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Population structure in Mediterranean islands and risk of genetic invasion in *Culex pipiens* L. (Diptera: Culicidae)

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The mosquito *Culex pipiens* is subjected to organophosphate treatments in both Corsica and southern Sardinia, but the resistance gene A2-B2, which is currently in a worldwide expansion, has only reached Sardinia. In order to understand this situation, the genetic structure of populations sampled in Sardinia and Corsica was assessed using 15 isozymes. Two loci (*HK1* and *HK2*) were not taken into account because of the possibility of selection. For the other loci, statistical independence was not rejected for all possible pairs, and no deviation from Hardy-Weinberg expectations was apparent. Low but significant genic differentiation was present between Corsica and Sardinia, as well as between northern and southern Sardinia, despite a large number of effective migrants per generation. These results are discussed in the context of the high probability of extinction/recolonization of breeding sites, the flight migration ability of this mosquito, and the pleiotropic cost of insecticide resistances genes. It is concluded that A2-B2 resistance is unlikely to reach Corsica from southern Sardinia, unless accidental human transportation occurs.

ADDITIONAL KEY WORDS:—gene flow – isozymes – genetic differentiation – drift – migration – mosquito.

CONTENTS

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INTRODUCTION

The wide use of organic insecticides to control medically and agriculturally important pest species has been a powerful agent of selection in natural populations of many insect species which have developed various degrees of resistance (Georghiou & Mellon, 1983; Georghiou & Lagunes-Tejeda, 1991). In a few species, such as the mosquito *Culex pipiens*, it is possible to identify each gene conferring resistance to organophosphate (OP) insecticides in single individuals (see e.g. Raymond & Marquine, 1994; Chevillon *et al.*, 1995a).

The dynamics of pesticide resistance genes in natural populations is dependent on the classical evolutionary factors: mutation, migration, drift and selection. For the mosquito *Culex pipiens*, it has been shown that mutation is a limiting factor (Raymond *et al.*, 1991; Raymond, Marquine & Pasteur, 1992). In contrast, the role of migration in the evolution of the resistance genes seems important, as illustrated by some resistance genes which have a unique origin and now have a wide distribution (Raymond *et al.*, 1991, 1992; Qiao & Raymond, 1995). Resistance genes are obviously advantageous in treated areas, so that their frequencies increase with selection. However, in non-treated areas or when control is scarce, resistance genes are at a disadvantage relative to susceptible ones (Raymond *et al.*, 1993; Chevillon *et al.*, 1995b). Therefore, the balance between positive and negative selection pressures, migration and drift, mainly determines the local dynamics of resistance genes.

One of the OP resistance factors, the association of A2 and B2 over-produced esterases (hereafter designated as A2-B2), is expanding world-wide and has reached three continents in less than 30 years (Raymond *et al.*, 1991). In the western Mediterranean, A2-B2 is present in Tunisia (Ben Cheikh & Pasteur, 1993), southern Sardinia (Marchi & Addis, 1990; A. Marchi, unpublished data), continental Italy (Bonning *et al.*, 1991), and southern France (Rivet *et al.*, 1993; Chevillon *et al.*, 1995a). However, it is absent from several OP-treated areas, like Corsica (Raymond & Marquine, 1994) and eastern Spain (Chevillon *et al.*, 1995a).

The fact that A2-B2 has reached Sardinia but not Corsica (a neighbouring island) could be because migrant mosquitoes have little chance of successfully reaching this island. How long A2-B2 will take to reach new areas depends on the levels of gene flow and the selective values in treated and untreated areas. Population structure and indirect estimates of gene flow can be

classically estimated by protein electrophoresis (see e.g. Hartl & Clarke, 1989).

The present work was undertaken to study the population structure of *Culex pipiens* mosquitoes on the islands of Sardinia and Corsica, addressing the following points:

Is gene flow restricted between Sardinia and Corsica? What is the balance between migration and drift in natural populations in Sardinia? Does the pattern of migration within Sardinia explain why A2-B2 is not present in the north (the closest place to reach Corsica)?

MATERIAL AND METHODS

Mosquito samples

Eleven Sardinian breeding sites were sampled as egg-rafts, larvae and pupae from 3 August to 25 November 1988 (Table 1, Fig. 1). They were reared in the laboratory until the adult stage, then deep frozen for further analyses. Two samples from Corsica, collected in June and July 1988 by Raymond & Marquine (1994) were also included.

