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COMPARATIVE KARYOLOGICAL STUDY OF CUPPED OYSTER SPECIES

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ABSTRACT

Chromosomes of six cupped oyster species were studied using karyometric analysis after conventional Giemsa staining, and silver staining. Karyotypes of *Crassotrea angulata* (nine metacentric and one submetacentric chromosome pairs), *C. sikamea* (nine metacentric and one submetacentric chromosome pairs), *C. virginica* (eight metacentric and two submetacentric chromosome pairs), *C. ariakensis* (eight metacentric and two submetacentric chromosome pairs), *C. gasar* (six metacentric and four submetacentric chromosome pairs), and *Saccostrea commercialis* (eight metacentric and two submetacentric chromosome pairs) are distinguishable by the number and position of the submetacentric chromosome pair and by the location of nucleolus organizer regions. Comparative karyological analysis of these six cupped oysters and of *C. gigas* was made using a Principal Component Analysis and a Hierarchical Clustering Analysis. *Crassostrea gasar* appears isolated from the other oyster species. Then, two clusters are separated. The first one groups *C. gigas, C. angulata* and *C. sikamea,* in which *C. gigas* is plesiomorphic. The second one consists of *C. ariakensis, C. virginica* and *S. commercialis.* Results are discussed with regards to oyster species relationships based on other genetic characters and to hybridization possibilities.

Key words: cupped oyster, chromosome, karyotype, NORs, cytotaxonomy, Bivalvia.

INTRODUCTION

Chromosomes of Ostreidae have been studied in 22 species (Nakamura, 1985; Vitturi et al., 1985; Ieyama, 1990). Cupped oyster species of the genera *Crassostrea* and *Saccostrea* show a common diploid chromosome number of 2n = 20, and their karyotypes include only metacentric and submetacentric chromosomes (Table 1). Interspecific differences consist of the occurrence and differing proportions of these morphological types, identified either by observation or after chromosome measurements. Karyotype differences may be seen within a species (e.g., *C. rhizophorae;* Table 1) which could be due either to intraspecific polymorphism or to the different techniques used.

Oyster species might have become differentiated through pericentric inversions of centric shifts. However, cytotaxonomic comparison needs to be based on karyological analysis carried out by the same technique and the same worker. For example, the concentration and time of incubation in the colchicine and in the hypotonic treatment, resulting in differential condensation or elongation of chromosomes (Sharma & Sharma, 1980), vary from one author to another. Karyometric analysis brings a more quantitative method to assess chromosome morphology, but still depends on the condensation or elongation of chromosomes.

Banding techniques have been found to be very useful for the identification of individual chromosomes and also of particular regions of chromosomes. Few studies have looked at banding patterns in the chromosomes of oysters (Rodriguez-Romero et al., 1979c; Insua & Thiriot-Quiévreux, 1991; Li & Havenhand, 1997). Fluorescence *in situ* hybridization has been tested in *Crassostrea gigas* (Clabby et al., 1996; Guo & Allen, 1997). Selective staining of the nucleolus organiser regions (NORs) has been shown to have potential as a cytotaxonomic tool (e.g., Amemiya & Gold, 1990). Patterns of specific NORs have been described in five species of oysters (Thiriot-Quiévreux & Insua, 1992; Insua & Thiriot-Quiévreux, 1993; Ladron de Guevara et al., 1994). Identification of structural chromosome features is useful in hybrid breeding programs and in oyster stock conservation.

In the present study, karyotypes and NORs

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TABLE 1. Chromosome data in cupped oysters.

