MORPHOLOGICAL, GENETIC AND CHROMOSOMAL CHARACTERIZATION OF CORSICAN AND SPANISH BACILLUS ROSSIUS (INSECTA PHASMATODEA)

F Tinti

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F. TINTI

Dipartimento di Biologia Evoluzionistica Sperimentale, Sede Zoologia, Università di Bologna, via S. Giacomo 9, 40126 Bologna, Italia

DISTANCES GENETIQUES
FUSION ROBERTSONIENNE
OOTAXONOMIE
RESTRUCTURATIONS
CHROMOSOMIQUES
SPANANDRIE

RESUME – L'ootaxonomie, l'électrophorèse des systèmes gène-enzyme et l'analyse chromosomique révèlent que le Phasmide *Bacillus rossius* de Corse, parthénogénétique, appartient à la sous-espèce *B. r. rossius*. Les distances génétiques et les caractéristiques chromosomiques, malgré une fusion Robertsonienne, indiquent une forte affinité avec les populations parthénogénétiques du Nord de la Sardaigne et de l'Ile d'Elbe; il est donc probable que toutes ces populations sont issues d'une dérive commune depuis le Tertiaire. L'analyse enzymatique de trois échantillons bisexués espagnols provenant de Catalogne (Tossa) et qui devraient être des *B. r. catalauniae*, indique au contraire qu'ils appartiennent également à la sous-espèce *B. r. rossius*.

GENETIC DISTANCES
OOTAXONOMY
REPATTERNED CYTOTYPES
ROBERTSONIAN FUSION
SPANANDRY

ABSTRACT – Chorionic pattern, allozymic and chromosomal analysis allow to assign the parthenogenetic Corsican *Bacillus rossius* to the *B. r. rossius* subspecies. Although some distinguishing chromosomal features have been detected (Robertsonian fusions), karyotype and general genetic characteristics indicate their high similarity to the parthenogenetic North-Sardinian and Elban demes, suggesting a common derivation during the Tertiary. Allozyme analysis of Spanish bisexual samples from *B. r. catalauniae* area (Tossa) reveals that these populations too belong to *B. r. rossius*.

INTRODUCTION

The holomediterranean genus *Bacillus* has been widely investigated through multidisciplinary approaches (Scali & Mantovani, 1989; Bullini & Nascetti, 1990; Mantovani *et al.*, 1991 b). In addition to several thelytokous taxa, it includes two bisexual species. The first one, *B. grandii* (2n = 34 XX, female; 33 X0, male), is strictly bisexual and differentiated into 3 subspecies : *B. g. benazzii*, from North-western Sicily and Levanzo (Egadi Islands), *B. g. maretimi*, endemic of Marettimo (Egadi Islands) and *B. g. grandii*, found in a very limited area of South-eastern Sicily (Nascetti & Bullini, 1982; Scali & Mantovani, 1990; Mantovani *et al.*, 1991 a; Scali, 1991). The second one, *B. rossius* (2n = 36 XX, female and 35 X0, male), spreads into Central and Western Mediterranean basin also with many facultatively parthenogenetic demes. *B. rossius* is differentiated into 8 subspecies, recognized on ootaxonomical and electrophoretical grounds : *B. r. tripolitanus* A, *B. r. tripolitanus* B, *B. r. lobipes*, *B. r. montalentii* and *B. r. medeae* (Northern Africa : Tunisia, Algeria); *B. r. catalauniae* (Spain : Catalonia); *B. r. rossius* (French and Western Italian coasts, Sardinia – except Sarrabus area – and Tuscan Archipelago); *B. r. redtenbacheri* (Sardinia – only in the Sarrabus area – Sicily, Eolie Islands, Southern Tyrrenian, Adriatic and Ionian coasts of Italy, Yugoslavia, Greece (see Mantovani *et al.*, 1991 b for a review).

Many facultative parthenogenetic demes of *B. r. rossius* and *B. r. redtenbacheri* are known. They are always genetically more similar to the bisexual populations of the same area than to the parthenogenetic ones of the others (Gasperi *et al.*, 1983; Nascetti & Bullini, 1983; Scali *et al.*, 1987).

Investigations carried out on populations from Central and Western Mediterranean basin have considerably increased our knowledge about distribution, ootaxonomy, genetic structure, chromosomal complements and reproductive biology of *B. rossius* subspecies (Gasperi *et al.*, 1983;

Nascetti & Bullini, 1983; Scali & Marescalchi, 1987; Scali *et al.*, 1987; Tinti & Scali, 1990; Manaresi *et al.*, 1991; Mantovani & Scali, 1991; Tinti *et al.*, 1992).

Ootaxonomical investigations on the chorionic features in *B. rossius*, support a subspecific level of differentiation in agreement with the genetic structure (Scali *et al.*, 1987).

The subspecific values of genetic distance, obtained from gene-enzyme systems analysis at about 20 loci, range between D = 0.116 (comparisons between Sardinian demes of *B. r. rossius* and *B. r. redtenbacheri*, Mantovani & Scali, 1991) and D = 0.543 (Italian, Yugoslavian and Greek samples of *B. r. redtenbacheri* compared to *B. r. montalentii*, Nascetti & Bullini, 1983).