Electrophoresis

Individual mosquitoes were homogenized in 10% sucrose, 1% Triton X-100 and 0.1% bromophenol blue. Vertical electrophoresis was performed on 6% (or 7.5% for ODH) polyacrylamide slab gels, with the addition of a non-ionic detergent (Photo-Flo, Kodak) at a final concentration of 0.5%. Twelve enzymes were assayed, based on the staining procedures described by Steiner & Joslyn (1979), and Shaw & Prasad (1970), and either using the TBE (Tris borate EDTA) or TC (Tris citrate) buffers described in Munstermann (1979). TBE buffer was used for AAT (aspartate aminotransferase, E. C. 2.6.1.1), ACO (aconitate hydratase, E. C. 4.2.1.3), FUM (fumarase, E. C. 4.2.1.2), GPI (glucose-6-phosphate isomerase, E. C. 5.3.1.9), HK (hexokinase, E. C. 2.7.1.1), ME (malic enzyme, E. C. 1.1.1.40), ODH (octanol dehydrogenase, E. C. 1.1.1.73), PGD (phosphogluconate dehydrogenase, E. C. 1.1.1.44) and PGM (phosphoglucomutase, E. C. 5.4.2.2). TC buffer was used for GPD (glycerol-3-phosphate dehydrogenase, E. C. 1.1.1.8), IDH (isocitrate dehydrogenase, E. C. 1.1.1.42) and MDH (malate dehydrogenase, E. C. 1.1.1.37). This allowed the identification of 15 loci. Allozymes were ranked alphabetically in order of increasing mobility from the origin. At least 26 individuals were analysed from each breeding site.

The nomenclature of alleles used here must not be compared with previous analyses using starch gel electrophoresis (e.g. Chevillon *et al.*, 1995a) since the correspondence had not been tested.

Hardy-Weinberg expectations

Hardy-Weinberg proportions were tested by the exact test proposed by Haldane (1954), using the algorithm of Louis & Dempster (1987) for up to four alleles. For fives alleles or more, an unbiased estimate of the exact

TABLE 1. Allelic frequencies for each locus in each sample (sample size in parenthesis). Alleles are coded by a letter. Exact probabilities or unbiased estimates of type-I error for departure from Hardy-Weinberg proportions are indicated for each locus in the last line (HW). 'All' refers to overall significance using the Fisher's combined probability test. Italics indicates cases not taken into account for overall testing (see Material and Methods for details). Probability values lower than 0.05 are in bold characters.

	Samples													
Loci	LUM	POG	OLB	CHI	CFR	SAN	BAL	VMA	SPR	CAG1	CAG2	GER	TEU	All
Att														
	(26)	(18)	(17)	(18)	(0)	(0)	(7)	(39)	(25)	(30)	(51)	(16)	(27)	
A	0.73	0.56	0.77	0.86			1	0.78	0.82	0.82	0.82	0.97	0.89	
B		0.03	0.03					0.03	0.02	0.10	0.17			
C	0.27	0.41	0.20	0.14				0.19	0.14	0.08	0.01	0.03	0.11	
D									0.02					
Hw	1	1	0.64	1	–	–	–	**0.0030**	1	0.31	**0.033**	–	1	0.25
Aco														
	(0)	(0)	(18)	(18)	(12)	(0)	(15)	(38)	(24)	(33)	(38)	(17)	(31)	
A			1	0.83	0.79		0.80	0.76	0.88	0.77	0.78	0.71	0.95	
B				0.14	0.21		0.17	0.24	0.10	0.20	0.21	0.27	0.05	
C				0.03			0.03		0.02	0.03	0.01	0.03		
Hw	–	–	–	1	1	–	0.46	0.16	0.021	1	0.73	0.66	*1*	0.57
Gpd														
	(41)	(26)	(43)	(28)	(34)	(20)	(35)	(48)	(36)	(43)	(73)	(32)	(38)	
A	1	1	0.98	1	0.97	1	0.99	1	0.96	0.98	0.99	1	0.99	
B			0.02				0.01							
C					0.03				0.04	0.02	0.01		0.01	
Hw	–	–	*0.12*	–	*1*	–	–	–	*1*	***0.12***	*1*	–	–	–
Gpi														
	(53)	(22)	(35)	(55)	(34)	(41)	(35)	(40)	(43)	(64)	(55)	(48)	(45)	
A	0.55	0.64	0.56	0.50	0.43	0.49	0.63	0.51	0.50	0.12	0.24	0.60	0.51	
B	0.42	0.34	0.40	0.46	0.54	0.51	0.36	0.46	0.47	0.80	0.71	0.33	0.47	
C	0.02	0.02	0.03	0.04	0.03				0.01	0.02	0.02	0.01	0.01	
D	0.01						0.01	0.03	0.02	0.05	0.03	0.03	0.01	
E			0.01							0.01		0.02		
Hw	0.53	0.57	**0.0037**	0.70	0.48	0.21	1	0.31	0.77	0.68	0.17	0.22	0.76	0.28
Hk-2														
	(40)	(26)	(29)	(55)	(34)	(21)	(29)	(40)	(43)	(64)	(66)	(42)	(40)	
A	0.48	0.56	0.69	0.77	0.72	0.60	0.69	0.64	0.74	0.58	0.49	0.57	0.71	
B	0.33	0.35	0.29	0.23	0.25	0.40	0.31	0.34	0.23	0.40	0.32	0.42	0.29	
C								0.01	0.03			0.01		
D	0.16	0.06	0.02					0.01		0.01	0.12			
E	0.03	0.03								0.01	0.07			
F					0.03									
Hw	$< 10^{-4}$	0.0076	0.77	0.12	0.41	0.37	**0.027**	0.29	0.53	**0.0054**	$< 10^{-4}$	0.26	0.70	$< 10^{-5}$
Idh-1														
	(38)	(26)	(43)	(29)	(28)	(19)	(32)	(39)	(27)	(53)	(74)	(32)	(33)	
A	0.99	0.98	0.99	0.98	1	0.97	1	1	1	1	1	0.99	0.99	
B			0.01	0.02		0.03						0.01	0.01	
C	0.01	0.02												
Idh-2														
	(41)	(26)	(43)	(39)	(28)	(19)	(35)	(45)	(35)	(56)	(74)	(29)	(51)	
A	0.90	0.87	0.84	0.89	0.84	0.84	0.90	0.88	0.81	0.97	0.90	0.90	0.91	
B	0.07	0.13	0.13	0.10	0.16	0.10	0.10	0.07	0.17	0.03	0.10	0.10	0.07	
C			0.03			0.03		0.04						
D								0.01	0.02				0.01	
E	0.03			0.01		0.03							0.01	
Hw	1	1	0.72	1	1	1	0.28	1	1	*1*	0.49	1	1	1
Mdh-1														
	(15)	(9)	(6)	(8)	(21)	(20)	(20)	(12)	(18)	(19)	(36)	(14)	(18)	
A	1	1	1	1	1	1	1	1	1	0.95	0.97	1	1	
B										0.05	0.03			
Hw	–	–	–	–	–	–	–	–	–	***0.027***	***0.014***	–	–	–
Mdh-2														
	(33)	(25)	(32)	(39)	(30)	(20)	(35)	(48)	(42)	(52)	(72)	(32)	(52)	
A	0.92	0.96	1	0.97	0.96	1	1	1	1	0.97	0.98	0.97	0.98	
B	0.03			0.03	0.02							0.03		