Species	2n	karyotype	Origin	Authors
Crassostrea				
C. amasa (Iredale)	20		Australia	Menzel, 1968
C. angulata (Lamarck)	20		Portugal	Menzel, 1968
	20	10m*	France (Barfleur)	Thiriot-Quiévreux, 1984
C. belcheria (Sowerby)	20	10 m-sm	Japan	Ieyama & Inaba, 1974
C. corteziensis (Hertlein)	20	7m-3sm*	Mexico	Rodriguez-Romero et al, 1979a
C. gigas (Thunberg)	20	8m-2sm	USA	Ahmed & Sparks, 1967
	20	10m*	France (Barfleur)	Thiriot-Quiévreux, 1984
C. glomerata (Gould)	20		West Pakistan	Ahmed, 1973
C. gryphoides (Scholteim)	20		West Pakistan	Ahmed, 1973
C. iredalei (Faustino)	20		Philippines	Menzel, 1968
C. rhizophorae (Guilding)	20	5m-5sm*	Mexico	Rodriguez-Romero et al., 1979b
	20	8m-2sm*	Venezuela	Marquez, 1992
C. "*rivularis* (Gould)"	20		West Pakistan	Ahmed, 1973
Syn. *C. ariakensis* (Fujita)	20	10m-sm	Japan	Ieyama, 1975
C. sikamea (Amemiya) (Kumamoto variety of *C. gigas*)	20		West Pakistan	Ahmed, 1973
C. virginica (Gmelin)	20	6m-4sm*	East coast USA	Longwell et al., 1967
	20	6m-4sm*	Mexico	Rodriguez-Romero et al., 1978
	20	6m-4sm*	Venezuela	Marquez, 1992
Saccostrea				
S. commercialis (Iredale & Roughley)	20		Australia	Menzel, 1968
S. cucullata (Born)	20	10m*	India	Goswami, 1992
S. echinata (Quoy & Gaimard)	20	10m-sm	Japan	Ieyama & Inaba, 1974
S. mordax (Gould)	20	10m-sm	Japan	Ieyama & Inaba, 1974

2n: diploid chromosome number
*: after chromosome measurements
m: metacentric; sm: submetacentric

were studied in six species of cupped oysters: *Crassostrea angulata, C. sikamea, C. virginica, C. ariakensis, C. gasar,* and *Saccostrea commercialis.* These species originating from different areas were imported and reared in common quarantine facilities. Comparative karyological analysis was made with reference to *C. gigas* (Thiriot-Quiévreux, 1984, and unpublished data, 1997).

MATERIALS AND METHODS

Species Studied

Five cupped oyster species of the genus *Crassostrea* and one of the genus *Saccostrea* were studied, none native to Europe. *Crassostrea gigas* and *C. angulata* have been introduced into the natural environment for decades (Grizel & Héral, 1991) or centuries (Boudry et al., 1998) respectively. The other species were recently imported into France as part of a genetic resources research program. They have been strictly confined to the quarantine facilities of the IFREMER hatchery in La Tremblade, Charente-Maritime, France, according to international recommendations. All the oysters studied were reared in the same environmental conditions for at least three months before sampling. The *C. angulata* oysters studied originate from the Rio Sado estuary, Setubal, Portugal. The taxonomic status of these oysters was assessed using mitochondrial DNA markers as described in Boudry et al. (1998). *Crassostrea sikamea* were imported from Bodega Marine Laboratory, University of California, USA. Their taxonomic status was confirmed using mitochondrial DNA markers as described in Banks et al. (1993). *Crassostrea virginica* were imported from a wild stock located in Shippagan, New Brunswick, Canada. *Crassostrea ariakensis* ("*C. rivularis,*" *auctt.*) were imported from the Shellfish Research Laboratory, Rutgers State University, New Jersey, USA. This species was introduced from Japan into the Northwest waters of the USA, and its aquaculture potential has been recently reviewed by Langdon & Robinson

(1996). Mangrove oysters, *C. gasar,* were imported from a wild stock located in Kafountine, Casamance, Senegal. *Saccostrea commercialis* were collected from the wild at Port Stephens, New South Wales, Australia.

Because of the low number of animals available, only two animals from each species (except three of *C. sikamea*) were used for this study.

Chromosome Preparations

Oysters were incubated for 7 h with 0.005% colchicine in sea water. The gills were then dissected out and treated for 30 min in 0.9% sodium citrate in distilled water. The material was then fixed in a freshly prepared solution of absolute ethanol and acetic acid (3:1), with three changes of 20 min duration each. Slide preparation was made using an air-drying technique (Thiriot-Quiévreux & Ayraud, 1982). For conventional karyotypes, slides were stained directly with Giemsa (4%, pH 6.8) for 10 min. Photographs of suitable mitotic metaphases were taken with a Zeiss III photomicroscope, and after karyotyping, chromosome measurements of ten metaphases in each species were made with a digitizer table (Summa Sketch II) interfaced with a Macintosh. Data analysis was performed with an Excel macro program. Terminology relating to centromere position follows that of Levan et al. (1964). NORs were silver-stained directly on unstained slides using the technique of Howell & Black (1980), modified by Gold & Ellison (1982).

