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Genetic structure of the palourde *Ruditapes decussatus* L. in the Mediterranean 1994

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Keywords: Bivalve, palourde, clam, *Ruditapes decussatus*, allozymes, population genetics, F-statistics, gene flow, Mediterranean

1-3 photographs

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Genetic structure of the palourde *Ruditapes decussatus* L. in the Mediterranean

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Abstract

The genetic structure of the bivalve *Ruditapes decussatus* was studied at several spatial scales: over the whole Mediterranean, among lagoons in the same region (Languedoc), among sites in the same lagoon (Thau), and at a temporal scale, among year cohorts at a given site, in order to estimate levels and patterns of gene flow among populations. Genetic divergence at each level was expressed by means of Cockerham's coancestry coefficient (θ), estimated from genotypic data at 6–9 polymorphic enzyme loci. Significant divergence found between regions at the scale of the whole Mediterranean ($\theta \pm SD = 0.0149 \pm 0.0072$) indicated that gene flow between regions is limited. There was no evidence for heterogeneity within a region ($\theta = 0.0015 \pm 0.0011$), nor within a lagoon ($\theta = 0.0006 \pm 0.0005$). However, the analysis of divergence between year cohorts at a given site revealed discrepancies among loci in single-locus θ values, indicating, perhaps, short-term selection or genetic drift.

2.3.1 Introduction

The planktonic larval stage has been interpreted as a means of dispersal and of maintenance of the homogeneity of the gene pool of a species over its distribution area (e.g. Scheltema, 1971). However, high potential dispersal does not necessarily imply gene flow. Genetic surveys have shown that gene flow between populations of marine invertebrates with a planktonic larval stage may be limited. Significant genetic heterogeneities among habitats (Koehn, Turano and Mitton, 1973), steep clines (Tracey *et al.*, 1975; Koehn *et al.*, 1984; McDonald and Siebenaller, 1989), and the maintenance of hybrid populations over generations (Skibinski, Beardmore and Cross, 1983) in spite of larval dispersal, have been reported. At the microgeographic level, patchiness in allele frequencies which may be due to drift or selection in swarms of larvae or early spat that were recruited by pulses has also been reported (Johnson and Black, 1982, 1984a,b; Watts, Johnson and Black, 1990). Thus, differentiation between local populations might be the rule rather than the exception (Burton, 1983; Hedgecock, 1986) and one is left with an apparently complex, multiple-scale array of population genetic patterns in marine invertebrates.

Here we report the results of a study of the genetic structure of *Ruditapes decussatus*, a bivalve of Mediterranean lagoons which has a planktotrophic, planktonic larval stage. The analysis was performed at several scales, from microgeographic to zoogeographic. Our objective was to infer levels of gene flow among populations or subpopulations from the estimation and comparison of genetic heterogeneities at each scale (i.e. temporal, microgeographic, regional and over the whole Mediterranean) in order to determine the boundaries of a population in *R. decussatus*. This should enhance our understanding of differentiation and speciation in marine invertebrates, a topic still subject to some debate (e.g. Palumbi, 1992; section 1.1). The present study includes a re-analysis of genotypic data from earlier surveys (Worms and Pasteur, 1982; Jarne, Berrebi and Guelorget, 1988), and extends the data of Borsa (1990) and Borsa, Zainuri and Delay (1991).

2.3.2 Materials and methods

(a) Zoogeographic and biological features of *R. decussatus*

The area of distribution of *R. decussatus* extends from the North Sea to the coasts of Senegal and along the coasts of the whole Mediterranean (Black Sea excepted), reaching as far east as the Red Sea (Mars, 1966; Fischer-Piette and Métivier, 1971). Its preferential habitats in the Mediterranean are coastal lagoons (Mars, 1966) and shallow-water, protected bays (H. Massé and O. Guelorget, personal communication). These habitats occur discretely all around the Mediterranean and are subject to anoxic crises which may cause local extinctions (Amanieu *et al.*, 1975; Borsa, Jousselein and Delay, 1992).