TABLE 1. *(Continued).*

Loci	Samples LUM	POG	OLB	CHI	CFR	SAN	BAL	VMA	SPR	CAG1	CAG2	GER	TEU	All
C		0.02									0.01		0.01	
D	0.05									0.03	0.01		0.01	
E		0.02			0.02									
Hw	*1*	*1*	–	*1*	*1*	–	–	–	–	*1*	*1*	*1*	*1*	–
Me														
	(24)	(16)	(26)	(35)	(32)	(0)	(29)	(36)	(31)	(54)	(50)	(25)	(31)	
A	1	0.97	0.86	0.99	0.97		0.97	0.98	1	0.99	1	0.98	0.92	
B			0.12	0.01	0.03		0.03	0.01		0.01		0.02	0.03	
C			0.02					0.01					0.02	
D													0.03	
E		0.03												
Hw	*1*	*1*	–	*1*	*1*	–	–	–	–	*1*	*1*	*1*	*1*	–
Odh														
	(0)	(0)	(4)	(4)	(7)	(0)	(7)	(19)	(13)	(10)	(27)	(2)	(13)	
A			0.87	1	0.86		0.79	0.95	0.85	1	0.89	1	1	
B			0.13				0.21	0.05	0.15		0.04			
C					0.14						0.07			
Hw	–	–	–	–	*1*	–	*1*	*1*	1	–	1	–	–	1
Pgd														
	(58)	(26)	(49)	(46)	(26)	(48)	(35)	(48)	(42)	(63)	(81)	(48)	(50)	
A	0.47	0.63	0.60	0.77	0.70	0.65	0.59	0.59	0.50	0.29	0.37	0.64	0.57	
B	0.52	0.33	0.39	0.23	0.30	0.32	0.40	0.40	0.50	0.70	0.62	0.35	0.43	
C	0.01	0.02	0.01			0.03	0.01					0.01		
D		0.02						0.01		0.01	0.01			
Hw	0.14	0.65	0.62	0.41	0.36	0.65	0.01	0.53	0.54	**0.0041**	0.91	0.58	0.77	0.12
Pgm														
	(27)	(20)	(37)	(53)	(30)	(27)	(34)	(40)	(43)	(64)	(65)	(43)	(40)	
A	0.92	0.87	0.82	0.83	0.80	0.68	0.77	0.84	0.81	0.84	0.83	0.86	0.87	
B	0.06	0.03	0.10	0.10	0.08	0.04	0.19	0.08	0.09	0.04	0.01	0.02	0.05	
C			0.01	0.01					0.01	0.01	0.04			
D		0.07	0.07	0.05	0.08	0.28	0.03	0.07	0.09	0.11	0.11	0.12	0.08	
E				0.01							0.01			
F							0.01							
G	0.02	0.03			0.04			0.01						
Hw	1	0.25	0.53	**0.032**	0.58	0.22	1	0.28	0.70	**0.023**	0.052	1	1	0.20
Fum														
	(40)	(24)	(38)	(53)	(21)	(40)	(20)	(40)	(43)	(52)	(75)	(46)	(52)	
A	0.95	0.98	0.91	0.97	0.98	1	1	0.99	1	0.96	0.96	0.99	1	
B	0.05		0.08	0.02	0.02					0.03	0.03	0.01		
C		0.02						0.01			0.01			
D			0.01	0.01						0.01				
Hw	1	–	1	1	–	–	–	–	–	1	0.10	–	–	0.91
All														
Hw	**0.013**	0.26	0.49	0.63	0.89	0.53	0.20	**0.047**	0.78	**0.021**	**0.0012**	0.80	1	**0.030**