Statistical Analysis

In order to evaluate the relationships between the six species studied here and *C. gigas,* a principal component analysis (PCA) was carried out. The data set is a matrix of 70 objects, that is, ten metaphases in seven species described by the centromeric index values of ten chromosome pairs. Means of centromeric index values for each species were considered as supplementary objects and were projected in the PCA space. The position (i.e., component score) of the ten metaphases around this mean point gives information of the scattering of each species. Their correlations give a criterion of their explanation by the PCA axes considered. As a second step, a hierarchical clustering analysis (HCA) was performed between the species described by their component scores on the first four axes of the PCA, using the Ward agglomeration method (Ward, 1963). This clustering offers the possibility of representing the distances between species by a dendogram. PCA and HCA were computed with the SPAD software (CESIA) (Lebart et al., 1995).

RESULTS

The results obtained for each species are summarised in Table 2.

Crassostrea angulata

The karyotype (Fig. 1A, Table 3) consists of nine metacentric and one submetacentric (no. 8) chromosome pairs. Ag-NORs were found terminally on the metacentric pair 10. The two homologous chromosomes showed heteromorphism involving apparent NOR activity. The most frequent case (69%) was one silver-stained NOR chromosome (Fig. 2A).

Crassostrea sikamea

The karyotype (Fig. 1B, Table 3) shows nine metacentric and one submetacentric (no. 6) chromosome pairs. Ag-NORs were found terminally on the metacentric pairs nos. 9 and 10 (Fig. 2B). A variable number of one to three Ag-NORs was observed. 54% of the silver stained metaphases only showed NORs on pair 10. The most frequent case (61%) was one silver-stained NOR chromosome in pair 10.

Crassostrea virginica

The karyotype (Fig. 1C, Table 3) has eight metacentric and two submetacentric (nos. 4 and 8) chromosome pairs. Ag-NORs were found terminally on the short arms of metacentric pairs nos. 1 and 5 (Fig. 2C). A variable number of one to three Ag-NORs was observed. The most frequent case (52%) was one silver-stained NOR chromosome in pair 1 and in pair 5.

Crassostrea ariakensis

The karyotype (Fig. 1D, Table 3) consists of eight metacentric and two submetacentric (nos. 4 and 8) chromosome pairs. Ag-NORs were found terminally on the metacentric pairs 9 and 10. A variable number of one to three Ag-NORs was observed (Fig. 2D). 68% of the silver stained metaphases showed Ag-NORs only on pair 10.

Crassostrea gasar

The karyotype (Fig. 1E, Table 3) includes six metacentric and four submetacentric (nos.

TABLE 2. Summary of karyological data of the six cupped oysters studied.

Species	No. metaphases studied		No. karyotypes studied			Chromosome type (no. chromo. pairs)		No. of (haploid) NOR-chromosomes
	Giemsa	NOR	Giemsa	NOR	2n	m	sm	
C. angulata	42	31	13	7	20	9	1	1 (pair 10)
C. sikamea	32	62	15	9	20	9	1	2 (pairs 9 and 10)
C. virginica	29	57	10	8	20	8	2	2 (pairs 1 and 5)
C. ariakensis	30	46	17	12	20	8	2	2 (pairs 9 and 10)
C. gasar	33	55	13	8	20	6	4	1 (pair 2)
S. commercialis	34	35	13	7	20	8	2	2 (pairs 9 and 10)

*after chromosome measurements of 10 metaphases
m: metacentric; sm: submetacentric

2, 8, 9, and 10) chromosome pairs. Ag-NORs were found terminally on the short arms of two homologous chromosomes of the metacentric pair 2 (Fig. 2E). Heteromorphism involving NOR-size occurred in 49% of the metaphases examined.

Saccostrea commercialis

The karyotype (Fig. 1F, Table 3) shows eight metacentric and two submetacentric (nos. 4 and 7) chromosome pairs. Ag-NORs were found terminally on the metacentric pairs 9 and 10. A variable number of one to three NORs were observed. 77% of the silver stained metaphases showed Ag-NORs only on pair 10 (Fig. 2F).