The duration of the planktonic larval stage of *R. decussatus* is 8–10 days at 24–25 °C (P. Borsa and A. Diter, unpublished data from hatchery experiments involving *R. decussatus* from Thau). This is approximately the temperature at which reproduction is known to occur in peri-Mediterranean populations of the species (Vilela, 1949; Gallois, 1977; Breber, 1980; Borsa and Millet, 1992). Since such temperatures occur in summer along the coasts of the Mediterranean (Robinson, 1973), and with coastal currents of a few centimetres per second, the distance that a larva is potentially able to cross ranges from about 10 to 100 km.

(b) Collection of samples

Surveys of genetic variation at electrophoretic loci of some of the samples have been reported earlier as indicated below. The samples studied here originated from the lagoons of Prévost, France (Worms and Pasteur, 1982; D. Monti and S. Salvidio, unpublished data), Ebro (Spain), Faro (South Portugal), Bizerte (Tunisia), Temsah (Egypt) (Jarne, Berrebi and Guelorget, 1988) and Thau, France (D. Monti and S. Salvidio, unpublished data; Borsa, 1990; Borsa, Zainuri and Delay, 1991; Borsa, Jousselein and Delay, 1992; P. Borsa, unpublished data). The list of samples, with sampling locations and dates, sample sizes and loci investigated, is presented in Table 2.3.1.

(c) Allozyme electrophoresis

Electrophoretic analyses of all samples, including those of earlier surveys, were conducted in the same laboratory, using the same protocols (except where noted below), i.e. media, buffers, trays, running conditions and staining procedures (Pasteur *et al.*, 1987).

When enzymes were encoded by genes at several presumptive loci, these were numbered consecutively, from slow to fast as in Borsa and Thiriout-Quévieux (1990). Note that the loci were numbered in the opposite direction by Worms and Pasteur (1982). Ten loci were scored in all: *Aat-1* (encoding the slower aspartate aminotransferase; EC 2.6.1.1), *Est-D* (4-methylumbelliferyl-specific esterase; EC 3.1.1.1), *Glo* (glyoxalase; EC 4.4.1.5), *Idh-1* and *Idh-2* (respectively, slower and faster isocitrate dehydrogenases; EC 1.1.1.42), *Lap-1* (slower leucine aminopeptidase; EC 3.4.11.1), *Mdh-1* and *Mdh-2* (respectively, slower and faster malate dehydrogenases; EC 1.1.1.37), *Me-1* (slower malic enzyme; EC 1.1.1.40) and *Pgm-1* (slower phosphoglucosmutase; EC 2.7.5.1).

The identities of electromorphs from different samples were ascertained by side-by-side runs (for all enzymes except LAP and EST-D). The buffer systems used for LAP and EST-D enzymes were not the same for all samples. For both enzymes, the discontinuous Tris-citrate-borate LiOH, pH 7.0 (TCBL) system was used for all Thau samples except TA-TD (Table 2.3.1), whereas for all other samples continuous Tris-maleate, pH 7.4, was used for EST-D extracts, and continuous Tris-EDTA-borate, pH 8.6, for LAP extracts. The use of

Table 2.3.1 List of samples of *Ruditapes decussatus* referred to in the present study, with sampling locality, sampling date, sample size (*N*) and loci scored per sample (*) that were used for the comparisons between populations