Nei's genetic distances between the *B. rossius* subspecies involved in the present paper are the following : *B. r. rossius* – *B. r. redtenbacheri* : 0.116 – 0.193; *B. r. rossius* – *B. r. catalauniae* : 0.161; *B. r. redtenbacheri* – *B. r. catalauniae* : 0.214 (Nascetti & Bullini, 1983; Mantovani & Scali, 1991; Tinti *et al.*, 1992). I would like to point out that no genotype or allele frequencies of *B. r. catalauniae* have been reported.

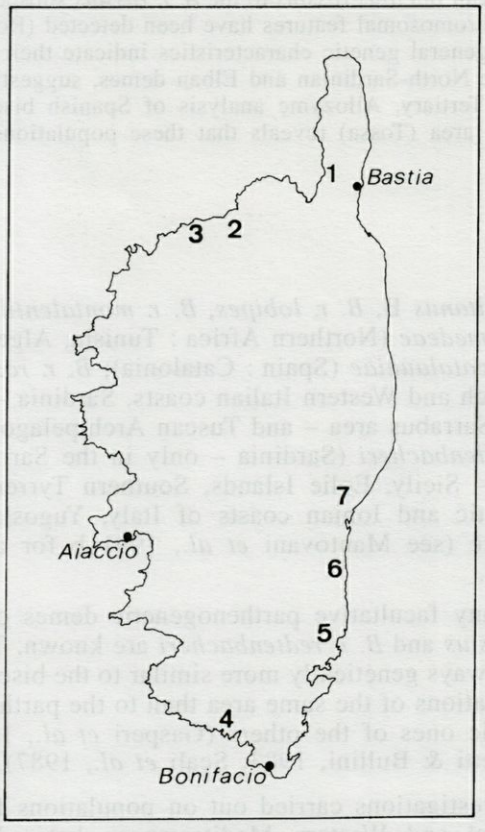

Fig. 1. – Map of Corsica showing the locations of the 7 analyzed *Bacillus rossius* demes (1 : Albo, 2 : Ile Rousse L, 3 : Ile Rousse B, 4 : Pianettoli, 5 : S. Giulie, 6 : Solenzara, 7 : Ghisonaccia).

Chromosomal analyses of *B. rossius* show 2n = 36, female and 35, male standard complement in all subspecies, with differences only in the number and position of secondary constrictions (Scali *et al.*, 1983; Scali & Marescalchi, 1987). However, instances of chromosomal repatterning (Robertsonian fusions or fissions, inversions and translocations) have been found to occur in some parthenogenetic demes from Sicily (Castelvetrano, 2n = 38; Scali & Marescalchi, 1987; Manaresi *et al.*, 1991) and from Northern Sardinia (Castelsardo, 2n = 32; Poglina, 2n = 32, 34; Tinti & Scali, 1990 and unpublished data).

No data about Corsica, the Balearic and others minor Mediterranean islands have been reported.

This paper deals with the systematic characterization of Corsican *B. rossius* through ootaxonomical, electrophoretical and karyological approaches, and the allozymic characterization of three samples from *B. r. catalauniae* area.

MATERIALS AND METHODS

Seven all-females samples of *B. rossius* were collected in Corsica during September 1990 (Table I, Fig. 1). Also three amphigonic samples collected near Tossa (Catalonia, Spain; Table I) were analyzed. Morphological, allozymic and karyological investigations were carried out as reported below.

Ootaxonomy

Only eggs of field collected females were used for SEM observations. After 80 % ethanol fixation, eggs were washed, ultrasonicated to eliminate debris, air dried and mounted to specimen-holders with Bio-Rad silver-conducting paint. The eggs were coated with gold in a Ed-

Table I. – Collecting sites, sample sizes, sex-ratios and natural food-plants of the 7 Corsican and 3 Spanish populations of *Bacillus rossius*.

POPULATIONS	FEMALES	MALES	FOOD-PLANTS
Corsican			
Albo	15	1	Bramble, lentisk
Ile Rousse L	3		Lentisk
Ile Rousse B	13		Bramble
Pianettoli	16		Bramble
S. Giulie	15	1	Bramble, wild rose
Solenzara	6		Bramble
Ghisonaccia	30		Bramble
Spanish			
Tossa 1	8	6	Lentisk
Tossa 2	7	7	Bramble, lentisk
Tossa 3	8	8	Bramble