Comparative Karyological Analysis

Figure 3 shows ideograms constructed from relative length and centromeric index values (Table 3) of the six oyster species studied here and of *Crassostrea gigas*. Chromosome measurements of this later species were taken from ten metaphases of animals collected at La Tremblade in 1997. Mean values of relative length and centromeric index are similar to those found in *C. gigas* from Barfleur (Thiriot-Quiévreux, 1984). *Crassostrea gasar* is distinguishable from the other species first, due to the occurrence of four submetacentric chromosome pairs. *Crassostrea angulata* and *C. sikamea* showed only one submetacentric chromosome pair, whereas *C. virginica, C. ariakensis* and *S. commercialis* have two submetacentric chromosome pairs. *Crassostrea angulata* and *C. sikamea* may be differentiated by the different positions of the submetacentric chromosome pair and by the Ag-NORs which appear on pair 10 and on pairs 9 and 10 respectively. *Crassostrea virginica* and *C. ariakensis* share a similar karyotype, but Ag-NORs are observed in different locations (pairs 1 and 5, and pairs 9 and 10, respectively). *Saccostrea commercialis* is close to *C. ariakensis*. Their karyotypes differ by the position of the second submetacentric pair and by the frequencies of Ag-NORs observed on pair 10. *Crassostrea gigas* has the most symmetrical karyotype, with only metacentric chromosome pairs.

Principal component analysis of the data set of 70 objects (ten metaphases for seven species described by centromeric index values of ten chromosome pairs) gives percentages of variance for the first five axes of 31.74, 20.06, 12.61, 11.17 and 7.77 respectively. The variance decreases progressively from 5th axis. We have thus only considered the information provided by the first four axes as relevant. The 1/2 plan (Fig. 4) explains 51.80% of the variance. It shows the separated position of *C. gasar* (correlation with 1/2 plan of 0.98) without continuity with the other species. The six other species overlap along a continuum. *Crassostrea gigas* (correlation of 0.87) is the most distant from this continuum. Then, *C. ariakensis* and *C. virginica* are very close and overlap a part of *S. commercialis* (correlations of 0.57, 0.43 and 0.52, respectively). *Crassostrea sikamea* (correlation of 0.38) shows a larger scattering. *Crassostrea angulata* is unexplained by this plan, as shown by its correlation of 0.01. The 3/4 plan explained less of the total variance: 23.78%. There is a trend of separation between *C. virginica, C. ariakensis* and *S. commercialis* (correlations of 0.40, 0.17 and 0.55 respectively). *Crassostrea angulata* and *C. sikamea* remain together (correlations of 0.48 and 0.55 respectively). Figure 5 shows the dendogram of a Hierarchical Clustering Analysis made using the information from the

FIG. 1. Giemsa-stained karyotypes of six cupped oysters. A: *Crassostrea angulata*, B: *Crassostrea sikamea*, C: *Crassostrea virginica*, D: *Crassostrea ariakensis*, E: *Crassostrea gasar*, F: *Saccostrea commercialis*. Arrows show submetacentric chromosome pairs. Scale bar = 5 μm

TABLE 3. Chromosome measurements and classification in ten cells of six cupped oyster species.