Name	Locality	Date	N	Aat-1	Est-D	Glo	Idh-1	Idh-2	Lap-1	Mdh-1	Mdh-2	Me-1	Pgm-1
	Prévoist (43°30'N, 3°53'E)												
P1 ^a	Prévoist	1982	47	*	*	*			*	*	*	*	*
P2 ^b	Prévoist	07 84	64		*	*			*	*	*		*
P3 ^b	Prévoist	08 84	52		*	*			*	*	*		*
P4 ^b	Prévoist	11 84	64		*	*	*		*	*	*		*
	Thau (43°20'N, 3°40'E)												
T1 ^c	Le Barrou	12 87	105	*	*	*	*	*	*	*			*
T2	Le Barrou	02 88	156		*	*	*	*	*	*			*
T3	Le Barrou	07 88	81		*	*	*	*	*	*			*
T4	Le Barrou	09 88	97		*	*	*	*	*	*			*
T5	Le Barrou	12 88	92		*	*	*	*	*	*			*
T6	Le Barrou	06 89	96		*	*	*	*	*	*			*
T7	Le Barrou	08 89	103		*	*	*	*	*	*			*
T8 ^c	Balaruc-Port	12 87	92	*	*	*	*	*	*	*			*
T9 ^c	Balaruc-Z.I.	02 89	92		*	*	*	*	*	*			*
T9bis	Balaruc-Z.I.	02 89	119		*	*	*	*	*	*			*
T10 ^{c,d}	Balaruc-Z.I.	06 87	27		*	*	*	*	*	*			*
T11 ^d	Balaruc	07 87	60		*	*	*	*	*	*			*
T12 ^c	Le Mourre	08 87	101	*	*	*	*	*	*	*			*
T13 ^c	Balaruc-Port	07 87	30		*	*	*	*	*	*			*
T14	Le Mourre	07 87	59		*	*	*	*	*	*			*
T15 ^c	Bouzigues	05 87	117	*	*	*	*	*	*	*	*	*	*
T16 ^{c,d}	Mourre-Blanc près Mèze	07 87	56		*	*	*	*	*	*			*
T17 ^c	Lido	08 87	74		*	*	*	*	*	*			*
T18	Le Barrou	11 89	84		*	*	*	*	*	*			*

Table 2.3.1 (cont) List of samples of *Ruditapes decussatus* referred to in the present study, with sampling locality, sampling date, sample size (*N*) and loci scored per sample (*) that were used for the comparisons between populations

Name	Locality	Date	N	Aat-1	Est-D	Glo	Idh-1	Idh-2	Lap-1	Mdh-1	Mdh-2	Me-1	Pgm-1
T19	Plan de Mèze	02 88	28		*	*	*	*	*	*	*		*
T20	Balaruc-Port	09 88	62		*	*	*	*	*	*			*
T21	Lido	07 87	20		*	*	*	*	*	*	*		*
TA ^b	Conque de Mèze	07 84	39		*	*			*	*			*
TB ^b	Conque de Mèze	07 84	39		*	*			*	*			*
TC ^b	Conque de Mèze	08 84	18		*	*			*	*			*
TD ^b	Conque de Mèze	11 84	61		*	*			*	*			*
Other localities													
	Faro ^c , Portugal (37°30'N, 8°00'W)	04 85	27		*	*	*					*	*
	Temsah ^c , Egypt (30°40'N, 32°15'E)	03 85	28		*	*	*					*	*
	Bizerte ^c Tunisia, (37°15'N, 9°49'E)	03 86	113		*	*	*					*	*
	Ebro ^c , Spain (40°40'N, 0°55'E)	03 86	32		*	*	*					*	*

^aSee Worms and Pasteur (1982).

^bSamples analysed by D. Monti and S. Salvidio.

^cSee Borsa, Zainuri and Delay (1991).

^dSee Borsa, Jousselin and Delay (1992).

^eSee Jarne, Berrebi and Guelorget (1988).

TCBL improved the resolution of patterns at the *LAP-1* and *EST-D* loci, but did not modify the migration ranks of the electromorphs. Because of variability in resolution among samples, some poolings of *EST-D* and *LAP-1* electromorphs were necessary (Table 2.3.2). For all other enzymes, buffer systems were the same as those used by Worms and Pasteur (1982) and Borsa and Thiriot-Quévieux (1990).

