Chromosome differences between European mussel populations (genus *Mytilus*)

A. Martínez-Lage[a], A. González-Tizón[a] & J. Méndez[a*]

[a]Departamento de Biología Celular y Molecular, Facultad de Ciencias, Universidad de La Coruña, 15071, La Coruña, Spain

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Abstract

We have analysed different populations of European mussels belonging to genus *Mytilus* in order to clarify the karyotype complements of these three species (*M. edulis, M. galloprovincialis* and *M. trossulus*). We have employed different cytogenetic techniques (C-banding, fluorochrome staining and Ag-NOR staining) and we have carried out chromosome measurements by using image analysis. Results obtained allow us to confirm the diploid number of these species and to detect different karyotype composition. Furthermore, we describe cytogenetical differences between larval chromosomes and gill tissue chromosomes.

Introduction

Initially, the taxonomic studies developed in order to clarify the systematic status of mussels belonging to the genus *Mytilus* were, basically, based on morphometric parameters and electrophoretic studies. In this sense, although none of the loci studied appear to be truly diagnostic and none of the morphometric characters analysed have allowed to assign a sample to a concrete species (KOEHN 1991; GARDNER 1992; GOSLING 1992; SEED 1992), they are virtually diagnostic. Multivariate analysis of some character provides evidence for the existence of three different taxa: *Mytilus edulis* (Linnaeus, 1758), *M. galloprovincialis* (Lamarck, 1819) and *M. trossulus* (Gould, 1850). Furthermore, it is proved that the genetic distance that separates these three taxa is characteristic of subspecies (SKIBINSKI et al. 1980; GRANT and CHERRY 1985; VÄINÖLÄ. and HVILSOM 1991).

In Europe, *M. edulis* is distributed from White and Baltic seas (McDONALD et al. 1990, 1991) to the south of Atlantic French coasts (SEED 1972, 1978; McDONALD et al. 1990). *M. galloprovincialis* appears distributed from Black sea along the Mediterranean coasts, great zones of the southwest of England (HEPPER 1957; LEWIS and SEED 1969) and the western French (SEED 1972) and Irish (SEED 1974) coast. *M. trossulus* is distributed by the Baltic sea (BULNHEIM and GOSLING 1988; VARVIO et al. 1988).

Studies of *Mytilus* cytogenetics have allowed to identify the karyotype of each one of the species distributed in Europe (MARTINEZ-LAGE et at. 1995). Genus *Mytilus* shows a diploid number of 28 chromosomes and there are a great number of studies which describe chromosome measurements (IEYAMA 1983, 1984; MOYNIHAN and MAHON 1983; THIRIOT-QUIEVREUX 1984; DIXON and FLAVELL 1986; PASANTES et at. 1990; lNSUA et at. 1994). *M. edulis* chromosome complement is constituted by six pairs of metacentric and eight pairs or submetacentric/subtelocentric chromosomes. *M. trossulus* shows the same chromosome complement (INSUA et at. 1994), but *M. galloprovincialis* shows different number of

metacentric chromosomes depending on the population analysed. So, while THIRIOT-QUIÉVREUX (1984) and lNSUA et at. (1994) observed that the kayotype is constituted by five pairs of metacentric chromosomes and nine pairs of submetacentric/subtelocentric chromosomes, DIXON and FLAVELL (1986) and PASANTES et at. (1990) found six pairs of metacentric and eight pairs of submetacentric/subtelocentric chromosomes (the same as *M. edulis* and *M. trossulus*). The population studied by lEYAMA (1983) in Seta (Japan) was iniatially described as *M. edulis*, but according to electrophoretic criteria it seems to be *M. galloprovincialis* (WILKINS et at. 1983), and their karyotype is constituted by five pairs of metacentric chromosomes and nine of submetacentric/subtelocentric chromosomes. Telocentric chromosomes have not been described in these species (MOYNIHAN and MAHON 1983; THIRIOT-QUIÉVREUX 1984; DIXON and FLAVELL 1986; lNSUA et at. 1994). lNSUA et al. (1994) suggest that these differences in the morphological composition could be due to i) the occurrence of chromosomal polymorphism, as suggested by AHMED and SPARKS (1970), ii) the different techniques employed by the workers, or iii) the insufficient sampling per species.