Chromosome pair No.	Relative length		Arm ratio		Centromeric index		Chromosome Type
	Mean	SD	Mean	SD	Mean	SD	
C. angulata							
1	12.81	0.91	0.79	0.08	43.79	2.50	m
2	11.34	0.41	0.84	0.09	45.22	2.45	m
3	10.75	0.41	0.83	0.09	45.02	2.65	m
4	10.33	0.63	0.64	0.09	38.46	2.89	m
5	10.12	0.74	0.82	0.06	44.79	1.86	m
6	9.82	0.60	0.62	0.07	38.01	2.56	m
7	9.53	0.82	0.88	0.07	46.47	1.94	m
8	9.25	0.68	0.59	0.07	36.84	3.01	sm
9	8.98	0.66	0.68	0.12	40.07	3.83	m
10	7.08	0.67	0.75	0.10	42.36	3.44	m
C. sikamea							
1	12.40	1.17	0.85	0.17	45.24	4.35	m
2	11.28	0.59	0.77	0.10	43.08	2.99	m
3	11.20	0.64	0.87	0.12	46.03	3.38	m
4	10.39	0.93	0.86	0.12	45.92	3.14	m
5	10.38	0.78	0.85	0.13	45.41	3.89	m
6	9.87	0.89	0.53	0.13	34.20	5.27	sm
7	9.50	0.76	0.79	0.13	43.72	4.36	m
8	9.27	1.19	0.79	0.08	43.52	2.48	m
9	8.61	0.89	0.82	0.20	44.28	6.39	m
10	7.10	1.05	0.95	0.18	47.75	4.98	m
C. virginica							
1	12.71	0.67	0.88	0.08	46.46	2.51	m
2	11.50	0.56	0.87	0.11	46.27	3.03	m
3	10.89	0.90	0.79	0.11	43.61	3.62	m
4	10.57	0.71	0.46	0.08	30.97	3.49	sm
5	10.33	0.47	0.82	0.08	44.63	2.34	m
6	9.62	0.61	0.74	0.09	42.18	3.19	m
7	9.38	0.92	0.79	0.16	43.52	5.43	m
8	9.16	0.69	0.43	0.10	29.34	4.57	sm
9	8.64	0.51	0.73	0.11	41.81	3.78	m
10	7 19	0.54	0.86	0.12	45.63	3.49	m
C. ariakensis							
1	12.10	0.63	0.82	0.07	44.65	2.09	m
2	11.34	0.43	0.77	0.10	42.98	3.47	m
3	10.67	0.46	0.78	0.07	43.71	2.10	m
4	10.54	0.59	0.51	0.05	33.77	2.32	sm
5	10.21	0.79	0.81	0.09	44.44	2.55	m
6	9.91	0.39	0.81	0.12	44.30	3.78	m
7	9.64	0.57	0.74	0.07	42.12	2.03	m
8	9.44	0.78	0.53	0.08	34.25	3.08	sm
9	9.09	0.90	0.79	0.17	43.41	5.00	m
10	7.07	0.43	0.76	0.12	42.64	3.86	m
C. gasar							
1	11.36	0.63	0.79	0.10	43.64	3.11	m
2	11.19	0.74	0.38	0.05	27.52	2.34	sm
3	11.04	0.41	0.85	0.08	45.62	2.47	m
4	10.62	0.76	0.62	0.07	37.80	2.31	m-sm
5	10.54	0.51	0.90	0.09	46.95	2.45	m
6	9.97	0.43	0.86	0.08	45.77	2.33	m
7	9.64	0.54	0.84	0.12	45.21	3.54	m
8	9.54	0.64	0.45	0.07	30.78	3.43	sm
9	8.82	0.60	0.41	0.06	28.65	3.01	sm
10	7.28	0.56	0.39	0.08	27.89	3.78	sm

TABLE 3. (*Continued*)

Chromosome pair No.	Relative length		Arm ratio		Centromeric index		Chromosome Type
	Mean	SD	Mean	SD	Mean	SD	
S. commercialis							
1	13.41	1.07	0.81	0.08	44.41	2.45	m
2	12.39	1.04	0.78	0.06	43.58	1.77	m
3	10.84	0.56	0.81	0.09	44.50	2.68	m
4	10.36	0.70	0.44	0.05	30.48	2.29	sm
5	9.89	0.74	0.80	0.08	44.07	2.58	m
6	9.82	0.66	0.81	0.11	44.32	3.48	m
7	9.13	0.98	0.48	0.07	32.24	2.98	sm
8	9.11	0.63	0.78	0.11	43.42	3.56	m
9	8.44	0.59	0.81	0.10	44.34	3.01	m
10	6.60	0.63	0.79	0.12	43.19	3.76	m

m: metacentric; sm: submetacentric

first four axes of the PCA. *Crassostrea gasar* appears clearly separated from the other species. Then, two clusters are differentiated, one with the grouping of *C. virginica, C. ariakensis* and *S. commercialis,* and the other with two close species *C. angulata, C. sikamea* and *C. gigas* at a higher distance.

DISCUSSION

Our chromosome study of these six cupped oyster species confirms the diploid chromosome number of 2n = 20 found up to now in all cupped oysters examined (Table 1).

The karyotype of *C. angulata* differs from that described in the animals reared at Barfleur (Thiriot-Quiévreux, 1984). This difference could be due to the origin of animals. Samples in this study came from the Bay of Setubal, Portugal, and are considered as pure *C. angulata* (Boudry et al., 1998). The origin of samples in the Barfleur study in unknown. The karyotype of *C. virginica,* showing two submetacentric chromosome pairs, differs from those with four submetacentric chromosome pairs described by Longwell et al. (1967), Rodriguez-Romero et al. (1978), and Marquez (1992). However, the position of these submetacentric chromosome pairs is different between these authors. Genetic discontinuity has been observed in this American oyster along the Atlantic coast and the Gulf of Mexico (Buroker, 1983; Reeb & Avise, 1990; Hare & Avise, 1996). The origin of our animals is close to those studied by Longwell et al. (1967). Therefore, the karyological variation observed could be due either to the effect of acclimation or to differences in the technique (e.g., different concentrations of colchicine: 0.02% in the Longwell et al. 1967 study and 0.005% in this study). Karyotypes made by the same scientist, with the same techniques carried out within a short period of time give a more valid comparison than karyotypes made by different authors.