(d) *Coancestry coefficient*

The coancestry coefficient θ , proposed by Cockerham (1969), equals F_{ST} in S. Wright's notation. This parameter has the properties of being affected neither by the numbers of alleles observed per locus, the numbers of individuals sampled per population, nor by the number of populations (Weir and Cockerham, 1984), thus allowing comparisons among surveys with different sample structures, which is the case here.

Multiallelic monolocus, and multilocus, θ values were estimated respectively as weighted averages over alleles and over loci (Equation 10 of Weir and Cockerham (1984)). Under the hypothesis, derived from the neutral model, that each locus can be used separately to estimate θ , its variance was estimated from the set of all monolocus estimates using the jackknife procedure as advocated by Weir and Cockerham (1984). Each multilocus θ was then compared to zero by a one-tailed *t*-test (Sokal and Rohlf, 1969).

(e) *Genetic distance*

A distance measure based on the coancestry coefficient, $D = -\ln(1 - \theta)$, of Reynolds, Weir and Cockerham (1983) was estimated. This distance is designed to measure divergence between populations under the model of genetic drift, so mutation is not included, unlike with other measures of genetic distance usually employed (Weir, 1990). We assume this to be an appropriate use in the case of *R. decussatus* populations around the Mediterranean, since no private allele was present in any population at any locus (see Table 2.32 for Thau, and Jarne, Berrebi and Guelorget (1988) for other peri-Mediterranean populations).

Table 2.3.2 Electromorph frequencies at 10 enzyme loci in *R. decussatus* from Thau (French Mediterranean). Electromorph mobilities are relative to the mobility of the commonest (100). *N* = number of individuals scored for each locus

Locus	<i>N</i>	Electromorph frequencies
<i>Aat-1</i>	218	030: 0.002, 100: 0.991, 220: 0.007
<i>Est-D</i> ^a	1649	060: 0.173, 088: 0.071, 100: 0.471, 116: 0.284
<i>Glo</i>	1665	070: 0.001, 100: 0.889, 135: 0.111
<i>Idh-1</i>	1721	075: 0.001, 100: 0.902, 125: 0.096, 142: 0.001
<i>Idh-2</i>	1590	064: 0.001, 085: 0.369, 100: 0.625, 118: 0.005
<i>Lap-1</i> ^a	1539	050: 0.012, 066: 0.030, 083: 0.002, 098: 0.002, 100: 0.488 102: 0.004, 133: 0.378, 135: 0.011, 167: 0.074, 169: 0.000
<i>Mdh-1</i>	1857	071: 0.008, 085: 0.128, 100: 0.861, 130: 0.002
<i>Mdh-2</i>	136	100: 0.993, 130: 0.007
<i>Me-1</i>	24	080: 0.146, 100: 0.854
<i>Pgm-1</i>	1772	080: 0.006, 100: 0.632, 124: 0.359, 140: 0.002

^aPoolings of electromorphs were made for comparing the Thau data with those of other lagoons: *Est-D* {088, 100}; *Lap-1* {050, 066, 083}, {098, 100, 102}, {135, 137}, {167, 169}

(f) Correspondence analysis (CA)

CA (Benzécri, 1973; Greenacre, 1984; Mathieu *et al.*, 1990) was performed on a contingency table, in which each sample was described by its gene counts at several loci. This analysis was made for comparing the populations at the regional scale, for which data from two neighbouring lagoons (e.g. Thau and Prévost) were available. Computations were run using the ECOLOGIX package by Roux (1982).

