Karyotypes and Ag-NORs of the mussels *Mytilus californianus* and *M. trossulus* from the Pacific Canadian coast

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Abstract

The karyotypes and the nucleolus organizer regions (NORs) of *M. californianus* and *M. trossulus* from the Pacific coast are reported here for the first time. The karyotypes show seven metacentric and seven submetacentric chromosome pairs. In *M. californianus*, nucleolar organizer regions appear located in terminal position on the short arm of one metacentric chromosome pair and of one submetacentric. A third Ag-NOR is located near to the telomere region on the short arm of one submetacentric. In *M. trossulus*, Ag-NORs appear terminally located on the short arms of two metacentric chromosome pairs and on the short and long arms of one submetacentric. The comparison of the karyotypes and Ag-NORs among the species of mussels from the Pacific and European coasts shows remarkable differences.

Keywords: *Mytilus californianus*; *Mytilus trossulus*; chromosome; karyotype; NOR.

Introduction

For several years, the taxonomy of the genus *Mytilus* has been the subject of an important controversy. Despite a great number of studies on morphological, electrophoretic (allozyme) and morphometric parameters, the taxonomic status of the genus *Mytilus* remains unclear. Morphological characters are remarkably variable in mussels, but electrophoretic analysis of different allozymes has allowed the recognition of five or six species belonging to the genus *Mytilus*: *M. edulis*, *M. galloprovincialis*, *M. trossulus*, *M. desolationis*, *M. californianus* and, perhaps, *M. coruscus* (McDonald and Koehn, 1988; McDonald et al., 1991; Gosling, 1992; Seed, 1992). The application of cytogenetic techniques has supplied information about the genetics of this genus, and it is obvious that it opens new possibilities to clarify the taxonomic status. Chromosome number and morphology have been described in *M. edulis, M. galloprovincialis, M.* trossulus and *M. desolationis* (Ahmed and Sparks, 1970; Ieyama, 1983; Moynihan and Mahon, 1983; Thiriot-Quiévreux, 1984; Dixon and Flavell, 1986; Pasantes et al., 1990; Insua et al., 1994).

Staining of nucleolar organizer regions (NORS) and/or C-banding were carried out in some of these *Mytilus* species (Dixon et al., 1986; Cornet, 1993; Insua et al., 1994; Martinez-Expósito et al., 1994). However, it is very complicated to obtain other chromosome banding patterns in molluscs. Méndez et al. (1990) described a G-banding pattern in *M. galloprovincialis* chromosomes employing a 2 x SSC solution. At the moment, only

Martínez-Lage et al. (1994) have widely characterized the karyotype of *M. galloprovincialis* after C-banding, fluorochrome and restriction endonuclease treatments. Furthermore, they classified and identified the chromosome complement of this species by means of G-banding obtained after digestion with trypsin enzyme. The same authors (Martínez-Lage et al., 1995) described the existence of changes in the constitutive heterochromatin of *M. edulis*, *M. galloprovincialis* and *M. trossulus* on the basis of C-, NOR and fluorescence banding patterns. The detection of different chromosome markers allows the identification of these three mussel species. We must point out that all these cytogenetic studies were carried out in mussels distributed throughout the European coasts, with the exception of *M. desolationis* samples (Kerguelen Isles) analyzed by Thiriot-Quiévreux, 1984 and the population of *M. galloprovincialis* studied by Ieyama (1983) in Seto (Japan).