Karyotypes of *C. sikamea, C. ariakensis, C. gasar* and *S. commercialis* are first described here.

Our observations on Ag-NORs are original in the six species studied. In *C. virginica,* Longwell & Stiles (1996) suggested that NOR sites could be located on the secondary constriction observed on the longest metacentric chromosome pair. Our results confirm the location of Ag-NORs on this pair 1, but another Ag-NOR was observed on pair 5. Heteromorphism involving apparent NOR activity and NOR-size is a common phenomenon in bivalves (Thiriot-Quiévreux & Insua, 1992; Insua et al., 1994; Martinez-Exposito et al., 1994). However, the number of Ag-NORs, their chromosomal location and their position within karyotypes are considered as species-specific characters (Sumner, 1990). In this study, the majority of species showed Ag-NORs on pair 9 or pairs 9 and 10, in a frequency that varies according to the species considered. The position of NORs was different in *C. virginica* and *C. gasar.* Ag-NORs allowed the separation of *C. angulata* and *C. sikamea,* and of *C. virginica* and *C. ariakensis* which have similar karyotypes.

Comparative karyological analysis (Figs. 3–5) highlights the isolation of *C. gasar.* Then two clusters are separated. The first cluster consists of *C. gigas, C. angulata* and *C. sikamea,* in which *C. gigas,* with the most symmetrical karyotype, could be considered as plesiomorphic. *Crassostrea gigas* and *C. an-*