probability was computed using the Markov chain method of Guo & Thompson (1992). The Markov chain was set to at least 800 000 steps, and 5000 steps of dememorization (see Guo & Thompson, 1992 for details) in order to obtain standard error estimates below 0.005. The GENEPOP software (version 1.2) was used for these computations (Raymond & Rousset, 1995b). The overall significance of multiple tests for each locus or for each sample was estimated by Fisher's combined probablity test (Fisher, 1970). This method assumes that P values for each of the several independent tests follow a uniform distribution between 0 and 1, and it is therefore slightly inaccurate (Yates, 1955). However, when sample size or allele numbers are high, the continuous approximation can be made because the number of

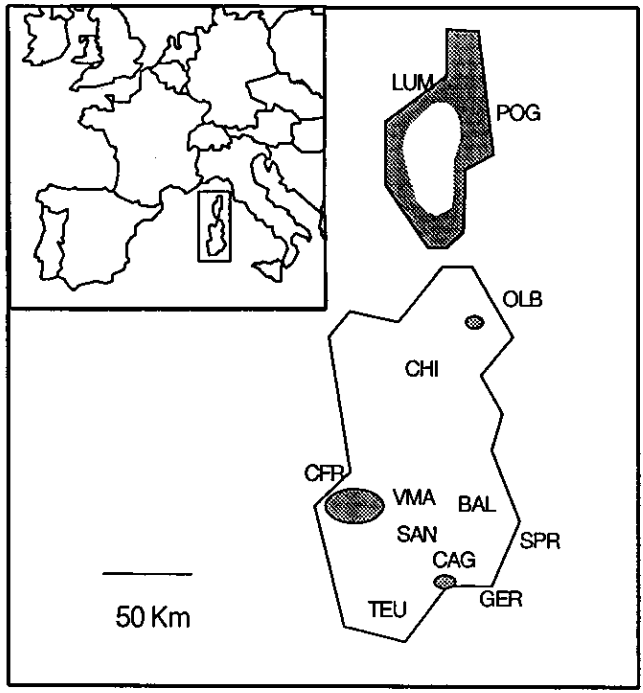

Figure 1. Samples collected in 1988. Treated areas are indicated in grey. From the north to the south, the sampling codes correspond to: two Corsican sites, LUM (Lumio, collected 23/06 from a ditch) and POG (Santa Maria di Poggio, 05/07, sewage station); eleven Sardinian sites, OLB (Olbia, 28/10, ground pool), CHI (Chilivani, 27/10, stream), CFR (Arborea, 26/10, drain), SAN (Furtei, 8/10, stream), BAL (Ballao, 4/11, river), VMA (Villamalla, 8/10, canal), SPR (San Priamo, 4/11, seepage), CAG1 (Caggliari, 15/11, tank), CAG2 (Caggliari, 14/10, canal), GER (Geremeas, 3/08, stream) and TEU (Teleuda, 27/08, well).

distinct genotypic tables (hence of probability values) considered becomes large (e.g. 7287 for locus *Hk-2* in sample LUM). Whenever this number was minimum, i.e. two possible tables as for *Mdh-2* in sample POG (AA = 50, AB = 1, AC = 1) or for *Mdh-1* in CAG2 (AA = 35, BB = 1), the corresponding probability was not included in overall testing.

F_{is} values were computed according to Weir & Cockerham (1984) using GENEPOP (version 1.2). Heterozygote deficits or excesses were tested using an exact test procedure. The test used is similar to the exact test of Haldane (1954), but the rejection region is built only with tables with a number of heterozygotes which is the same or lower for deficit, or the same or higher for excess (the N_{Het} test of Rousset & Raymond, 1995).