The Pacific coast of Canada and North America is inhabited by three species of the mussel *Mytilus*: *M. galloprovincialis* Lamarck, 1819, *M. californianus* Conrad, 1837 and *M. trossulus* Gould, 1850. *M. californianus* is easily identified by the presence of radiating ribs on the shell (Soot-Ryen, 1955) and because it inhabits exposed shores along the coast (Seed, 1976). There is little information about *M. californianus*. To our knowledge, only Ahmed and Sparks (1970) have studied the chromosome number and morphology from primary oocytes and embryos of this species. We have not found references to electrophoretic data. Perhaps, because the specific status of *M. californianus* has never been in question (Gosling, 1992). Furthermore, Ahmed and Sparks (1970) have analyzed the chromosome number and morphology of *M. edulis* (ecotype *M. trossulus*). Allozyme data about *M. trossulus* from the Pacific coast were reported by McDonald and Koehn (1988); McDonald et al. (1991) and Sarver and Foltz (1993).

In this work, we analyzed the karyotypes of *M. californianus* and *M. trossulus* from the Canadian Pacific coast, by means of karyotyping and Ag-NOR staining in an attempt to provide new data on the taxonomy of these species.

Materials and methods

Mussels were collected in two localities of Vancouver Island, British Columbia, (Canadian Pacific coast): *M. trossulus* from Esquimalt Lagoon and *M. californianus* from Point No Point. In the laboratory, animals were fed with *Isochrisis galbana* microalgae for 10 days. Then, mussels were treated with 0.005% colchicine for 6-8 h; gills were dissected and 0.56% KC1 solution was added for 15 min. Fixation was carried out in ethanol: glacial acetic acid (3:l) involving four changes of 10, 10, 20 and 20 min at 4°C. Fixed cells were dissociated in a 45% acetic acid: water solution and were dropped onto heated slides at 43°C. Metaphases were stained with 4% Giemsa in phosphate buffer pH 6.8 and NOR silver staining was performed according to Howell and Black (1980). Finally, the metaphases were photographed with a Nikon Optiphot microscope.

Chromosome analysis

Karyotypes were arranged by decreasing size and classified according to the centromeric index (Levan et al., 1964). Measurements of the total chromosome length and chromosome long arms length were carried out employing a Magiscan image analysis. Total chromosome length and chromosomal long arm length were measured in 10 metaphases of gill tissue from each one of the population studied. Mean value of the length of the chromosome long arms and the mean value for their total chromosome length were calculated for each one of the chromosome pairs. The relative length (100 X chromosome length/ total haploid length) and the centromeric index (100 X length of short arm/ total chromosome length) were also calculated. Finally, we calculated the mean value and the standard error (standard deviation/number of individuals) $^{1}/^{2}$ of the relative lengths and centromeric index.