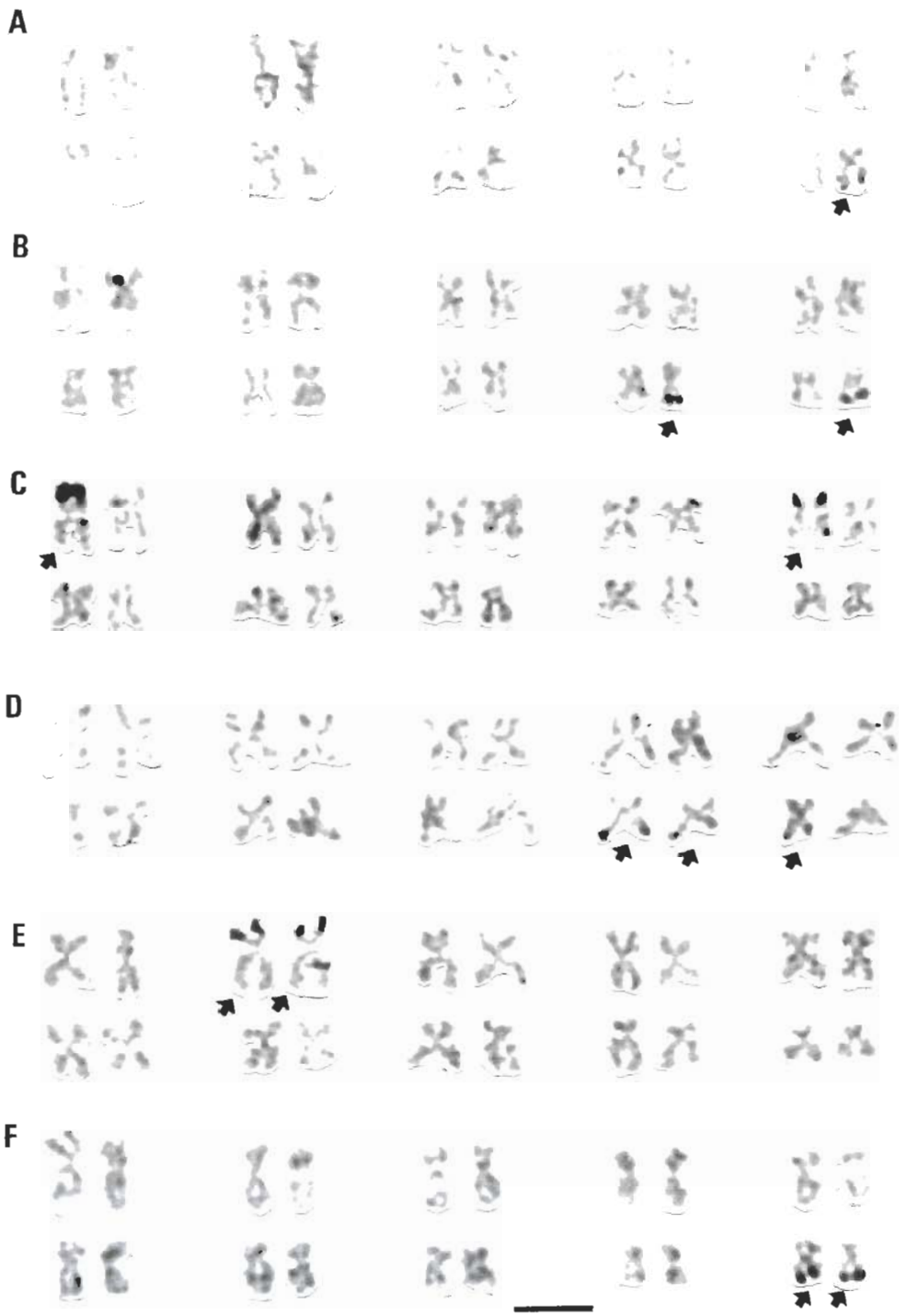

FIG. 2. Silver-stained karyotypes of six cupped oysters. A: *Crassostrea angulata,* B: *Crassostrea sikamea,* C: *Crassostrea virginica,* D: *Crassostrea ariakensis,* E: *Crassostrea gasar,* F: *Saccostrea commercialis.* Arrows show Ag-NORs. Scale bar = 5 μm.

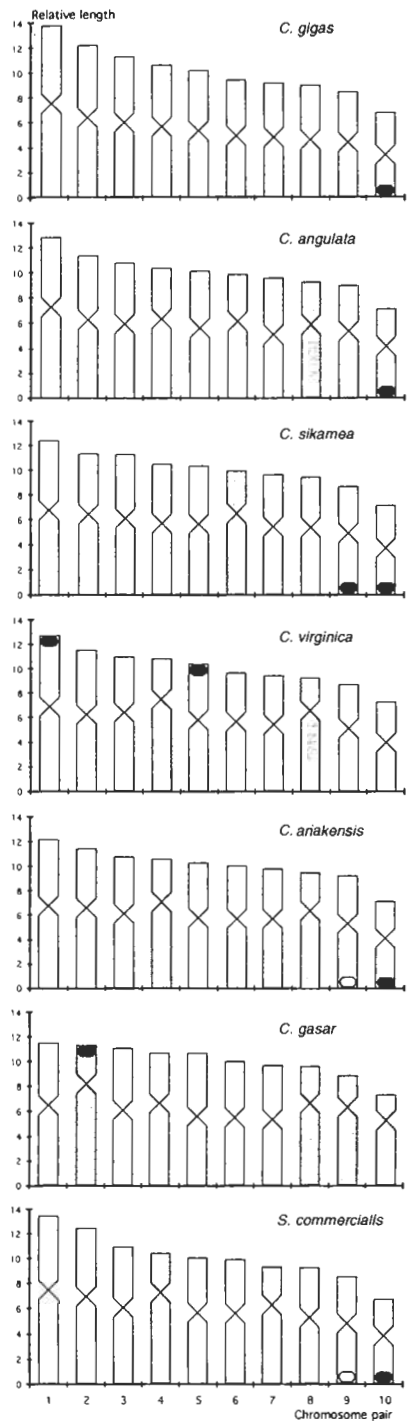

FIG. 3. Ideograms of seven cupped oysters constructed from relative length and centromeric index values. White chromosome: metacentric, grey chromosome: submetacentric. Circles indicate Ag-NORs, dark circles the most frequent case.

gulata are often considered as a same species (Menzel, 1974), as are *C. gigas* and *C. sikamea,* of which the latter has sometimes been considered as the "Kumamoto variety" (Ahmed, 1973). Recent molecular genetic studies have displayed differences between *C. gigas* and *C. sikamea* (Banks et al., 1994), and between *C. gigas* and *C. angulata* (Boudry et al., 1998; Ó Foighil et al., 1998). Our karyological study confirms these genetic differences. The second cluster put together *C. ariakensis, C. virginica* and *S. commercialis.* Molecular phylogenetics of cupped oysters (Littlewood, 1994) distinguished two lineages: (1) *C. gigas, C. belcheri,* "*C. rivularis*" (= *C. ariakensis*) and (2) *C. virginica, C. rhizophorae* and *S. commercialis.* Ó Foighil et al. (1995) using mitochondrial 16S ribosomal gene sequences confirm a genetic divergence between *C. virginica* and two Asian congeners *C. gigas* and *C. ariakensis.* Ladron de Guevara et al. (1996) suggested that *C. virginica* showed the most primitive karyological features when compared with *C. rhizophorae* and *C. corteziensis.* Our study is in agreement with the relationship between *C. virginica* and *S. commercialis,* but does not agree on the position of *C. ariakensis.* Multidisciplinary approaches would help in understanding evolutionary relationships of oyster taxa.

