	0.757	0.219
MPI-T1	0.411	0.430	0.033
PGM-T1	0.297	0.351	0.076
EST-T2	0.468	0.478	0.019
EST-T3	0.507	0.514	0.013
Mean	0.418	0.450	0.053
SD	0.051	0.057	0.027
95% CI ^a	0.322–0.513	0.347–0.561	0.015–0.113

^aInferior and superior limits of confidence intervals at 95%.

differentiation of the subpopulations has a geographic basis. This was confirmed by the results of the four-way ANOVA and the factorial correspondence analyses carried out on the allelic frequencies. In the ANOVA, p values for all factors (island, year, season, and altitude) and interactions were >0.816 . The first three discriminant axes of the factorial correspondence analyses include 62.9% or 62.8% of the variability when the subpopulations are grouped according to altitude or season. These analyses clearly showed that the subpopulations are similar and do not separate out by origin or season. On the other hand, there were no significant differences between males and females collected on São Miguel and Faial (Fisher's exact test, $p > 0.05$).

Discussion

It is clear from all of the different parameters measured in this study that there is no significant and consistent differentiation between the continental and island populations of the armyworm *P. unipuncta*. There are two possible explanations for the low differentiation we obtained using F_{ST} values and private alleles to estimate gene flow. It is possible that because of the migratory nature of this species, the rate of gene flow between sites is high enough to maintain panmictic matings, and thus constitutes only one population. Alternatively, the Azorean populations are isolated, with very limited gene flow from continental populations, but they have not been separated for a sufficiently long period of time to have differentiated genetically. This explanation has been proposed for the diamondback moth, *Plutella xylostella* (L.) (Plutellidae), populations in the Hawaiian islands (Caprio and Tabashnik 1992; Chang et al. 1997), and for the monarch butterfly, *Danaus plexippus* (L.) (Nymphalidae), populations in Trinidad and Tobago (Brower and Boyce 1991).

However, there are significant differences with respect to morphology (Franelemont 1951; J.N. McNeil, V. Vieira, and J. Tavares, unpublished data), reproductive biology, and underlying physiology (McNeil et al. 1996, 2000; Koladich et al. 2002) between North American and Azorean populations. Adults of Azorean origin consistently reach sexual maturity at a younger age following emergence (about 3 vs. 7 days at 25 °C) and despite being of significantly lower body mass,

Table 7. Matrix of Nei's genetic distances for six populations (field samples) of *P. unipuncta* from islands in the Azores, calculated from allele frequencies at seven polymorphic loci.

	1997			1998	
	Santa Maria	São Miguel	Faial	Santa Maria	São Miguel
1997					
São Miguel	0.013	—			
Faial	0.026	0.016	—		
1998					
Santa Maria	0.014	0.010	0.019	—	
São Miguel	0.020	0.013	0.032	0.003	—
Faial	0.022	0.010	0.015	0.010	0.019

produce more eggs than their North American counterparts. These differences strongly reflect the differences normally seen between migrant and nonmigrant populations (Roff and Fairbairn 1991). Furthermore, these differences persist over generations when individuals are reared under identical laboratory conditions, and reciprocal crosses clearly demonstrate a genetic basis for these traits (J.N. McNeil, unpublished data). This suggests that the enzyme systems examined in this study are associated with fundamental processes that are essential for all individuals and thus have not been subjected to the same selection pressures as those associated with body size and reproduction. Interestingly, the *D. plexippus* populations in Trinidad and Tobago are sufficiently morphologically distinct from those in continental North America to be considered a subspecies, *Danaus plexippus megalippe*, yet there are no significant differences in mitochondrial DNA between the populations (Brower and Boyce 1991).

In the absence of differences between continental and island populations, it is not particularly surprising that there are no evident temporal differences either within or between island populations. There is considerable temporal variability in the numbers of adults captured in light and pheromone traps at different altitudes on São Miguel (Anunciada 1983; Tavares 1989; Tavares et al. 1992; Vieira et al. 1994), suggesting that adults move up and down in response to habitat quality, especially in summer, when the pastures at lower altitudes become very dry. On occasion, as on São Miguel and Faial in 1998, very high adult densities have been observed on the sides of buildings, even during daylight. Pheromone-trap catches did not reflect the high densities observed and most of the females examined were virgins. These aggregations could be associated with intra- or inter-island migration during the 2–3 days from emergence to sexual maturation, similar to the scenario described for the African armyworm, *Spodoptera exempta*, with respect to local habitat deterioration (Wilson and Gatehouse 1993).

Based on early descriptions of outbreaks it would appear that *P. unipuncta* has been in the Azores for several centuries (Vieira 1997, 1999). Given general wind patterns, it is possible that actively migrating individuals from either continent were accidentally blown to the Azores. On other hand, passive transport by man cannot be excluded, in which case the more probable source would be Europe. However, it is clear that more specific genetic analyses will be needed to deter-

mine whether the Azorean populations are of European or North American origin.

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