On the other hand the use of chromosome banding techniques applied to molluscs has been scarce. So, nucleolar organizer regions (NORs) and C-bands were described by different authors (DIXON et al. 1986; CORNET 1993; lNSUA et al. 1994; MARTINEZ-EXPOSITO et al. 1994) and NORs, C-, fluorescence and restriction endonuclease banding were described by MARTINEZ-LAGE et al. (1994-1995).

In this work, we analyse different populations of *M. edulis*, *M. galloprovincialis* and *M. trossulus* in order to study the differences related to morphological composition of the karyotypes by means of C-banding, chromomycin Al (CAl) fluorochrome and NOR staining.

Materials and methods

Metaphases

Mussel populations analyzed in this work were collected from different places along the European coasts as shows Fig. 1. In the laboratory individuals were placed in tanks containing filtered sea water in photoperiodic cameras at 18 ± 1 °C (for mussels from Spain and Portugal) and 15 ± 1 °C (for mussels from Zoutelande, Harlesiel and Dahme). Individuals from Dahme population were placed in filtered sea water with a 10‰ saline concentration, meanwhile the others populations were placed in filtered sea water with a saline concentration of 35‰. Each one of the populations was fed continuosly on a suspension of *Isochrisis galbana* and *Tetraselmis suecica* microalgae for 10-15 days. Then colchicine (0.005%) was added during 6-8 hours; gills were dissected and treated with 0.56% KCl for 15 min. The gill tissue was fixed in ethanol: glacial acetic acid (3:1) involving four changes of 10, 10, 20 and 20 min. at 4°C. Fixed samples were dissociated in 45% acetic acid:water solution. Cells were dropped onto heated slides at 43°C.

Figure 1. Location of mussel populations analysed.

To obtain metaphases from mussel larvae we employed the method described by MARTINEZ-LAGE et al/. (1994).

Metaphases were stained with 4% Giemsa in phosphate buffer pH 6.8 and photographed with a Nikon optiphot microscope.

Chromosome analysis

Karyotypes were carried out according to PASANTES et al. (1990). Measurements of the total chromosome length and chromosome long arms length were carried out employing a Magiscan image analysis system. Total chromo-some length and chromosomal long arms length were measured in 20 metaphases of gill tissue from Cullera mussels, 10 metaphases of larvae and gill tissue from Fisterra mussels, 10 metaphases of larvae from Zoutelande and 3 metaphases of larvae from Dahme mussels. Measurements of chromatids were carried out when it was possible, i.e., when they appeared separated along chromosome structure (low condensated chromosomes). Then, we calculated the mean value of the length of the chromosome long arms and the mean value for their total chromosome length for each one of the chromosome pairs. The relative length (100 X chromosome length I total haploid length) and the centromeric index (100 X length of short arm I 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.

C-banding, NOR silver stainingh and CA3 staining were carried out as described MARTINEZ-LAGE {1994, 1995).

Results

The diploid number (2*n* = 28) has been confirmed in each one of the populations studied (Fig. 2). *M. edulis* and *M. trossulus* karyotypes show 6 metacentric chromosomes pairs and 8 submetacentric/subtelocentric pairs (Figs, 2a, b; Table 1). However, we have observed some differences in *M. galloprovincialis* populations. So, while Atlantic mussel karyotypes (Ribadeo, Fisterra, Baiona, Nazaré) are uniform in their composition (Figs. 2e, f, 3c), mussel karyotypes from Mediterranean coasts show differences in the number of metacentric chromosomes (Figs. 2c, d; 3a, b; Table 1); we have observed metaphases with 5 metacentric chromosomes pairs and 9 submetacentric/subte-locentric, and metaphases with 6 metacentric pairs and 8 submetacentric/subte-locentric pairs. This feature is individual specific, i.e., each one of the metaphases is constituted by the same number of metacentric chromosome pairs. We can observe a progressive increase in the number of metaphases with 5 metacentric pairs as we move forward to the south of the Iberian Peninsula (Table 1).

In order to investigate such differences, we carried out the measurements of the karyotypes from four different populations: Cullera, Fisterra, Zoutelande and Dahme. Individuals from Cullera populations were subdivided in two subpopulations which were named Cullera-5 and Cullera-6 (individuals with karyotypes showing 5 and 6 metacentric pairs respectively). We carried out the measurements of 10 metaphases from Fisterra populations obtained from gill tissue and 10 metaphases from larvae. In metaphases obtained from larvae we could analyse the role of the chromosome condensation because these metaphases always show lower condensation degree than metaphases obtained from adult tissues (Fig. 3c). Furthermore, we measured 10 metaphases from one of the *M. edulis* populations and 3 metaphases from *M. trossulus* population.