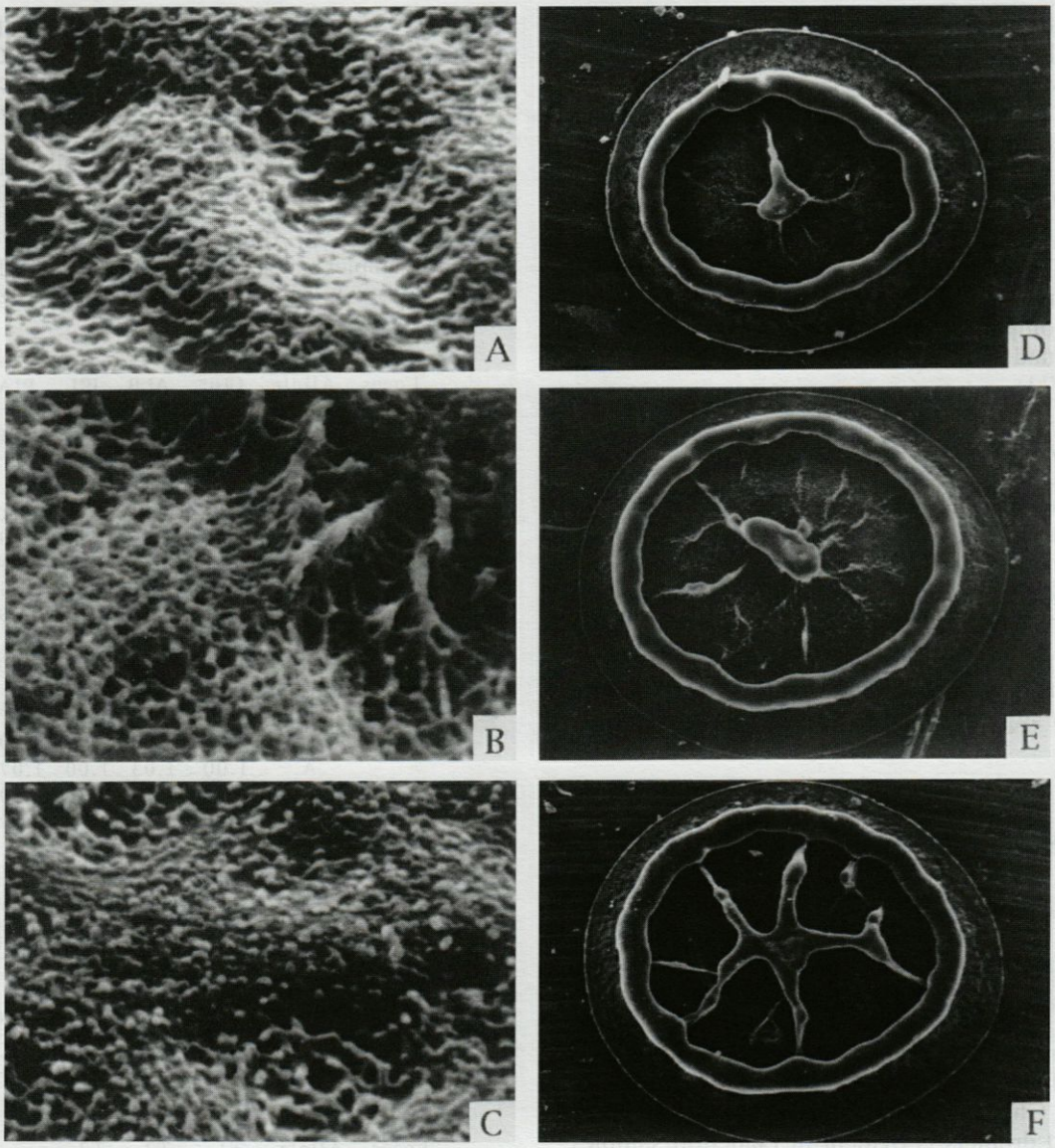

Fig. 2. – Fine capsule patterns (A : Solenzara, B : Ghisonaccia, C : Pianettoli; x 1300) and operculae (D : Ile Rousse L, E : S. Giulie, F : Pianettoli; x 60) of eggs laid by field-collected Corsican females.

wards S 150 A sputter coater and observed with a Philips 515 scanning electron microscope. The used terminology is according to Scali *et al.* (1990).

Allozyme analysis

Sample homogenates, electrophoretic runs and staining procedures were performed according to Mantovani *et al.* (1991 a). The following enzymes gave interpretable patterns in all analyzed populations : α 1 – GPDH (glycerophosphate dehydrogenase, E.C. : 1.1.1.08), MDH (malate dehydrogenase, E.C. : 1.1.1.37), IDH (isocitrate dehydrogenase, E.C. : 1.1.1.42), 6PGDH (6-phosphogluconate dehydrogenase, E.C. : 1.1.1.44), GOX (glucose oxidase, E.C. : 1.1.1.47), G6PDH (glucose 6-phosphate dehydrogenase, E.C. : 1.1.1.49), G3PDH (glyceraldehyde 3-phosphate dehydrogenase, E.C. : 1.2.1.12), GOT (glutamate-oxaloacetate transaminase, E.C. : 2.6.1.1), HK (hexokinase, E.C. : 2.7.1.1), ADK (adenilate kinase, E.C. : 2.7.4.3), PGM (phosphoglucomutase, E.C. : 2.7.5.1), ALD (aldolase, E.C. : 4.1.2.13), FH (fumarase, E.C. : 4.2.1.2), MPI (mannose 6-phosphate isomerase, E.C. : 5.3.1.8), PGI (phosphoglucose isomerase, E.C. : 5.3.1.9). For MDH, IDH, GOT, HK, and ADK two systems were identified so that a total number of 20 loci were therefore studied.