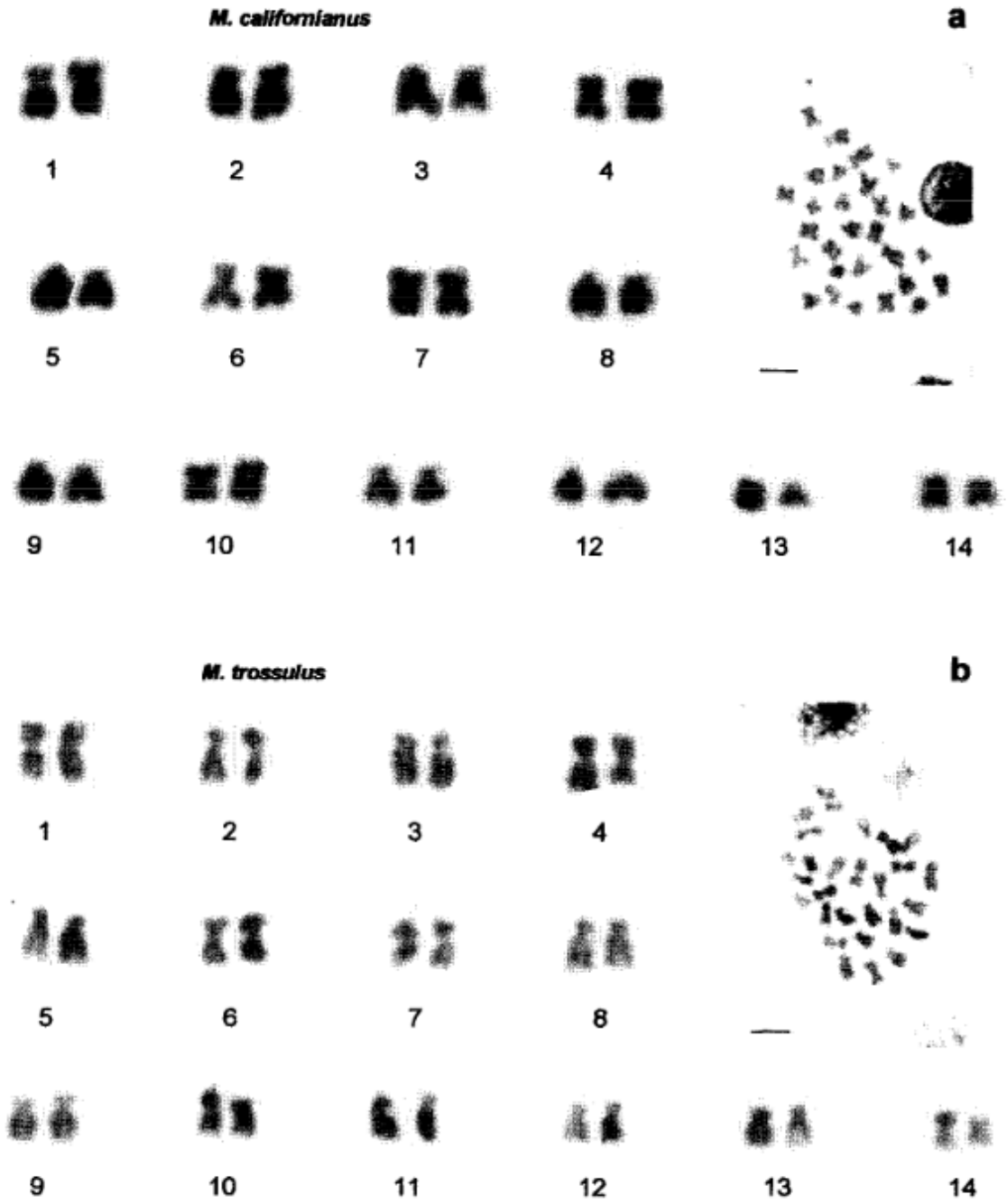

Figure 1. Giemsa stained karyotypes of (**a**) *Mytilus californianus* and (**b**) *M. trossulus*. Bar = 5 µm

Results

A diploid complement of 28 chromosomes was confirmed for *M. californianus* and *M. trossulus* from the Canadian Pacific coast (Fig. 1). Table 1 gives relative length and centromeric index values. Relative length values varied from 9.37 to 5.60 for *M. californianus* and 9.53 to 5.61 for *M. trossulus*. There is only one chromosome pair (chromosome No. 6 from both species) with a centromeric index value which varies between the submetacentric and subtelocentric categories. In each one of the metaphases analyzed for *M. californianus* and *M. trossulus*, karyotypes show seven metacentric and seven submetacentric chromosome pairs (Fig. 1, Table 1). Rarely, in some metaphases, we have observed subtelocentric chromosomes, but we have never detected any telocentric chromosomes.

NORs were analyzed in 103 metaphases observed from a total of 23 individuals of *M. californianus* (Table 2). We have observed three silver-stained NORs located on chromosome pairs Nos. 3, 5 and 12 (Fig. 2*a*). Terminal NORs appear on the short arm of chromosome Nos. 3 and 12; the NOR located on chromosome No. 5 appears near to the telomere of the short arm (subterminal position) (Fig. 3*a*). A variable number of one to six Ag-NORs per cell was observed (Fig. 4, Table 2), with two NORs the most frequent case. The analysis of 37 metaphases on 11 individuals allowed us to detect that the Ag-NOR which shows high activity is located on chromosome No. 12. The NOR activity of chromosomes Nos. 3 and 5 is very similar and lower than the activity of the NOR located on chromosome No. 12 (Fig. 5*a*).