2.3.3 Results

All results of the computations of single-locus and multilocus θ at several scales, temporal (among year cohorts within a sample) and spatial (among sites within a lagoon, among lagoons within a region, and among regions in the periphery of the Mediterranean), are presented in Table 2.3.3. Globally, the larger single-locus θ values were observed at the scale of the whole Mediterranean and the lower at the very local scale, i.e. among year cohorts within a site or among sites within a lagoon. However, two exceptions were noted: large θ values were observed at locus *Idh-1* among presumptive year cohorts in the Bouzigues sample, and at locus *Glo* among year cohorts in the Balaruc-Z.I. sample. A comparison of the different sets of single-locus θ values at different scales was made by means of a Kruskal–Wallis test (Sokal and Rohlf, 1969). No significant difference appeared between the within-sample scale and that of the lagoon ($p = 0.375$), nor between the scale of the lagoon and the regional scale ($p = 0.674$). A significant difference was observed between the regional scale and that of the whole Mediterranean ($p = 0.014$). Multilocus θ values were not significantly different from zero, either among cohorts, among sites, or among lagoons at the regional scale. The value observed among lagoons at the scale of the Mediterranean was significant ($p < 0.05$). Thus, the data in Table 2.3.3 do not yield any indication of population structuring at any scale below that of the whole Mediterranean, at which the null hypothesis of homogeneity among populations could be rejected.

A multidimensional treatment (CA) of multiple-locus (*Est-D*, *Glo*, *Lap-1*, *Mdh-1* and *Pgm-1*) data was run for samples from Thau ($n = 13$) and from Prévost ($n = 4$) which were characterized at all five loci simultaneously, and were of relatively large sizes ($N \geq 47$) (Table 2.3.1). By using this approach, we expected to be able to point out possible multiple-locus associations that would allow a distinction, if any, between the populations of these two lagoons. We found that the variability among the 13 samples from Thau encompassed that of all four samples from Prévost (Figure 2.3.1).

The matrix of genetic distances based on the multilocus coancestry coefficient (D), between populations at the peri-Mediterranean scale is given in Table 2.3.4. Genetic distances ranged from 0.0000 (Thau/Ebro) to 0.0590 (Bizerte/Temsah). Only one value (Thau/Bizerte) could be considered significant by using a t -test as for multilocus θ (one-tailed $t = 2.83$; 5 d.f.; $p < 0.025$). This is probably due to the fact that sample sizes in this case were relatively important (see Table 2.3.1). The non-significance of the D values, all larger, between populations that are separated by larger geographic distances (Table 2.3.4) is likely to be related to their smaller sample sizes. Nevertheless, no significant correlation of genetic distance with geographic distance was found (Mantel's test for the association of parameters in a matrix with internal correlation (Manly, 1985); one-tailed; $p = 0.125$).

Table 2.3.3 Single-locus and multilocus values of coancestry coefficient (θ) (Weir and Cockerham, 1984) among populations of *R. decussatus* at different scales, temporal and spatial

Locus	(k)	Scale				
		Among cohorts within sample		Among sites within lagoon (Thau)	Regional (Thau versus Prévoist)	Mediterranean
<i>Bouzigues^a Balaruc Z.I.</i>						
<i>Aat-1</i>	(3)	—	—	0.0069	-0.0019	—
<i>N</i>		—	—	218	265	—
<i>n</i>		—	—	4	2	—
<i>Est-D</i>	(4)	-0.0070	-0.0013	0.0007	0.0041	0.0055
<i>N</i>		108	209	1649	1753	1827
<i>n</i>		3	2	8	2	5
<i>Glo</i>	(3)	-0.0107	0.0136	0.0029	-0.0013	0.0571
<i>N</i>		105	206	1665	1891	1850
<i>n</i>		3	2	9	2	5
<i>Idh-1</i>	(4)	0.0316	-0.0039	-0.0013	0.0071	0.0303
<i>N</i>		106	211	1721	1777	1922
<i>n</i>		3	2	8	2	5
<i>Idh-2</i>	(4)	0.0055	-0.0049	0.0011	—	—
<i>N</i>		107	211	1590	—	—
<i>n</i>		3	2	8	—	—
<i>Lap-1</i>	(4)	-0.0069	-0.0045	-0.0009	0.0004	—
<i>N</i>		110	211	1886	2113	—
<i>n</i>		3	2	9	2	—
<i>Mdh-1</i>	(4)	0.0039	-0.0039	0.0015	-0.0004	0.0287
<i>N</i>		107	211	1857	2084	2054
<i>n</i>		3	2	9	2	5
<i>Mdh-2</i>	(2)	—	—	-0.0057	-0.0029	—
<i>N</i>		—	—	136	263	—
<i>n</i>		—	—	3	2	—
<i>Me-1</i>	(2)	—	—	—	—	0.0113
<i>N</i>		—	—	—	—	212
<i>n</i>		—	—	—	—	5
<i>Pgm-1</i>	(4)	-0.0118	0.0068	0.0012	0.0000	-0.0001
<i>N</i>		110	212	1772	1998	1968
<i>n</i>		3	2	9	2	5
Multilocus		-0.0014	0.0001	0.0006	0.0015	0.0149*
<i>SD</i> (jackknife)		0.0070	0.0021	0.0005	0.0011	0.0072