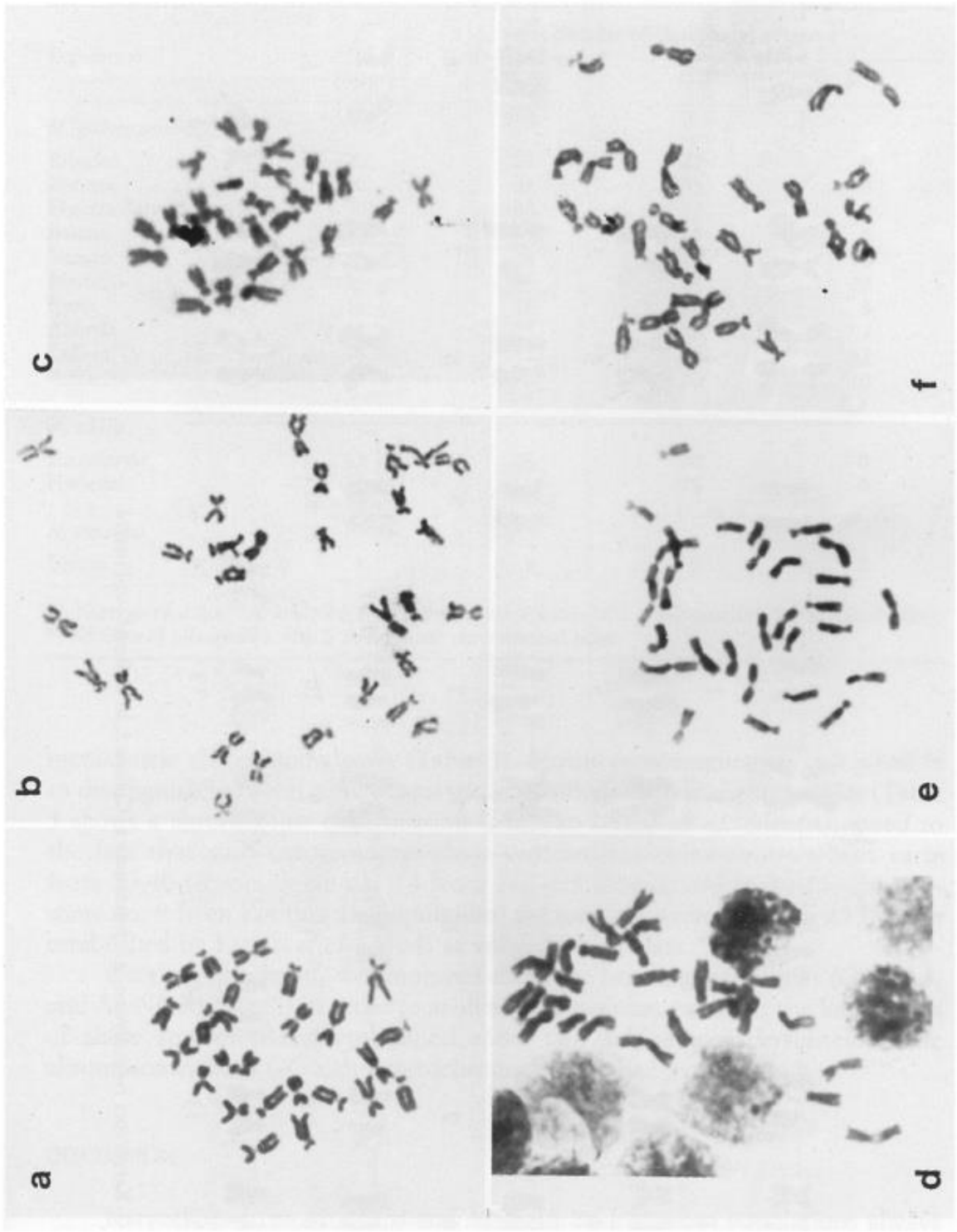

Figure 2. Metaphases from gill tissue **a**) *Mytillus edulis* (Zoutelande), **b**) *M. trossulus* (Dahme), **c**) *M. galloprovincialis* (Cullera-5), **d**) *M. galloprovincialis* (Cullera-6), **e**) *M. galloprovincialis* (Fisterra). **Fig. 2f** shows larve chromosomes from *M. galloprovincialis* (Fisterra)