Table 1. Chromosome measurements and classification of *M. californianus* and *M. trossulus* populations

	M. californianus			*M. trossulus*		
C.N.	**Rel. Leng.**	**Cen. Ind.**	**Clas.**	**Rel. Leng.**	**Cen. Ind.**	**Clas.**
1	9.37 ± 0.21	40.36 ± 1.50	m	9.53 ± 0.24	43.82 ± 0.98	m
2	8.41 ± 0.21	28.96 ± 0.97	sm	8.44 ± 0.30	29.03 ± 0.89	sm
3	8.37 ± 0.17	37.88 ± 0.91	m	7.93 ± 0.23	44.12 ± 1.69	m
4	7.80 ± 0.11	44.87 ± 1.07	m	7.63 ± 0.18	43.20 ± 1.03	m
5	7.57 ± 0.16	28.28 ± 1.24	sm	7.54 ± 0.21	26.99 ± 0.86	sm
6	7.36 ± 0.16	26.08 ± 1.75	sm/st	7.35 ± 0.18	25.25 ± 1.12	sm/st
7	7.21 ± 0.18	44.10 ± 1.15	m	7.00 ± 0.20	26.16 ± 0.91	sm
8	7.15 ± 0.21	42.24 ± 1.32	m	6.92 ± 0.20	27.97 ± 1.18	sm
9	6.57 ± 0.12	43.64 ± 1.30	m	6.90 ± 0.24	43.16 ± 1.38	m
10	6.54 ± 0.12	27.71 ± 0.70	sm	6.85 ± 0.19	43.79 ± 0.85	m
11	6.25 ± 0.12	28.69 ± 0.74	sm	6.33 ± 0.18	42.67 ± 1.95	m
12	6.06 ± 0.14	31.33 ± 1.05	sm	6.27 ± 0.24	30.01 ± 1.38	sm
13	5.74 ± 0.25	43.38 ± 0.72	m	5.71 ± 0.17	28.69 ± 0.94	sm
14	5.60 ± 0.16	28.46 ± 1.01	sm	5.61 ± 0.25	45.94 ± 0.72	m

C.N.: Chromosome number; **Rel. Leng.**: Relative length; **Cen. Ind.**: Centromeric Index; **Clas.**: Classification; **m**: metacentric; **sm**: submetacentric; **st**: subtelocentric.

Table 2. Summary of Ag-NORs in *M. californianus* and *M. trossulus*

Species	**No. Ag-NORs per metaphase**							**Metaphases**	**Individuals**
	0	1	2	3	4	5	6		
M. californianus									
Pair 3	14	15	8	-	-	-	-	37	11
Pair 5	15	17	5	-	-	-	-	37	11
Pair 12	6	18	13	-	-	-	-	37	11
T. no. NOR	-	9	38	28	22	2	4	103	23
M. trossulus									
Pair 3	15	22	1	-	-	-	-	38	10
Pair 7 short arm	10	18	10	-	-	-	-	38	10
Pair 7 long arm	32	6	0	-	-	-	-	38	10
Pair 9	16	6	6	-	-	-	-	38	10
T. no. NOR	-	8	23	27	3	3	1	69	23