^aSource: Borsa, Zainuri and Delay (1991)* $p < 0.05$; one-tailed *t*-test.*N* = total number of individuals; *n* = number of samples; *k* = number of electromorphs per locus.

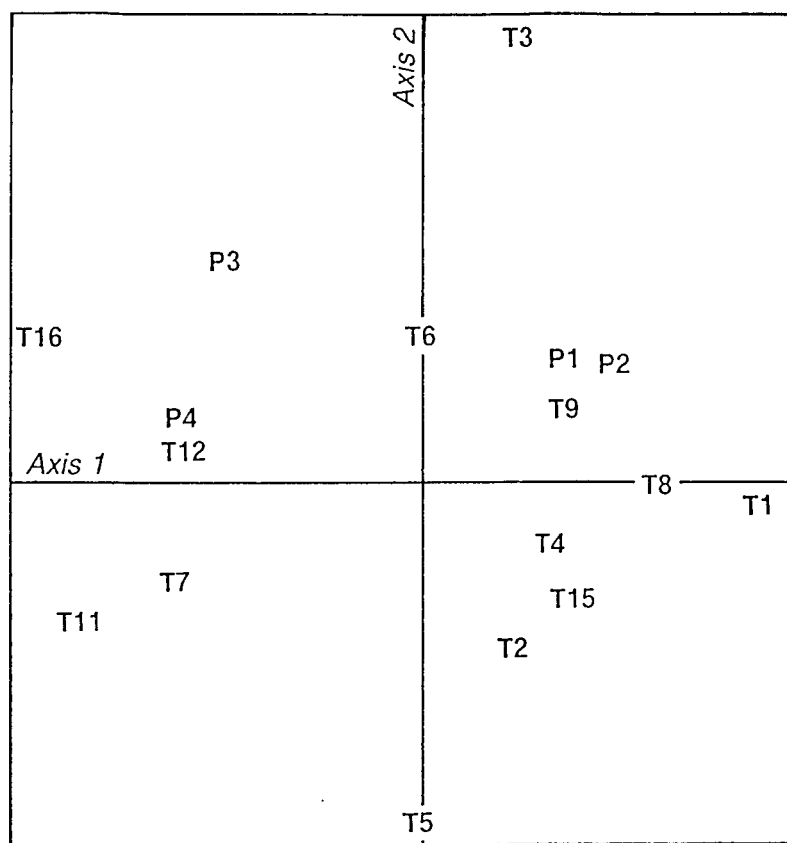


Figure 2.3.1 Correspondence analysis: projection on the plane defined by axes 1 and 2 (respectively, 28.4% and 21.4% of total inertia) of samples of *R. decussatus* from Thau ($n = 13$) and Prévost ($n = 4$), each defined by its genotypic frequencies at loci *Est-D*, *Glo*, *Lap-1*, *Mdh-1* and *Pgm-1*. Symbols for samples as in Table 2.3.1.