The allozyme polymorphism indexes $\overline{H}obs$ (mean observed heterozygosity), A (mean effective number of allele per locus) and P (frequency of polymorphic loci) were obtained following Mantovani *et al.* (1991 a).

Corsican and Spanish populations were compared with the two nearest Central Mediterranean *B. rossius* subspecies : *B.r. rossius* (samples from Sardinia, Elba Island, Tuscany, Latium) and *B.r. redtenbacheri* (samples from Sicily and Ionian-Adriatic coasts of Italy) (Mantovani & Scali, 1991; Tinti *et al.*, 1992).

Genetic distances (D) were calculated according to Nei's method (1972) on 20 loci for Corsican and Spanish samples and on 18 loci in intersubspecific comparisons. A dendrogram, based on D, was obtained with an UPGMA method (Sneath & Sokal, 1973).

Karyology

Chromosome preparations of females and males were obtained from follicular cells and spermatogonia respectively, following Tinti & Scali (1991). Slides were stained with Giemsa stain (3 % Giemsa solution in 0.1 M phosphate buffer pH 7.0) and mounted in Euparal.

For chromosome characterization, the criteria and terms suggested by Levan *et al.* (1964) were followed.

RESULTS

Ootaxonomy

In all samples, the capsule pattern is undulated. The net of indented ribbons present in South-eastern samples (S. Giulie, Solenzara, Ghisonaccia) becomes more faintly dented in the Western coastal ones; well developed cristae are found only in S. Giulie and Ghisonaccia (Fig. 2 A-B). A pattern given by isolated droplets is evident in Pianettoli samples (Fig. 2 C). The operculum is very similar in all demes and shows a well defined peripheral ring with variously developed internal cristae (Fig. 2 D-F); the differential developmental patterns of cristae does not follow any geographical cline.

Electrophoretic analysis

Table II A gives the allelic frequencies of the Corsican demes at the 5 polymorphic loci (*6Pgdh, Got*– 1, *Hk*– 1, *Hk*– 2, *Fh*), the other 15 loci being monomorphic for the same allele in all samples. Ile Rousse B, S. Giulie, Ghisonaccia, Solenzara demes showed an identical allele pattern and therefore they were pooled into one sample called GHI*. At each locus, all samples show a homozygous genotype ($\overline{H}obs = 0.00$); consequently, the low polymorphism is also expressed by the low A and P values (1.00 – 1.07 and 0.00 – 0.10, respectively).

It is also to be noted that all populations show at all loci the 100 allele either as unique or most common, the only exception being the Ile Rousse L population having the alternative 105 allele at the *6Pgdh* locus.

Table II. – A, Allelic frequencies at the 5 polymorphic loci of the Corsican *Bacillus rossius* demes (GHI* : Ile Rousse B, S. Giulie, Solenzara and Ghisonaccia; ALB : Albo; IRL : Ile Rousse L; PIA : Pianettoli). Sample sizes (n) and polymorphism indexes ($\overline{H}obs$: mean observed heterozygosity; A : mean effective of allele per locus; P : frequency of polymorphic loci) are also reported. B, Nei's genetic distances (D) between Corsican *Bacillus rossius* samples. C, Allelic frequencies of the 3 bisexual Spanish samples at the 8 polymorphic loci (T1 : Tossa 1; T2 : Tossa 2; T3 : Tossa 3) and sample sizes (n).

A

Locus	Allele	GHI*	ALB	IRL	PIA
6Pgdh	100	1.00	1.00	-	1.00
	105	-	-	1.00	-
Got-1	95	-	0.14	-	-
	100	1.00	0.86	1.00	1.00
Hk-1	100	1.00	1.00	1.00	0.71
	102	-	-	-	0.29
Hk-2	100	1.00	1.00	1.00	0.71
	102	-	-	-	0.29
Fh	97	-	0.14	-	-
	100	1.00	0.86	1.00	1.00
	n	53	14	3	14
	$\overline{H}obs$	0.00	0.00	0.00	0.00
	A	1.00	1.03	1.00	1.07
	P	0.00	0.10	0.00	0.10

B

	GHI*	ALB	IRL	PIA
GHI*	- -	0.002	0.051	0.008
ALB		- -	0.054	0.011
IRL			- -	0.061
PIA				- -

C

Locus	Allele	T1	T2	T3
6Pgdh	96	0.84	0.50	0.63
	100	0.16	0.50	0.37
Gox	100	0.65	0.75	0.81
	105	0.35	0.25	0.19
G6pdh	98	0.17	1.00	1.00
	100	0.83	-	-
Adk-1	97	0.50	0.79	0.56
	100	0.50	0.21	0.44
Adk-2	96	-	-	0.13
	100	1.00	1.00	0.81
Fh	97	0.14	0.06	0.19
	100	0.86	0.94	0.81
Mpi	98	-	0.13	-
	100	1.00	0.87	1.00
Pgi	97	0.93	0.63	0.44
	100	0.17	0.37	0.56
	n	14	16	16

The high homogeneity deriving from the allele frequencies (Table II A), as well as the resulting low genetic distances ($0.002 < D < 0.061$, Table II B), clearly indicate a differentiation of only interpopulation level.