T. no. NOR: total number of NORs per metaphase

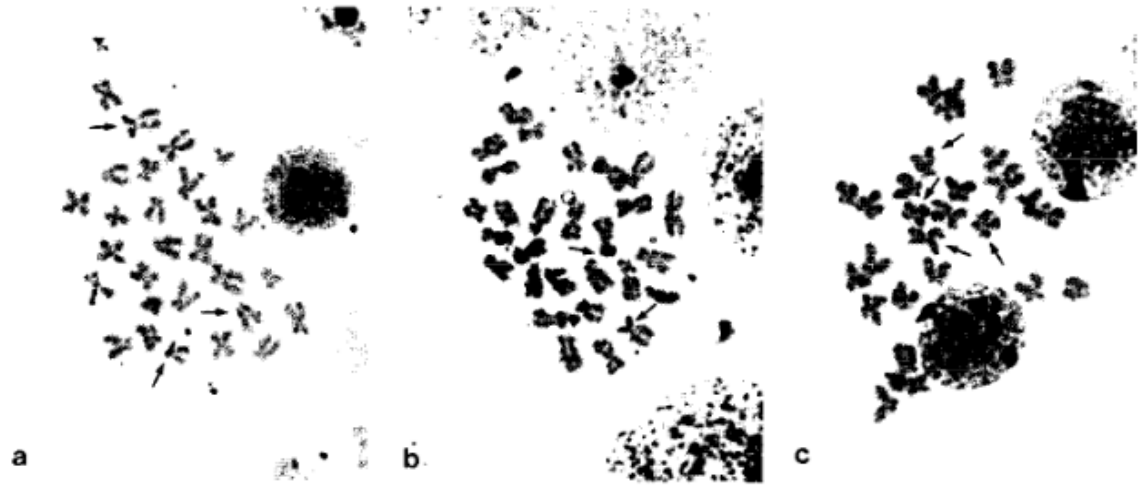

Figure 2. Silver-stained metaphases of (**a**) *M. californianus*; (**b** and **c**) *M. trossulus*. Arrows show the Ag-NOR regions.