Table 2.3.4 Below diagonal: values of pairwise genetic distance among five peri-Mediterranean populations of *R. decussatus*. D = genetic distance based on multilocus coancestry coefficient (Reynolds, Weir and Cockerham 1983); SD = standard deviation (jackknife). Above diagonal: approximate geographic distances (km) measured following summer coastal surface currents (after Nielsen, 1912)

		<i>Thau</i>	<i>Ebro</i>	<i>Faro</i>	<i>Bizerte</i>	<i>Temsah</i>
Thau		–	400	1500	2800	5500
Ebro	D	0.0000	–	1100	2400	5100
	SD	0.0041				
Faro	D	0.0099	0.0002	–	1800	4500
	SD	0.0107	0.0030			
Bizerte	D	0.0181	0.0030	0.0084	–	2700
	SD	0.0064	0.0058	0.0110		
Temsah	D	0.0233	0.0336	0.0421	0.0590	–
	SD	0.0188	0.0359	0.0425	0.0553	

2.3.4 Discussion

The present study, which was based on the estimation of coancestry coefficients between populations at different scales, spatial and temporal, provided an insight into the genetic structure of *R. decussatus*.

Genetic differences were found between populations of *R. decussatus* from different regions of the Mediterranean, separated by an effective distance of several thousand kilometres. At that scale, no correlation between genetic distance and geographic distance was observed, which is consistent with an island model of population structure (Wright, 1969). The estimates of coancestry coefficients at the scale of the Mediterranean were homogeneous among loci. Since gene flow is expected to affect all loci to the same extent (Slatkin, 1985), homogeneity among single-locus θ values is consistent with the expectation of differentiation having arisen solely through genetic drift.

No heterogeneity could be detected at the within-region scale, i.e. between neighbouring lagoons. Therefore, all individuals at a regional scale should be considered as belonging to the same, genetically homogeneous population. In other words, high gene flow occurs, or has occurred recently, at that scale. The geographic limits of the population could even be extended to neighbouring regions: there was no indication for divergence, albeit small, between Thau and Ebro, both located along the northwestern coast of the western Mediterranean, about 400 km apart. Gene flow among lagoons at the regional scale is possible through larval transport (with the conditions that larvae are released from one lagoon and brought by currents into the next lagoon, where they are recruited and develop to the reproductive stage for participating as parents to the next generation), and may be enhanced by the recolonization of habitats left vacant after an extinction. These conditions for massive migration might not be met very often. However, under the current models of population genetic structure, an average of more than one successful migrant per generation is sufficient to prevent alternative alleles being fixed in geographically separated local populations (Slatkin, 1985).

The comparison of year cohorts within a local population revealed some heterogeneity among loci: large θ values were found at one locus in each of the two samples for which separate year-cohort data were available. Discrepancies among single-locus coancestry coefficient values suggest that selection or possibly genetic drift affects a particular enzyme locus, or a locus which is in gametic disequilibrium with it. In the face of gene flow, such differentiation, if real, can only be short-term.

A recent report based on a comparison of results from different studies of genetic structure of the American oyster, using nuclear RFLPs, mitochondrial DNAs and allozyme genes (Karl and Avise, 1992), stressed the presumption that allozyme genes are subjected to balancing selection, at least in bivalve molluscs, a hypothesis also consistent with the reported correlations of allozymic heterozygosity with reproductive performance or survival (e.g. Zouros *et al.*, 1983). This could be true in *R. decussatus*, in which correlations of allozymic heterozygosity with survival in stress conditions have also been reported (Borsa, 1990; Borsa, Jousselein and Delay, 1992). In order to get a more complete picture of the genetic structure of Mediterranean *R. decussatus*, and to eliminate doubts as to the existence of composition that is masked by balancing selection at allozyme loci, as cautioned by Karl and Avise (1992), this study should be completed using other genetic markers.

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