The same applies to the three Spanish samples which show a quite similar allelic structure at the 8 polymorphic loci (*6Pgdh, Gox, G6pdh, Adk– 1, Adk– 2, Fh, Mpi* and *Pgi*, Table II C); the remaining 12 loci (see Materials and Methods) are monomorphic. Their low genetic distances ($0.009 < D < 0.020$, clearly of interpopulation degree) again suggest to pool them (TOSSA).

The allelic frequencies at the 14 polymorphic loci of *B. r. rossius* and *B. r. redtenbacheri* references samples (split into amphigonics and parthenogenetics), pooled Corsican and Spanish *B. rossius* samples are presented in Table III. The α-

Table III. – Allelic frequencies at the 14 polymorphic loci of *Bacillus rossius rossius* reference samples (ROSUC, unisexual continental demes; ROSBC, bisexual continental populations; ROSEL, Elban populations; ROSUS, unisexual Sardinian demes; ROSBS, bisexual Sardinian populations), pooled Corsican (CORSI), pooled Spanish (TOSSA) *B. rossius* samples and *B. r. redtenbacheri* reference samples (REDU, unisexual demes; REDB, bisexual populations). Allelic frequencies of *B. rossius* reference samples were desumed from Mantovani & Scali, 1991 and Tinti *et al.*, 1992.

Locus	Allele	ROSUC	ROSBC	ROSEL	ROSUS	ROSBS	CORSI	TOSSA	REDU	REDB
Mdh-1	100	-	-	-	-	-	-	-	1.00	0.85
	110	1.00	1.00	1.00	1.00	0.95	1.00	1.00	-	0.15
	116	-	-	-	-	0.02	-	-	-	-
	125	-	-	-	-	0.03	-	-	-	-
Mdh-2	100	1.00	1.00	1.00	0.84	1.00	1.00	1.00	1.00	1.00
	111	-	-	-	0.16	-	-	-	-	-
Idh-1	96	-	-	-	-	0.01	-	-	-	-
	100	1.00	1.00	1.00	1.00	0.97	1.00	1.00	1.00	1.00
	103	-	-	-	-	0.01	-	-	-	-
	107	-	-	-	-	0.01	-	-	-	-
6Pgdh	96	-	-	-	-	-	-	0.64	-	0.10
	100	1.00	1.00	1.00	1.00	0.92	0.93	0.36	1.00	0.88
	103	-	-	-	-	0.03	-	-	-	-
	105	-	-	-	-	0.05	0.07	-	-	-
	107	-	-	-	-	-	-	-	-	0.02
Gox	94	-	0.06	-	-	0.06	-	-	-	0.02
	100	1.00	0.66	1.00	1.00	0.88	1.00	0.76	1.00	0.86
	102	-	-	-	-	-	-	-	-	-
	105	-	-	-	-	0.03	-	0.24	-	0.01
	107	-	0.26	-	-	-	-	-	-	-
	110	-	-	-	-	0.03	-	-	-	0.09
	115	-	-	-	-	-	-	-	-	0.02
	121	-	0.02	-	-	-	-	-	-	-
G6pdh	98	-	-	-	-	-	-	0.05	-	0.02
	100	1.00	1.00	1.00	1.00	0.96	1.00	0.95	1.00	0.98
	105	-	-	-	-	0.04	-	-	-	-
Got-1	93	-	0.04	-	-	-	-	-	-	-
	95	-	-	-	-	-	0.02	-	-	-
	100	1.00	0.96	1.00	1.00	1.00	0.98	1.00	1.00	1.00
Got-2	96	-	-	-	-	0.04	-	-	-	-
	100	1.00	0.88	1.00	1.00	0.96	1.00	1.00	1.00	1.00
	103	-	0.12	-	-	-	-	-	-	-
Hk-1	100	1.00	0.98	1.00	1.00	1.00	0.95	1.00	1.00	1.00
	102	-	-	-	-	-	0.05	-	-	-
	104	-	0.02	-	-	-	-	-	-	-
Hk-2	100	1.00	0.98	1.00	1.00	1.00	0.95	1.00	1.00	1.00
	102	-	-	-	-	-	0.05	-	-	-
	104	-	0.02	-	-	-	-	-	-	-
Adk-1	94	-	-	-	-	0.03	-	-	0.04	0.40
	97	1.00	0.95	1.00	1.00	0.87	1.00	0.53	0.03	0.11
	100	-	0.05	-	-	0.10	-	0.47	0.90	0.49
	103	-	-	-	-	-	-	-	0.03	-
Adk-2	96	-	-	-	-	-	-	0.05	-	-
	100	0.97	1.00	0.83	1.00	0.94	1.00	0.95	1.00	1.00
	104	0.03	-	0.17	-	-	-	-	-	-
	110	-	-	-	-	0.06	-	-	-	-
Mpi	96	-	-	-	-	0.25	-	-	-	0.04
	98	-	-	-	-	-	-	0.04	-	-
	100	1.00	1.00	1.00	1.00	0.69	1.00	0.96	1.00	0.86
	105	-	-	-	-	0.03	-	-	-	0.03
	108	-	-	-	-	-	-	-	-	0.02
	109	-	-	-	-	0.03	-	-	-	-
	110	-	-	-	-	-	-	-	-	0.02
	112	-	-	-	-	-	-	-	-	0.02
Pgi	84	-	-	-	-	0.02	-	-	-	-
	95	-	-	-	-	0.03	-	-	-	0.02
	97	0.22	0.83	1.00	1.00	0.70	1.00	0.66	-	0.04
	100	0.78	0.17	-	-	0.25	-	0.34	1.00	0.94