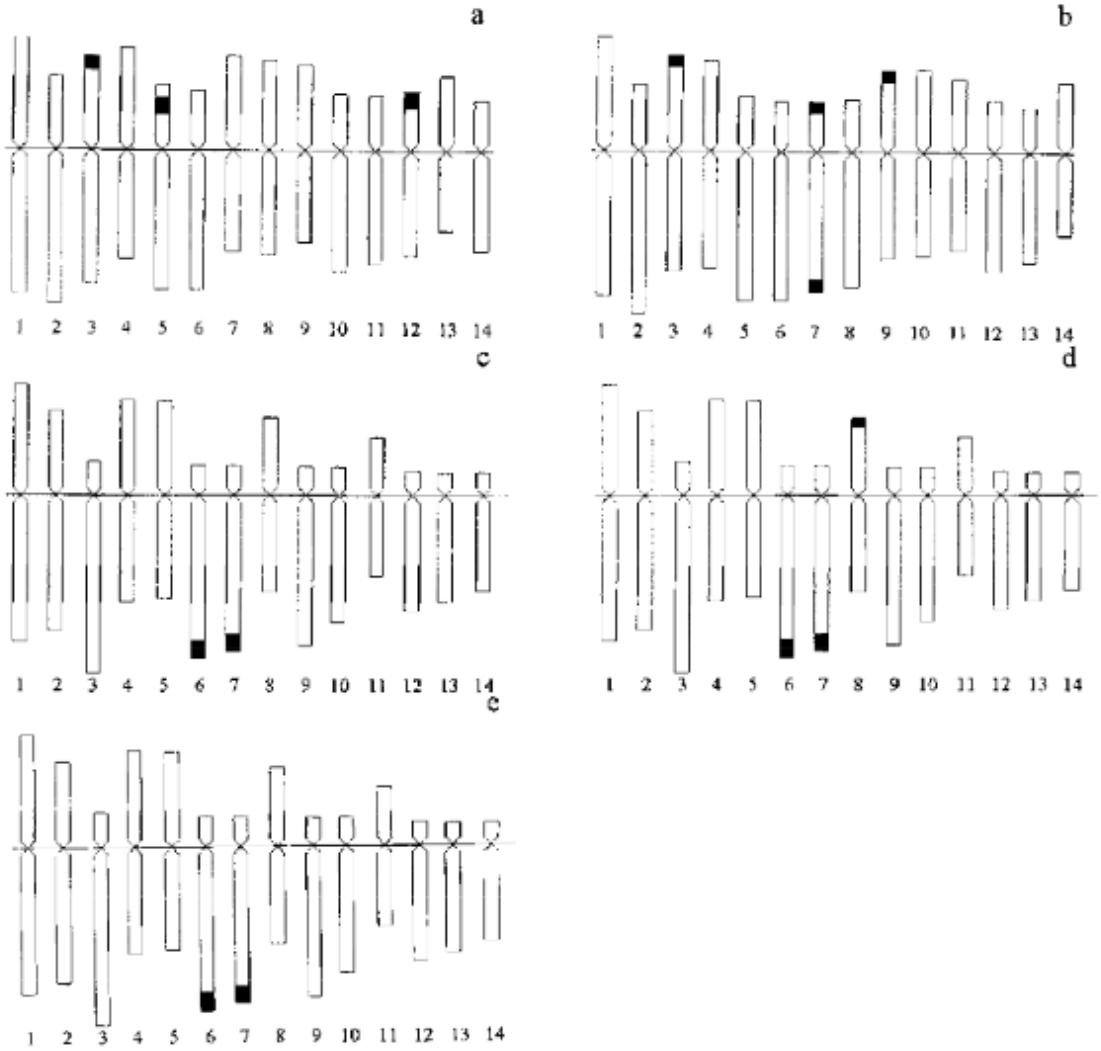

Figure 3. Idiograms of Ag-NORs in (**a**) *M. californianus* from the Pacific Canadian coast; (**b**) *M. trossulus* from the Pacific Canadian coast; (**c**) *M. edulis* from the Atlantic Dutch coast; (**d**) *M. trossulus* from the Baltic German coast and (**e**) *M. galloprovincialis* from the Atlantic Spanish coast.

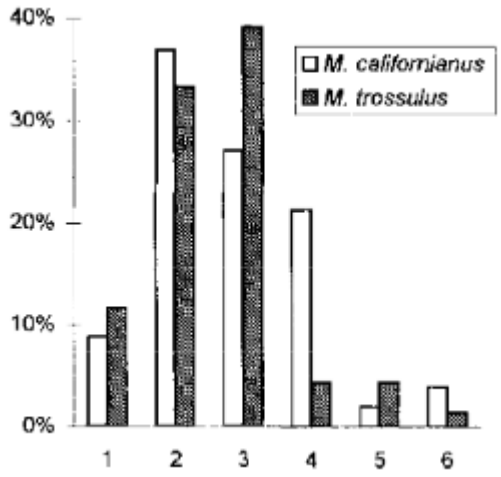

Figure 4. Frequency of metaphases with one to six active NORs in the species studied.

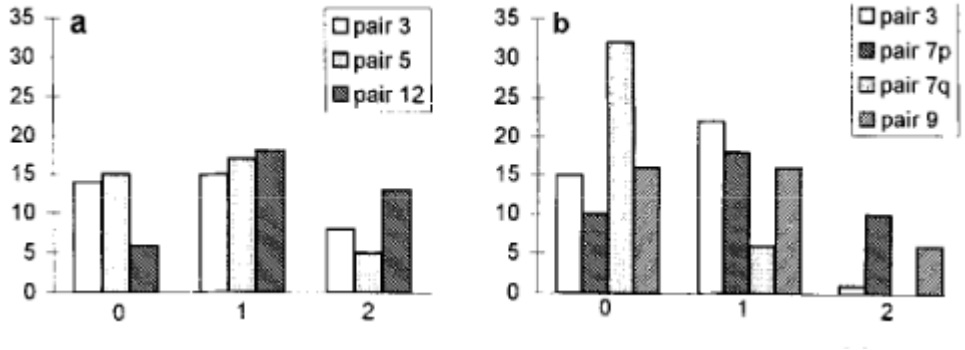

Figure 5. Number of metaphases with 0, 1 or 2 active NORs per chromosome pair in (**a**) *M. californianus* and (**b**) *M. trossulus*.