Differentiation among populations

Genetic differentiation between populations, or groups of populations, was tested using Fisher's exact test on $R \times C$ contingency table for each locus. An unbiased estimate of the exact probability was obtained with a Markov chain method (Raymond & Rousset, 1995a), using the GENEPOP (version 1.2) software (Raymond & Rousset, 1995b). For all tests, the Markov chain was set to at least 200 000 steps, and 5000 steps of dememorization (see

Raymond & Rousset, 1995a for details). The overall significance of multiple tests for each locus was estimated by Fisher's combined probability test (Fisher, 1970). F_{st} parameter was computed according to Weir & Cockerham (1984).

Linkage disequilibrium and D statistics

For each population, the global disequilibrium between pairs of loci was estimated using the common correlation coefficient (Weir, 1990: 111–113), and tested using Fisher test on $R \times C$ contingency table (see above). For each pair of loci, a global measure was obtained by averaging the correlation coefficients across populations, and a global test was obtained by combining (Fisher's method) the probability for each population.

Either genetic drift or directional selection pressures acting on pairs of loci can create a linkage disequilibrium between two alleles i and j. To discriminate between the two situations, Ohta (1982) proposed to decompose the gametic associations observed on the whole data set (D_{it}) in four indices which estimate the parts created within (D_{is} and D'_{is}) and between populations (D_{st} and D'_{st}). The discrimination is based on the comparisons of D_{is} and D_{st} values, on the one hand, and of D'_{is} and D'_{st} values, on the other. These indices were computed using the LINKDOS program (Garnier-Gere & Dillman, 1992).

Effective migrants and isolation by distance

The number of effective migrants (Nm) was estimated by two methods. First, it was estimated from the F statistics of each locus according to the equation $Nm = (1/F_{st} - 1)/4$ (Wright 1969). This formula assumes the neutrality of the polymorphic genes and an island model of migration (see e.g. Hartl & Clark 1989). Second, Nm was estimated by the method of private alleles described by Slatkin (1985). Isolation by distance was analysed as described by Slatkin (1993). Geographical distances between samples were the shortest measurements on a map, except when putative barriers to migration were present (e.g. Corsican mountains); in this case, distance was measured along valleys.

Multiple tests

The significance level for each test was adjusted to take into account the other tests using the sequential Bonferroni method as described by Holm (1979).

RESULTS

Polymorphism recorded

A total of 702 mosquitoes were analysed for the 15 loci. In some cases, genotypes were not recorded because of technical reasons or because some enzymes were not tested in a few samples (e.g. *Odh*). Overall, a total of

TABLE 2. Analysis of departure from Hardy-Weinberg proportions for locus HK2. Type-I error probabilities of rejecting Hardy-Weinberg proportions for all possible reasons (General) or for only heterozygote excess or deficit. F_{is} according to Weir & Cockerham (1984) is indicated. Total refers to Fisher's combined probability test across samples. Bold characters indicate significant ($P < 0.05$) values.

		Rejection region for HW test		
Samples:	F_{is}	General	Heterozygote Deficit	Excess
LUM	+0.27	**0.00001**	**0.012**	1
POG	+0.13	**0.0076**	0.26	0.86
OLB	+0.073	0.77	0.45	0.77
CHI	+0.23	0.12	0.091	0.98
CFR	−0.11	0.41	0.81	0.30
SAN	−0.26	0.37	0.95	0.23
BAL	−0.44	**0.024**	1	**0.020**
VMA	+0.23	0.29	0.084	0.96
SPR	−0.18	0.53	0.95	0.17
CAG1	−0.12	**0.0054**	0.87	0.19
CAG2	+0.25	$<$**10**$^{-6}$	**0.003**	1
GER	−0.18	0.26	0.91	0.14
TEU	+0.097	0.70	0.40	0.85
Total	+0.008	$<$**10**$^{-6}$	0.067	0.50

6018 genotypes were available for analyses (Table 2). The number of alleles detected varied between two (*Mdh*) and seven (*Pgm*). For four loci (*Gpd, Idh-1, Mdh-1*, and *Fum*), the frequency of the most common allele was greater than 95% in all samples.

Statistical independence among loci

Genotypic association between each pair of loci was measured by the common correlation coefficient and was tested with an exact test. Independence of *Hk-1* and *Hk-2* was rejected in each population ($P < 10^{-5}$) and for the whole data set (Common correlation: 0.963, $P < 10^{-5}$). The close linkage between these two loci (Tabachnick & Howard, 1982) partially explains this statistical association. Among all the other pairs of loci, non-independence was only rejected for the pair *Aco-Fum* (Common correlation: 0.233, $P = 0.027$). This non-independence does not remain significant at the 0.05 level when multiple testing was taken into account.