Gpdh, Idh– 2, *G3pdh* and *Ald* loci are monomorphic. Within all samples, the number of alleles ranges from 2 (*Mdh–* 2) to 8 (*Gox* and *Mpi*). All samples share the 100 allele at 10 out of the 14 polymorphic loci either as the unique or the most common allele (*Mdh–* 2, *Idh–* 1, *Gox, G6pdh, Got* –1, *Got* –2, *Hk* –1, *Hk* –2, *Adk* –2, *Mpi*). However, while at *Mdh* –1 and *Adk* –1 loci, *B. r. rossius* samples present the same allele (*Mdh–* ^{110} and *Adk* -1^{97}) as the unique/most common one, the *B. r. redtenbacheri* show alternative alleles (*Mdh* -1^{100} and *Adk* -1^{94} or *Adk* -1^{100}). Corsican and Spanish *B. rossius* generally show either as the unique/most frequent allele that of the reference *B. r. rossius*, the only exception being the *6Pgdh* ^{96} allele of the Spanish sample, where it is the most common one.

Genetic distances (Table IV) clearly indicate an interpopulation level of differentiation between reference *B. r. rossius* samples and pooled Corsican demes ($0.002 < D < 0.037$). The same applies to Spanish samples ($0.038 < D < 0.053$). On the other hand, D values obtained from the comparisons *B. r. rossius – B. r. redtenbacheri* ($0.085 < D < 0.178$), Corsican samples – *B. r. redtenbacheri* ($0.142 < D < 0.178$) and Spanish samples – *B. r. redtenbacheri* ($0.103 < D < 0.132$), clearly fall into a subspecific differentiation range.

Table IV. – Nei's genetic distances (D) between of *Bacillus rossius rossius* reference samples, pooled Corsican samples, pooled Spanish samples and *B. r. redtenbacheri* reference samples (for captions see Table III).

	ROSUC	ROSBC	ROSEL	ROSUS	ROSBS	CORSI	TOSSA	REDU	REDB
ROSUC	--	0.028	0.036	0.037	0.021	0.036	0.053	0.115	0.085
ROSBC		--	0.010	0.010	0.011	0.009	0.044	0.161	0.126
ROSEL			--	0.003	0.012	0.002	0.049	0.178	0.144
ROSUS				--	0.012	0.002	0.050	0.178	0.144
ROSBS					--	0.011	0.038	0.141	0.106
CORSI						--	0.046	0.178	0.142
TOSSA							--	0.132	0.103
REDU								--	0.012
REDB									--

The most likely phyletic relationships among analyzed samples are figured in the dendrogram (Fig. 3).

Karyology

Females from Ghisonaccia, Solenzara, S. Giulie and Pianettoli share the same standard 2n = 36 (XX) *B. rossius* complement. The large elements of the karyotype (1st pair, large metacentrics; 2nd pair, acrocentrics; 3rd pair, submetacentrics – sexual chromosomes –) and also others small chromosomes, such as the smallest, are clearly recognizable; minor differences related to secondary constrictions (see f.i. pairs 13 and 16) are however noticeable (Fig. 4 A).

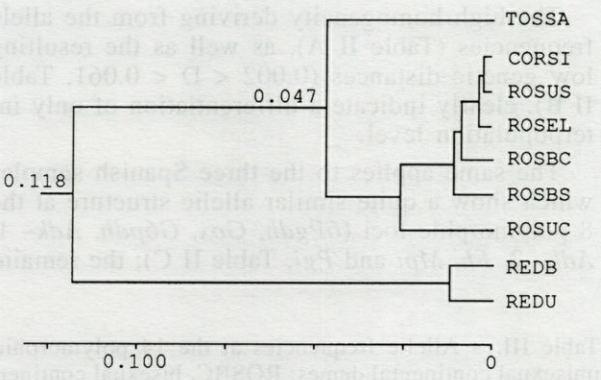

Fig. 3. – Dendrogram showing the most likely phyletic relationships among the here analyzed Corsican and Spanish *Bacillus rossius, B. r. rossius* and *B. r. redtenbacheri* reference samples (for captions see Table III).