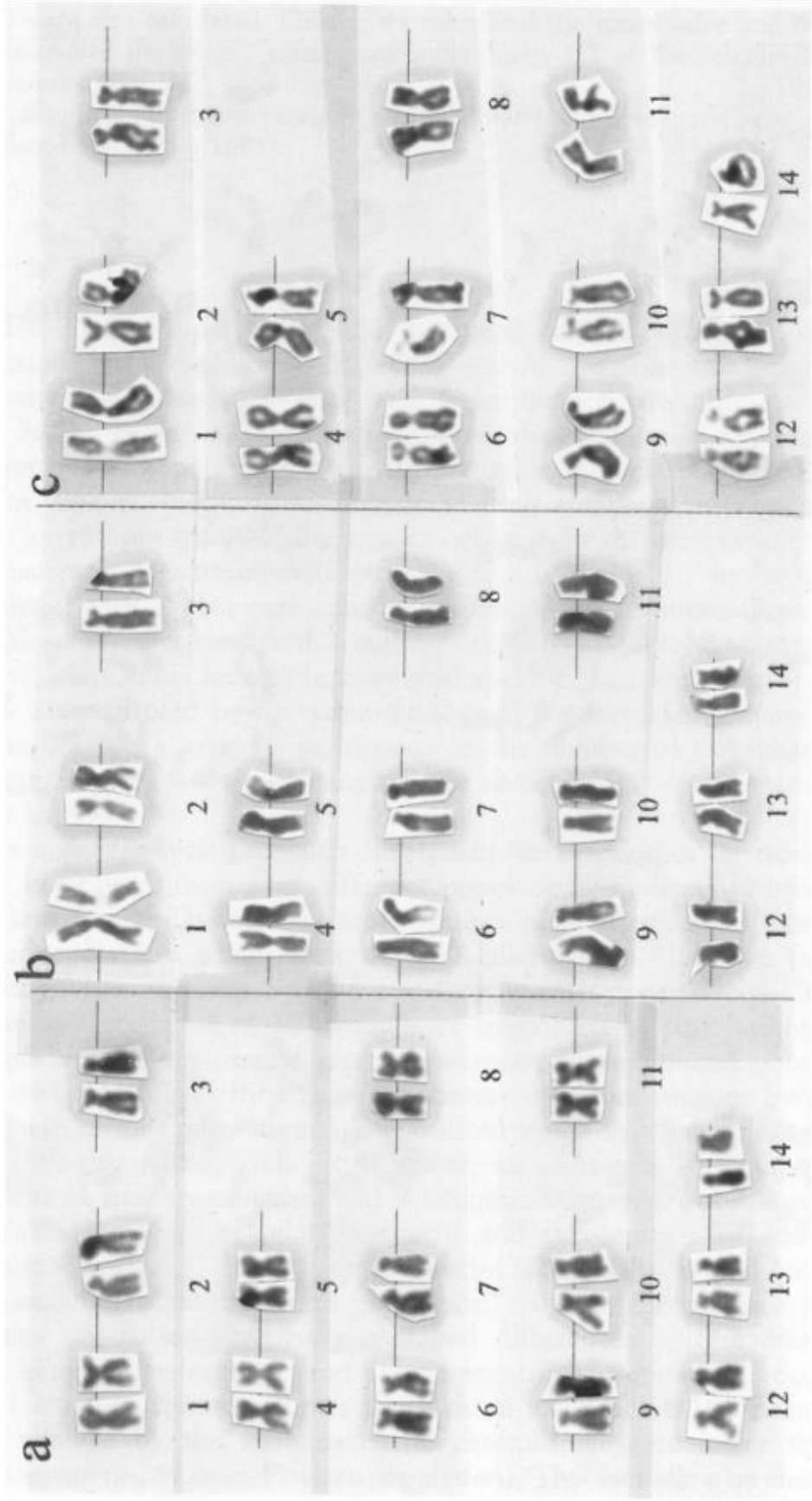

Figure 3. Karyotypes from *Mytillus galloprovincialis* **a**) Cullera-5, **b**) Cullera-6, **c**) Fisterra.