The analysis of the Ohta indices is consistent with the hypothesis that the observed gametic associations were only due to the action of drift except for the pair *Hk1-Hk2* which is possibly subjected to selection. Due to the statistical dependence between *Hk-1* and *Hk-2*, only the most polymorphic one (*Hk2*) will be considered further.

Hardy-Weinberg proportions

The hypothesis of Hardy-Weinberg equilibrium was rejected ($P < 0.05$) in 16 out of 116 tests (Table 2). When testing across samples for each locus,

TABLE 3. Allelic differentiation between sets of populations: probability of Fisher exact test on contingency table (SE in parenthesis). Bold characters indicate significant ($P < 0.05$) values. Total refers to the Fisher's combined probability test.

Locus	Populations	Intra islands		Inter islands
	All	Sardinia	Corsica	
Aat	**<0.001**	**<0.001**	0.13	**<0.001**
	(<0.001)	(<0.001)	(0.0033)	(<0.001)
Aco	**0.0015**	**0.0027**	–	–
	(0.00048)	(0.0010)	–	–
Gpd	0.14	0.16	–	0.60
	(0.0063)	(0.0061)		(0.0023)
Gpi	**<0.001**	**<0.001**	0.73	**0.018**
	(<0.001)	(<0.001)	(0.0040)	(0.0014)
Idh-1	0.17	0.20	1.00	**0.023**
	(0.0070)	(0.0042)	(0.00)	(0.00087)
Idh-2	**0.0035**	**0.0039**	0.35	0.34
	(0.0017)	(0.0014)	(0.0029)	(0.0072)
Mdh-1	0.63	0.58	–	1.00
	(0.0058)	(0.0055)		(0.00)
Mdh-2	**0.011**	0.11	0.12	**0.011**
	(0.0023)	(0.0079)	(0.0024)	(0.0011)
Me	**0.0011**	**<0.001**	0.40	0.14
	(0.00073)	(<0.001)	(0.0014)	(0.0044)
Odh	0.068	0.063	–	–
	(0.0041)	(0.0042)		
Pgd	**<0.001**	**<0.001**	**0.020**	0.46
	(<0.001)	(<0.001)	(0.0019)	0.0099)
Pgm	**<0.001**	**<0.001**	0.17	0.060
	(<0.001)	(<0.001)	(0.0023)	(0.0037)
Fum	**0.023**	**0.032**	0.12	0.38
	(0.0031)	(0.0035)	(0.0017)	(0.0047)
Total	**<0.00001**	**<0.00001**	0.055	**<0.00001**

Hardy-Weinberg equilibria were only rejected for *Hk2* ($P < 10^{-5}$). There was no systematic heterozygote excess or deficit (Table 3), which suggests that this locus is directly or indirectly under non-heterotic selection. *Hk2* will not be further considered. When testing across loci (excluding *Hk-2*) within each sample, Hardy-Weinberg equilibria were only rejected for sample VMA ($P = 0.045$). However, this was not significant when multiple tests (over 13 samples) were taken into account. In addition, the overall test for all loci (excluding *Hk-2*) in all populations indicated that Hardy-Weinberg equilibria were not rejected ($P > 0.8$).

Genetic differentiation

The overall differentiation among populations was highly significant ($P < 10^{-5}$) and corresponded to a F_{st} value of 0.048 (Tables 4 and 5). In order to investigate the differentiation hierarchically, several groupings were considered. First, the inter-island differentiation, tested by the allelic composition of Corsican vs. Sardinian populations, was significant ($P < 10^{-5}$) with a corresponding F_{st} value of 0.028 (Tables 4 and 5). Second, intra-island differentiation was tested within each island. A significant differentiation

TABLE 4. Values of the theta parameter of Weir & Cockerham (1984) for each locus for different population sub-divisions. 'All' refers to the multi-locus estimate. Bold characters indicate significant allelic differentiation.

Locus	None	Subdivision Intra islands Sardinia	Corsica	Inter islands
Aat	**0.063**	**0.030**	0.034	**0.11**
Aco	**0.029**	**0.028**	–	–
Gpd	−0.00091	−0.0022	–	0.0016
Gpi	**0.082**	**0.087**	−0.0029	**0.028**
Idh-1	−0.0018	−0.0012	−0.0152	0.0081
Idh-2	**0.0072**	**0.0094**	0.00	−0.0036
Mdh-1	−0.0286	−0.026	–	−0.018
Mdh-2	**0.0092**	0.0032	0.0047	**0.030**
Me	**0.029**	**0.031**	0.013	0.0024
Odh	0.033	0.033	–	–
Pgd	**0.070**	**0.077**	**0.049**	−0.0031
Pgm	**0.014**	**0.012**	−0.003	0.014
Fum	**0.014**	**0.016**	0.0087	0.0001
All	**0.048**	**0.046**	0.019	**0.028**

TABLE 5. Number of migrants for different population subdivisions, estimated from both Wright's F_{st} and Slatkin's private allele methods.