On the other hand, all the Ile Rousse L females share a 2n = 34 cytotype, which differs from the standard one for a submetacentric pair, ranking as the 2nd in size (Fig. 4 B). It appears to be derived from the Robertsonian fusion of acrocentric pairs 4 and 5, since they are missing as separate elements from this cytotype. Ile Rousse B deme shares both the standard and the repatterned cytotype.

The two odd males collected at Albo and S. Giulie (one each from among otherwise all females demes, see Table II), show a standard 2n = 35 (X0) karyotype, regular meiotic processes and normal spermatogenesis (Fig. 4 C-E).

DISCUSSION

Ootaxonomical and electrophoretical results indicate a remarkable level of homogeneity within Corsican *B. rossius* demes, although some minor differences occur in Pianettoli and Ile Rousse L samples.

The undulated net-like capsule pattern, sometimes faintly dented, observed in all demes, excepting Pianettoli, is actually the same as the already described for *B. r. rossius* (Scali *et al.*, 1987). The chorionic features of Pianettoli, already evidencied in a few Sardinian demes (Scali *et al.*, 1983 and unpublished data) suggest a certain degree of variability in ootaxonomic details, even within subspecific taxa, which make the Pianettoli deme a *redtenbacheri* – like one, from the chorionic point of view.

Allelic constitution, genetic structure and distances unequivocally assign Corsican *B. rossius* to the *B. r. rossius* subspecies. Furthermore, the low