Table 1. Number of metaphases analysed in each one of the populations studied.

		Number of metaphases analysed		
Population	**N**	**Total**	**N-6**	**N-5**
M. galloprovincialis				
Ribadeo	20	27	27	0
Fisterra	30	45	45	0
Fisterra (larvae)	30	30	30	0
Baiona	30	45	45	0
Nazaré	10	14	14	0
Marbella	25	36	14	22
Nerja	15	18	10	8
Almería	18	23	16	7
Cullera	30	41	29	12
Peñíscola	27	30	20	10
M. edulis				
Zoutelande	10	20	20	0
Harlesiel	15	15	15	0
M. trossulus				
Dahme	2	3	3	0

N: Number of individual analysed; **6**: Number of metaphases with 6 metacentric chromosomal pairs; **5**: Number of metaphases with 5 metacentric chromosomal pairs.

Table 2 shows the relative lengths and the centromeric index of the populations analysed. Relative lengths varies from 9.86 (chromosome no. 1 in adults individuals from Fisterra population) to 4.82 (chromosome no. 14 for the same population); we have observed differences in centromeric index values between metacentric and submetacentric chromosomes. So, chromosome 1 from *M. trossulus* shows a minimum value of 38.18, meanwhile the values showed by the submetacentric chromosomes are lower than 29.40 (chromosome no. 14 from Fisterra population). This fact allow us clearly detect metacentric chromosome pairs (Table 3). However, sometimes is not possible to distinguish between submetacentric and subtelocentric chromosomes (Table 3 shows a chromosome classification based on LEVAN et al. criteria), owed to the fact that such chromosomes show centromeric index values which varie from 29.40 (chromosome no. 14 from Fisterra population) to 20.75 (chromosome no. 9 from Zoutelande population). These values overlap with 25.0 value established by LEVAN et al. (1964) as value limit of class.

On the other hand, we employed different banding techniques (C-, CA3, and Ag-NOR) (Fig. 4) in order to analyse, at cytogenetical level, the karyotypes of these species. Results obtained show two submetacentric/subtelocentric chromosomes with GC rich heterochromatic regions.

Table 2. Relative length and centromeric index values for each one of chromosome pairs in *Mytilus galloprovincialis*, *M. edulis* and *M. trossulus*