In *M. trossulus*, NORs were analyzed in 69 metaphases from 23 individuals (Table 2) and we have observed four Ag-NORs terminally located on the short arms of chromosome pairs Nos. 3 and 9. On chromosome No. 7, Ag-NORs appear located on the telomeres of the short and long arms (Fig. 2*b*, Fig. 3*b*). The number of Ag-NORs varies from one to six (Fig. 4, Table 2), with three NORs being the most frequent case. To analyze 38 metaphases from 10 individuals we can observe that Ag-NORs on the telomeres of the short arms of the chromosome Nos. 7 and 9 show high activity. The Ag-NOR on the telomere of chromosome No. 7 shows low activity (Fig. 5*b*).

Discussion

We confirm that *M. californianus* and *M. trossulus* show a diploid chromosome number of $2n = 28$. Metaphases above or under 28 chromosomes appear in a very lower proportion. When we determined the amount of DNA in mussel cells, employing flow cytometry, we showed that the amount of DNA is very constant (Méndez et al., unpublished data).

The values obtained for the relative length of individual chromosomes are very similar between the two species. No differences were observed when we compared these values with those obtained in *M. edulis*, *M. galloprovincialis*, *M. trossulus* or *M. desolationis*. Maximum values are close to 10 and minimum above 5 (Moynihan and Mahon, 1983; Thiriot-Quiévreux, 1984; Dixon and Flavell, 1986; Pasantes et al., 1990; Insua et al., 1994). The karyotypes of both populations show seven metacentric chromosome pairs and seven submetacentric. These results reflect clear differences in relation to the other populations we have examined.

In this sense, the karyotypes of different populations of *M. trossulus* from the Baltic Sea consist of six metacentric chromosome pairs and eight submetacentric-subtelocentric (Insua et al., 1994; Martínez-Lage et al., 1995).

The variability observed in the centromeric index values, which are close to 25 (value established by Levan et al., 1964 as limit between submetacentric and subtelocentric chromosomes) could be due to the different degree of condensation of the chromosome arms (amongs other causes). Such variability is accompanied by variations in the classification of the chromosomes from one metaphase to another. So, in *M. californianus*, generally, the chromosome pairs Nos. 2, 5, 6, 10, 11, 12 and 14 are submetacentric; however, in some metaphases they may appear as subtelocentric; as with chromosome pairs Nos. 2, 5, 6, 7, 8, 12 and 13 of *M. trossulus.*

On the other hand, *M. californianus* Ag-NORs were found located on the short arms of three chromosome pairs. These locations do not show any similarity with the positions of the Ag-NORs detected in populations of *Mytilus* from European coasts. Two of the four positions of the Ag-NORs detected in *M. trossulus* from Canada, were not found in European mussels. In this sense, Insua et al. (1994) and Martínez-Lage et al. (1995) found three Ag-NORs regions in *M. trossulus* from Baltic Sea. Such regions were terminally located on the long arms of two medium-size chromosome pairs submetacentric-subtelocentric and on the short arm of one medium-size metacentric chromosome pair. As suggested by Martínez-Lage et al. (1995) these chromosome pairs could be Nos. 6, 7 and 8.