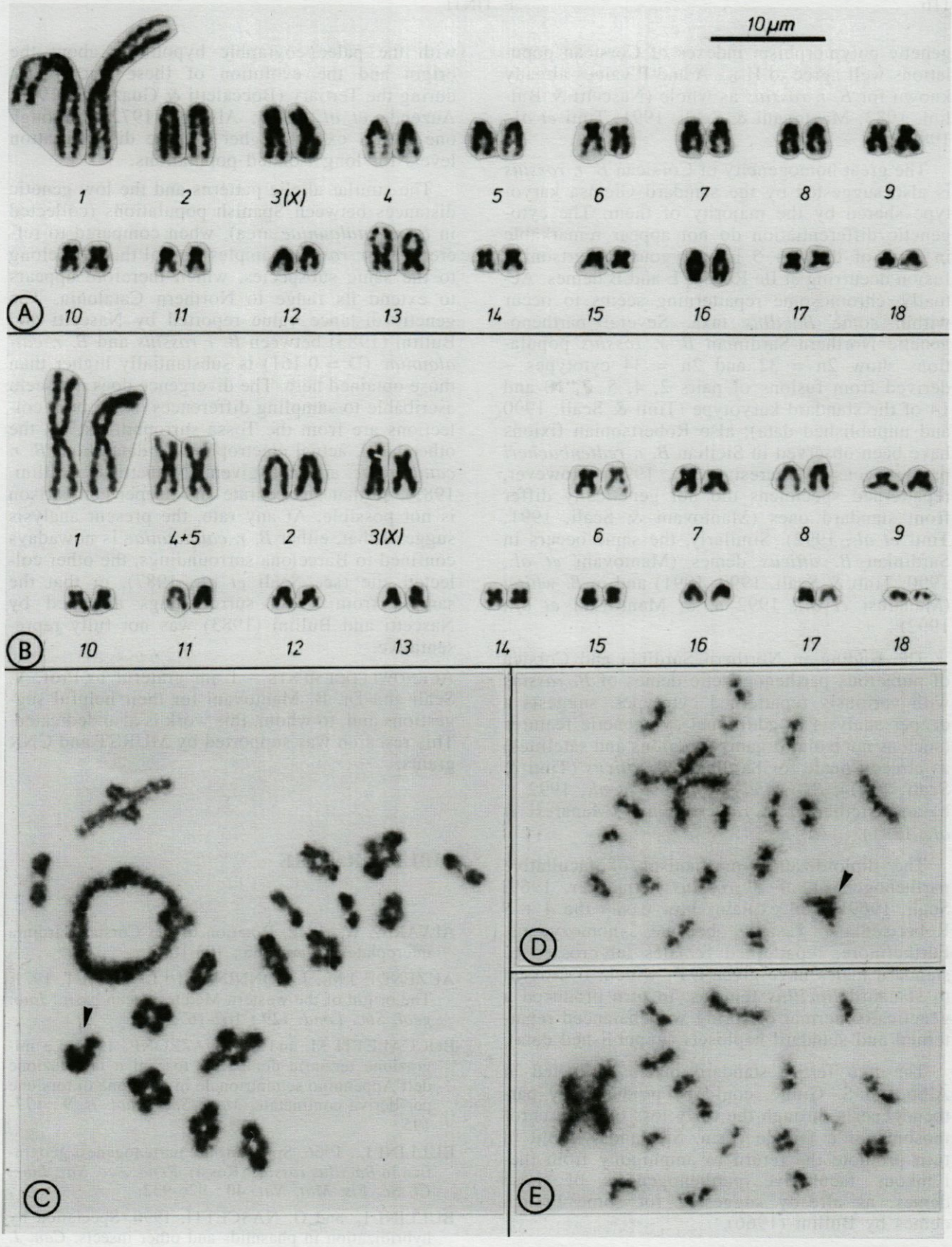

Fig. 4. – A : The standard karyotype (2n = 36) shared by the majority of Corsican demes. In this karyotype (Solenzara), two evident homozygous satellites are present on the pairs 13 and 16. B : The 2n = 34 cytotype found in Ile Rousse L and Ile Rousse B demes. Note the large submetacentrics (2nd pair in size) derived from the homozygous Robertsonian fusion of pairs 4 and 5, here missing. C : A regular metaphase I, with 17 bivalents and 1 univalent (X, arrowhead) found in Albo male. D and E : Regular metaphases II showing 18 (X, arrowhead) and 17 diads, respectively, found in S. Giulie male.

genetic polymorphism indexes of Corsican populations well agree to H_{obs}, A and P values already known for *B. r. rossius* as whole (Nascetti & Bullini, 1983; Mantovani & Scali, 1991; Tinti *et al.*, 1992).

The great homogeneity of Corsican *B. r. rossius* is also suggested by the standard Giemsa karyotype shared by the majority of them. The cytogenetic differentiation do not appear remarkable in spite of the 4 + 5 homozygous Robertsonian fusion occurring at Ile Rousse L and B demes. Actually, chromosome repatterning seems to occur within some *Bacillus* taxa. Several parthenogenetic Northern-Sardinian *B. r. rossius* populations show 2n = 32 and 2n = 34 cytotypes – derived from fusions of pairs 2, 4, 5, 7, 10 and 14 of the standard karyotype (Tinti & Scali, 1990 and unpublished data); also Robertsonian fixions have been observed in Sicilian *B. r. redtenbacheri* parthenogens (Manaresi *et al.*, 1991). However, repatterned specimens did not genetically differ from standard ones (Mantovani & Scali, 1991; Tinti *et al.*, 1992). Similarly, the same occurs in Sardinian *B. atticus* demes (Mantovani *et al.*, 1990; Tinti & Scali, 1990, 1991) and in *B. whitei* (Manaresi *et al.*, 1992 a, b; Mantovani *et al.*, 1992).

The finding in Northern Sardinia and Corsica of numerous parthenogenetic demes of *B. rossius* with variously repatterned cytotypes, suggests a deeper analysis of additional cytogenetic features (such as nucleolar organizer regions and satellites) as already made for Sardinian *B. atticus* (Tinti & Scali, 1991), *B. whitei* (Manaresi *et al.*, 1992 a, b) and Sicilian *B. r. redtenbacheri* (Manaresi *et al.*, 1991).

The diploidization mechanism of facultative parthenogenesis of *B. rossius* (Pijnacker, 1969; Scali, 1969), well explains how easily the 4 + 5 Robertsonian fusion became homozygous. Furthermore, repatterned females lab-crossed to standard males have given a F_1 which, if crossed to standard *Bacillus* females, in turn produced a genetically normal offspring with balanced repatterned and standard haplosets (unpublished data).

The two fertile standard males, collected at Albo and S. Giulie, could be produced by parthenogenesis through the early loss of an X chromosome of a female germ. Spanandry could in turn promote the return to amphigony from thelytokous facultative parthenogenesis of these demes, as already suggested for some French demes by Bullini (1966).

On the whole, Corsican *B. r. rossius* appear – either on chromosome and allozymic grounds – more similar to the geographically close Sardinian and Elban parthenogenetic demes than to other *B. r. rossius*, suggesting that these samples may have had a parallel evolution. This observation is in line with the paleogeographic hypothesis about the origin and the evolution of these microplates during the Tertiary (Boccaletti & Guazzone, 1970; Auzende *et al.*, 1971; Alvarez, 1972), although one would expect higher genetic differentiation levels for long isolated populations.

The similar allelic patterns and the low genetic distances between Spanish populations (collected in *B. r. catalauniae* area), when compared to reference *B. r. rossius* samples, reveal that all belong to the same subspecies, which therefore appears to extend its range to Northern Catalonia. The genetic distance value reported by Nascetti and Bullini (1983) between *B. r rossius* and *B. r. catalauniae* (D = 0.161) is substantially higher than those obtained here. The divergence does not seem ascribable to sampling differences, since both collections are from the Tossa surroundings. On the other hand, actual electrophoretic data about *B. r. catalauniae* are not given (Nascetti & Bullini, 1983), so that an accurate and deeper comparison is not possible. At any rate, the present analysis suggests that, either *B. r. catalauniae* is nowadays confined to Barcelona surroundings, the other collected site (see Scali *et al.*, 1987), or that the sample from Tossa surroundings analyzed by Nascetti and Bullini (1983) was not fully representative.

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