C.N	*Mytilus galloprovincialis*								*M. edulis*		*M. trossulus*	
	Fisterra (larvae)		Fisterra		Cullera-5		Cullera-6		Zoutelande		Dahme	
	R.L.	**C.I.**	**R.L.**	**C.I.**	**R.L.**	**C.I.**	**R.L.**	**C.I.**	**R.L.**	**C.I.**	**R.L.**	**C.I.**
1	9.48 ± 0.12	39.67 ± 0.91	9.86 ± 0.23	42.59 ± 1.38	9.51 ± 0.23	39.38 ± 1.06	9.54 ± 0.29	42.09 ± 0.15	9.61 ± 0.15	40.94 ± 1.40	9.57 ± 0.36	38.18 ± 1.11
2	8.30 ± 0.13	39.59 ± 1.35	8.35 ± 0.30	41.28 ± 1.25	8.67 ± 0.15	22.43 ± 1.49	8.79 ± 0.36	40.87 ± 0.11	8.67 ± 0.25	41.22 ± 1.42	8.17 ± 0.09	45.33 ± 1.52
3	8.03 ± 0.16	23.38 ± 0.98	8.17 ± 0.27	24.76 ± 1.62	7.91 ± 0.12	25.94 ± 2.02	8.29 ± 0.20	23.17 ± 0.11	8.18 ± 0.16	21.78 ± 1.54	8.14 ± 0.13	24.79 ± 1.31
4	7.73 ± 0.16	45.75 ± 1.21	7.72 ± 0.16	43.84 ± 0.98	8.17 ± 0.21	41.17 ± 1.01	8.29 ± 0.14	43.37 ± 0.68	7.88 ± 0.07	42.11 ± 1.58	8.07 ± 0.11	43.69 ± 1.24
5	7.26 ± 0.11	44.64 ± 1.02	7.63 ± 0.12	44.41 ± 1.03	7.52 ± 0.10	44.38 ± 0.81	7.42 ± 0.17	46.08 ± 0.98	7.39 ± 0.11	41.02 ± 1.66	7.58 ± 0.17	41.01 ± 1.54
6	7.63 ± 0.15	21.52 ± 0.68	7.46 ± 0.21	22.67 ± 1.39	7.48 ± 0.10	22.27 ± 1.44	7.69 ± 0.09	24.92 ± 1.07	7.63 ± 0.12	23.43 ± 1.15	7.56 ± 0.19	21.50 ± 2.05
7	7.24 ± 0.07	23.42 ± 0.97	7.19 ± 0.14	25.15 ± 1.58	7.03 ± 0.08	22.35 ± 1.20	6.99 ± 0.08	23.46 ± 0.88	7.38 ± 0.14	24.15 ± 1.78	7.09 ± 0.22	21.11 ± 1.64
8	6.77 ± 0.09	42.99 ± 0.73	7.20 ± 0.24	42.62 ± 1.50	7.12 ± 0.08	44.12 ± 1.09	6.84 ± 0.19	43.98 ± 1.82	6.86 ± 0.13	43.07 ± 1.40	6.57 ± 0.33	40.86 ± 1.14
9	7.08 ± 0.06	23.13 ± 1.67	6.70 ± 0.30	28.74 ± 1.34	6.80 ± 0.07	23.78 ± 0.73	6.94 ± 0.16	25.41 ± 1.61	6.94 ± 0.15	20.75 ± 2.69	6.88 ± 0.17	22.20 ± 1.54
10	6.68 ± 0.10	25.63 ± 0.56	6.61 ± 0.13	25.08 ± 1.12	6.53 ± 0.06	22.45 ± 1.40	6.54 ± 0.13	23.76 ± 0.86	6.76 ± 0.14	24.06 ± 1.73	6.71 ± 0.23	28.22 ± 1.58
11	6.10 ± 0.16	40.46 ± 1.45	6.41 ± 0.19	41.72 ± 1.21	6.33 ± 0.18	42.75 ± 1.37	5.92 ± 0.27	41.97 ± 0.65	6.07 ± 0.23	41.35 ± 1.11	6.04 ± 0.09	40.50 ± 1.18
12	6.44 ± 0.07	25.47 ± 0.72	6.07 ± 0.20	26.53 ± 1.17	6.11 ± 0.12	25.92 ± 1.60	6.30 ± 0.12	26.54 ± 1.64	6.13 ± 0.09	27.32 ± 1.10	6.45 ± 0.09	24.07 ± 0.48
13	5.93 ± 0.11	26.36 ± 1.24	5.65 ± 0.13	24.94 ± 1.30	5.67 ± 0.13	23.57 ± 1.26	5.41 ± 0.25	25.78 ± 1.54	5.49 ± 0.23	24.90 ± 2.22	5.94 ± 0.14	26.11 ± 0.98
14	5.34 ± 0.07	28.87 ± 0.87	4.82 ± 0.10	29.40 ± 1.17	5.14 ± 0.14	25.80 ± 1.31	5.05 ± 0.12	25.31 ± 1.61	5.01 ± 0.25	25.37 ± 2.38	5.23 ± 0.31	24.51 ± 0.59

C.N.: Chromosome number; **R.L**.: Relative length; **C.I.**: Centromeric index

Table 3. Chromosome pairs morphology from Fisterra and Cullera populations (*Mytilus galloprovincialis*), Zoutelande (*M. edulis*) and Dahme (*M. trossulus*).

C.N.	*Mytilus galloprovincialis*				*M. edulis*	*M.trossulus*
	Fisterra (lar.)	Fisterra	Cullera 5	Cullera 6	Zoutelande	Dahme
1	m	m	m	m	m	m
2	m	m	st	m	m	m
3	st	st/sm	sm/st	st	st	st/sm
4	m	m	m	m	m	m
5	m	m	m	m	m	m
6	st	st	st	st/sm	st	st
7	st	sm/st	st	st	st/sm	st
8	m	m	m	m	m	m
9	st	sm	st	sm/st	st	st
10	sm	sm/st	st	st	st/sm	sm
11	m	m	m	m	m	m
12	sm/st	sm	sm/st	sm/st	sm	st
13	sm	st/sm	st	sm/st	st/sm	m
14	sm	sm	sm/st	sm/st	sm/st	st/sm

C.N.: Chromosome number.; **m**: metacentric chromosome; **sm**: submetacentric chromosome; **st**: subtelocentric chromosome.