Subdivision	Nm estimated by F_{st}	Private allele
Overall	5.0	5.4
Inter islands	8.7	7.0
Intra islands:		
Corsica	13	4.0
Sardinia	5.2	6.9
Intra Sardinia:		
South vs North	14	7.3

was found ($P < 10^{-5}$) for Sardinia but not for Corsica ($P > 0.05$), with associated F_{st} values of 0.046 and 0.014, respectively. Third, possible intra-Sardinia differentiation due to (1) insecticidal control or (2) geographical distances was investigated. The group of intensively treated populations (OLB, CAG1, CAG2, VMA and CFR) was significantly differentiated ($P < 10^{-5}$; $F_{st} = 0.025$) from the group composed of the other populations. Similarly, the group represented by the samples from the north (OLB and CHI) had an allelic composition significantly ($P < 10^{-5}$) different ($F_{st} = 0.018$) from that of the group composed of the other samples. Several other groupings taking into account various ecological factors gave similar results (details not shown).

Number of migrants and isolation by distance

The number of migrants between populations or groups of populations was equal or above four for all subdivisions considered (Table 5). Wright's

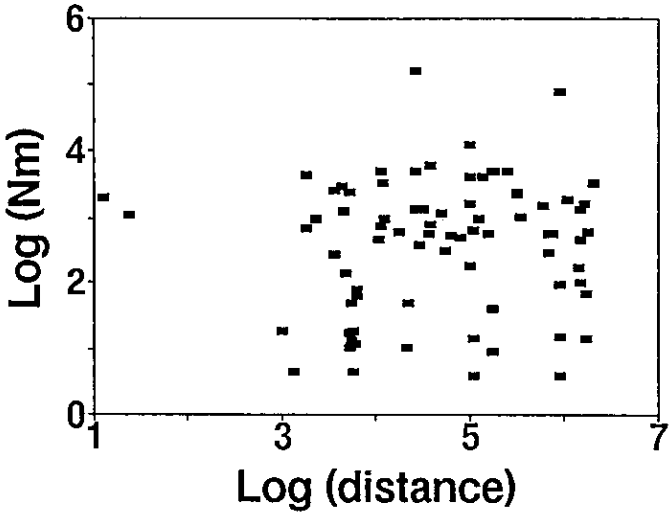

Figure 2. Isolation by distance using Slatkin (1993) method.

F_{st} or Slatkin's private allele methods of estimating the number of migrants, provided similar values (Table 5).

When the number of migrants was plotted against geographical distances on a log scale (Slatkin, 1993), no isolation by distance was detected on the whole data set (Fig. 2), inside Sardinia or within the various Sardinian subdivisions considered (data not shown).

DISCUSSION

Is gene flow restricted between Sardinia and Corsica?

The hierarchical analysis of differentiation revealed that Sardinian and Corsican populations have significantly diverged ($F_{st} = 0.028$, $P < 10^{-5}$). This significant differentiation remains when northern Sardinia and Corsica are compared. Thus, despite the short distance between the two islands (about 12 km), differences in gene frequencies are apparent. As a comparison, for a mosquito of the same species complex, no genic differentiation was found between Tahiti and Moorea, two islands of the Society archipelago (south Pacific) which are 17 km apart (Pasteur *et al.*, 1995). Transport by human activities (Highton & Van Someren, 1970) is certainly responsible for these situations: it is much lower between Corsica and southern Sardinia (which belong to distinct countries, and are not connected by air traffic) than between Tahiti and Moorea which are connected by more than 14 500 flights each year (Pasteur *et al.*, 1995).

However, the high estimate of effective migrants between Corsica and Sardinia ($Nm > 7$) indicates that sufficient migration exists to overcome genetic drift, and thus to prevent further divergence.

What is the balance between migration and drift in natural populations of this mosquito?

The Sardinian situation is characterized by a low but significant genic differentiation ($F_{st} = 0.046$), associated with high estimates of gene flow

(Nm > 4). If the detected polymorphism is neutral, then drift should have a large effect in order to explain the existence of a non-zero F_{st} value. Ecological studies indicate that the breeding sites of this mosquito are generally ephemeral (e.g. Riley & Johannsen, 1938), which suggests that extinction and colonization events are frequent. This stochastic phenomenon could enhance F_{st} values (Wade & McCauley, 1988; Whitlock & McCauley, 1990).

It could be proposed that the differentiation due to drift during each colonization event is limited by extensive migration across large distances. This model, which needs to be further tested by specific field experiments and theoretical work, is supported by the following points.

(1) The two methods used to estimate gene flow have provided a high number of migrants between Sardinian populations, as have other studies carried out in southern Europe and in America, for populations sampled within a few hundred kilometres (Chevillon *et al.*, 1995a; Cheng *et al.*, 1982).