Taking this into account, we could suppose that the chromosome No. 9 of *M. trossulus* from Canada is equal to the chromosome No. 8 of *M. trossulus* from Baltic Sea. Similarly, the telomeric region of the long arm of chromosome No. 7 of *M. trossulus* from Canada could be chromosome Nos. 6 or 7 from European populations. However, there is a different location among the populations of *M. trossulus* from Canada in relation to the other Ag-NOR regions detected in the chromosomes (i.e. located on the short arms of chromosomes 3 and 7). These characteristics are accompanied of different chromosome morphology too. So, although the allozymic data relating to *M. trossulus* from North Pacific coast and from Baltic Sea suggest that they are a same taxon (Bulnheim and Gosling, 1988; Varvio et al., 1988; McDonald et al., 1991; Väinölä and Hvilsom, 1991), our results suggest that both populations of *M. trossulus* are clearly different. In this sense, as suggested by Geller et al. (1993) if we assume that the rate of nucleotide substitution in the 16S rDNA gene is similar in the different species of *Mytilus*, *M. trossulus* is an older species than *M. edulis* and *M. galloprovincialis*, and, subsequently, could be supposed that *M. edulis* on the Atlantic shore of North America may derive from Pacific population of *M. trossulus* which dispersed through the Arctic Sea in an interglacial or postglacial period (Geller et al., 1993). This idea is supported by the apparent North Pacific origin of much of the North Atlantic marine fauna (Vermeij, 1991, 1992; Reid, 1990). Our results suggest that new chromosome changes may have taken place after mussels settled in new ecological niches from North America to the North Atlantic. These changes were fixed giving rise to the different European taxa. A strong preservation of the primitive chromosome morphology (chromosome Nos. 7 and 8) would take place in the Baltic Sea population. They show a high similarity in the allozyme frequencies, too. However, when *M. trossulus* frequencies are compared between regions, e.g. the Baltic Sea (Tvärminne) and the west coast of North America (Tillamook), there are large differences between them at most of the analyzed loci (Gosling, 1992). Marked allele frequency differences (no significant values given) between Baltic and Canadian maritime *M. trossulus* have also been observed at four out the five loci analyzed by Varvio et al. (1988). By all these reasons, we suggest that the mussel populations from Baltic Sea may be considered as a different taxon from *M. trossulus*.

On the other hand, we must point out that Dixon et al. (19861, in *M. edulis*, found one Ag-NOR on the telomere of a large metacentric chromosome. Martínez-Expósito et al. (1994), analyzing different *M. galloprovincialis* populations from the northwest of Spain, describe the existence of one Ag-NOR (in nine metaphases out of 411) in a large metacentric chromosome. We think that such Ag-NORs could be the same as chromosome No. 3 from *M. trossulus*. Furthermore, in mussels from Baltic Sea, we have detected two metaphases with a medium size submetacentric-subtelocentric chromosome showing one Ag-NOR on the

telomere of the short arm (unpublished data). This chromosome could be equivalent to chromosome No. 7 of *M. trossulus* from Pacific coast.

There is a clear similarity between *M. californianus* and *M. trossulus*. Their karyotypes are constituted by the same number of metacentric and submetacentric chromosomes; chromosome No. 3 in both species show great similarity, and the chromosome No. 12 from *M. californianus* and the chromosome No. 7 from *M. trossulus* are very similar, too. These data suggest a greater similarity between *M. californianus* and *M. trossulus* from Pacific coast than exists among European *Mytilus* species.

In relation to the Ag-NOR activity, it is very similar in both species (Fig. 4), although slightly higher in *M. trossulus*. The values are similar to those reported in the European mussels by Insua et al. (1994) but higher than the values detected in adult mussels populations from northwest of Spain and lower than larvae from the same population (Martínez-Expósito et al., 1994). We must point out that, occasionally, we have detected the existence of six actives Ag-NORs per metaphase (four metaphases of *M. californianus* and one of *M. trossulus*, Table 2).

This analysis shows remarkable differences in the chromosome morphology of mussel *Mytilus* from Pacific and North Atlantic coasts. It would be very important to analyze the chromosome morphology of *Mytilus* from American Atlantic coast. Furthermore, we hope that the analysis of satellite DNA, by means of in situ hybridization, which we are performing at present, will provide additional data to clarify the phylogeny of these species of mussel.

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