Discussion

Karyotypes from *M. edulis* and *M. trossulus* European populations always show 6 pairs of metacentric and 8 pairs of submetacentric-subtelocentric chromosomes. The proportion of submetacentric and subtelocentric chromosomes varies from 8 submetacentric pairs (MOYNIHAN and MAHON 1983) to 1submetacentric and 7 subtelocentric pairs (INSUA et al. 1994). Only THIRIOT-QUIEVREUX (1984), analysing a population from Charron (France), described the presence of two chromosome pairs which were very difficult to classify as metacentric or submetacentric. In most published papers there is not any difficult to distinguish between metacentric and submetacentric chromosomes. However, most of authors had difficulties to classify submetacentric and subtelocentric chromosomes. Such as previosly suggested AHMED and SPARKS (1970), MOYNIHAN and MAHON (1983) and DIXON and FLAVELL (1986), it is due to a chromosome polymorphism. INSUA et al. (1994) pointed out that this polymorphism is the consequence of a variation in the centromeric index values, which may be greater in chromosomes with unequal arms. This is because chromosome condensation is a dynamic process that proceeds at different rates along the length of each chromosome (FRANCKE and OuviER 1978; YuNIS 1980). In chromosomes with a subterminal centromere, the short arm may contract only moderately, whereas the long arm contracts in much greater extent (DROUIN et al. 1991). This problem is owed to centromeric index values, which are very near to 25.0. Acording to LEVAN et al. criteria (1964) such value represents the limit of classes. Then, in order to avoid possible errors, we suggest that both categories should be joined in only one called submetacentric/subtelocentric.