(2) No isolation by distance was detected on the whole island or for the various subdivisions. This is also consistent with other studies on the same species. For example, in southern France, no isolation by distance could be detected over 800 km (Chevillon *et al.*, 1995a).

(3) The genic differentiation could not be unambiguously attributed to one environmental factor. Geographical distribution seems to be involved, as northern and southern samples displayed significant allelic differences. OP insecticide control seems also to influence gene frequencies, as treated and non-treated populations were significantly differentiated. But these factors cannot be tested independently and the presence of confounding variables cannot be ruled out (arbitrary grouping also gave significant differentiation; data not shown). No general trend is apparent and the differentiation observed is congruent with random effects across samples.

Does the pattern of migration within Sardinia explain why A2-B2 is not present in northern Sardinia?

An estimate of about 10 effective migrants per generation between southern and northern Sardinia does not necessarily mean that an average of 10 mosquitoes physically migrate between the two parts of the island. It simply indicates that an estimated mean of 20 genes from the first place, *or their copies*, are arriving each generation in the second place.

Migrations over several hundred kilometres, such as in those described in *C. tritaeniorynchus* (Ming *et al.*, 1993), are unknown in *C. pipiens*. Ecological studies indicate that *C. pipiens* can travel several kilometres, up to 11 km in 10 days (Reisen, Milby & Meyer, 1992). It is therefore probable that several generations are needed for a neutral gene, migrating from southern Sardinia to have copies of itself in northern Sardinia. For a selected gene like A2-B2, the number of generations is certainly shorter if OP insecticide control is widespread because it will rapidly increase in frequency in each new colonized site, and thus increase its chances of being in a future colonizing mosquito.

Resistance genes from the treated area in southern France have invaded

another French treated area situated 300 km away between Lyon and Chambery (Rivet *et al.*, 1993; Chevillon *et al.*, 1995a). It appears that A2-B2 has followed the Rhone valley in migrating from Marseille airport (or harbour) to Lyon/Chambery (Rivet *et al.*, 1993). The migration of insecticide resistance genes is facilitated because OP insecticide control is common along the Rhone valley (Chevillon *et al.*, 1995a). In contrast, the French Atlantic treated area, north of Bordeaux, is separated from the nearest treated area by more than 450 km and is still free of resistance genes (Rivet *et al.*, 1993; unpublished data). This is probably due to the extent of the distance between these areas, and also to the absence of other treated regions in between.

There is other indirect evidence of restricted migration of resistance genes despite the absence of obvious barriers to migration (such as mountains and sea). In southern France, resistance gene frequencies decrease when populations are sampled further away from the treated area (Pasteur *et al.*, 1981; unpublished data). It seems that, 150 km away from the treated area, resistance gene frequencies fall below a detectable level, and the observed cline is the result of a large migration and a high fitness cost (Chevillon *et al.*, in prep.). In *Culex pipiens*, with respect to costly resistance genes and on the condition that no barrier to migration exists, 150 km can be considered as the minimum distance required to maintain a relatively independent evolution of resistance within two treated areas.

If there is little insecticide usage, as is the case in Sardinia (except in the south), a resistance gene will often be at a disadvantage due to its pleiotropic cost. The greater the disadvantage, the lower the probability that a migrant mosquito is resistant. Pleiotropic cost can be particularly severe in natural populations, and declines in fitness of up to 60% have been described for OP resistance genes (McKenzie, 1994; Chevillon *et al.*, 1995b). A pleiotropic cost associated with A2-B2 probably exists, as indicated by a significant decrease in its frequency following the interruption of OP insecticide control in Lucca (Severini *et al.*, 1993).

CONCLUSION

The invasion by A2-B2 of northern Sardinia (and furthermore to Corsica) from southern Sardinia is apparently precluded by the large distance separating the two treated areas (about 200 km), which requires several generations of active migration in order to be crossed. Meanwhile, the counter selection against resistance individuals will operate in such untreated areas, rapidly reducing its chance to be in a migrating mosquito of the next generation.

The recent world-wide spread of A2-B2 is probably due to passive transportation from one treated area to another since recently colonized areas are always near harbours or airports (Raymond *et al.*, 1987, 1991; Rivet *et al.*, 1993). *Culex pipiens* mosquitoes have been repeatedly found on planes (e.g. Highton & van Someren, 1970; Carneiro de Mendonça & Cerqueira, 1947) or boats (e.g. Van Dine, 1904; Andreadis, 1988), and a *C. pipiens* with A2-B2 has been caught from a plane arriving in London from Africa (Curtis & White, 1984). Thus, future passive migration from southern Sardinia to Corsica cannot be excluded if sanitary regulations are not enforced.

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