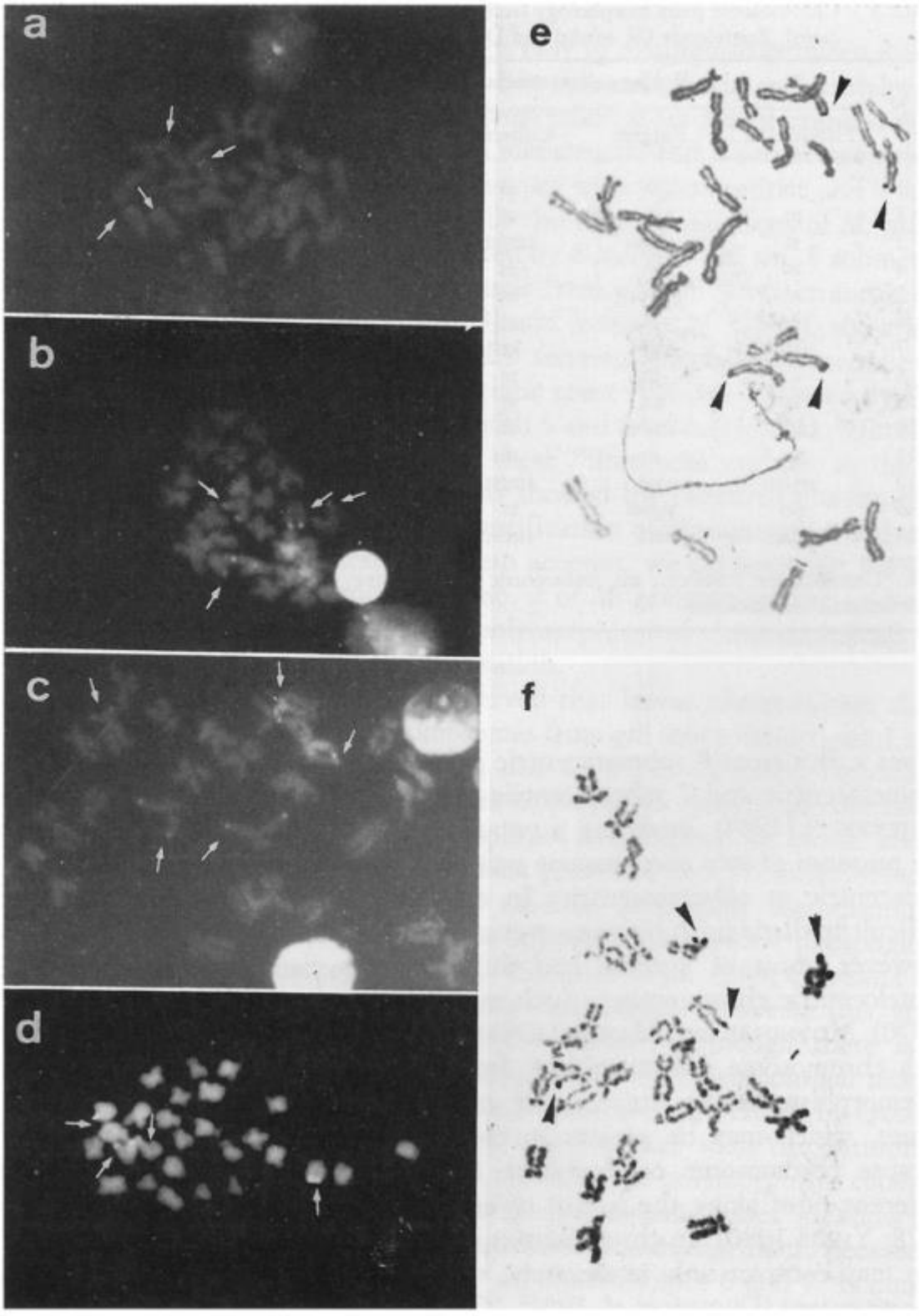

Figure 4. C-banding in **a**) Cullera-5 and **b**) Cullera-6 populations. CA3 banding in Fisterra population **c**) from larvae and **d**) from gill tissue. NOR banding in Fisterra population **e**) from larvae and **f**) from gill tissue

Similarly, in *M. galloprovincialis* it is easy to distinguish between metacentric and submetacentric chromosomes, but submetacentric and subtelocentric are not clearly differenciated. *M. galloprovincialis* from Mediterranean populations show karyotypes constituted by 6 metacentric and 8 submetacentric/subtelocentric chromosome pairs and karyotypes with 5 metacentric and 9 submetacentric/subtelocentric pairs. Meanwhile the other populations of *M. galloprovincialis* only show karyotypes constituted by 6 metacentric and 8 submetacentric/subtelocentric pairs. However mussels from French Mediterranean coast, analysed by THIRIOT-QUIEVREUX (1984) and INSUA et al. (1994), show karyotypes constituted by 5 metacentric and 9 submetacentric/subtelocentric chromosomes pairs, while mussels from Adriatic coast (Venice), analysed by DIXON and FLAVELL (1986), show karyotypes with 6 and 8 submetacentric/subtelocentric pairs. These authors suggest that these differences are due to the poor quality of the chromosome preparations showed by THIRIOT-QUIEVREUX and AYRAUD (1982), resulting in the misidentification of chromosomes and consequent mispairing. Taking these facts into account, we suppose that there is a polymorphism related to chromosome no. 2 of *M. galloprovincialis*, and we do not think that the centromeric index polymorphism of chromosome no. 2 be due to an unequal chromosome condensation.

On the other hand, we have observed that larvae chromosomes show a greater number of bands than chromosomes from gill tissue (MARTINEZ-LAGE et al. 1995). We think that it could be due to i) the different chromosome condensation degree, ii) the temperature employed to spread gill chromosome metaphase, and/or iii) the method employed for fixation of larvae and gill tissue chromosomes. In this sense we must point out that *M. galloprovincialis*, from de the southern Mediterranean exhibits an abrupt discontinuity in allozyme frequencies of the loci octopine dehydrogenase (Odh), phosphglucose isomerase (Pgi) and aminopeptidase (Ap) (SANJUAN et al. 1994; QUESADA et al. 1995). We suppose that may exist any relationship between this abrupt discointinuity and the chromosome polymorphism. Although there is not concordance between the allozyme variability and the chromosomal morphology in the two zones of *M. galloprovincialis* which were described by QUESADA et al. (1995), it is very remarkable to observe that such discontinuity is coincident in the only region where this chromosome polymorphism appears.

The lack of heterochromatic regions in chromosome 2 does not allow us to analyse the existence of any type of chromosome rearrangement, because C-, CA3 and Ag-NOR banding appear only on chromosomes 6 and 7. It must be clarified by means of satellite DNA and *in situ* hybridization studies.

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* fina@udc.es
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