Interspecific hybridizations have been produced in cupped oyster species (Gaffney & Allen, 1993, provide a review). *Crassostrea gigas* is known to hybridize with *C. angulata, C. sikamea* and, rather less successfully, with *C. ariakensis.* These observations are in agreement with our study. Looking at karyological features, *C. ariakensis* and *S. commercialis* would be good candidates for hybridization, although this cross has apparently never been tried. Ag-NORs observed in *C. virginica* isolate this species from the others. This could explain inviability of hybrids of *C. gigas* and *C. ariakensis* with *C. virginica* (Allen & Gaffney, 1993). In the future, it will be of great interest to study the mitotic and meiotic chromosomes of interspecific hybrids such as *C. gigas* × *C. sikamea* or *C. gigas* × *C. ariakensis* and their backcross offspring.

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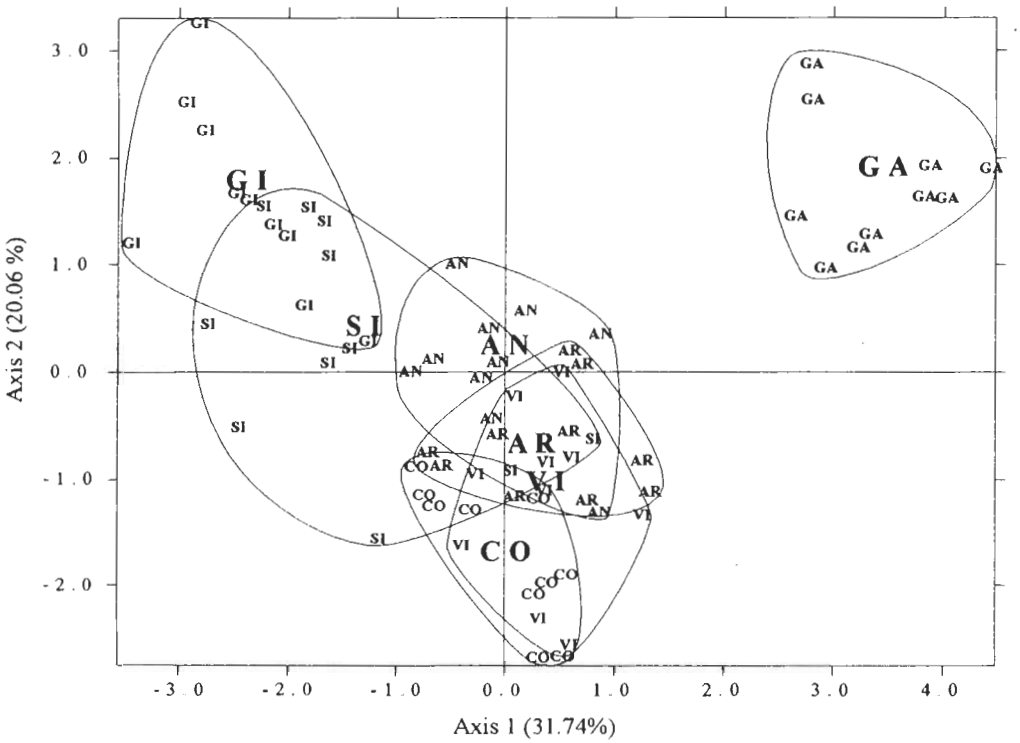

FIG. 4. 1/2 plan determined by Principal Component Analysis of chromosome data. Small characters represent active objects, large characters indicate the mean for each species. A line is drawn around each species to show the dispersion within species. AN: *Crassotrea angulata*, CO: *Saccostrea commercialis*, GA: *Crassostrea gasar*, GI: *Crassostrea gigas*, AR: *Crassostrea ariakensis*, SI: *Crassostrea sikamea*, VI: *Crassostrea virginica*.

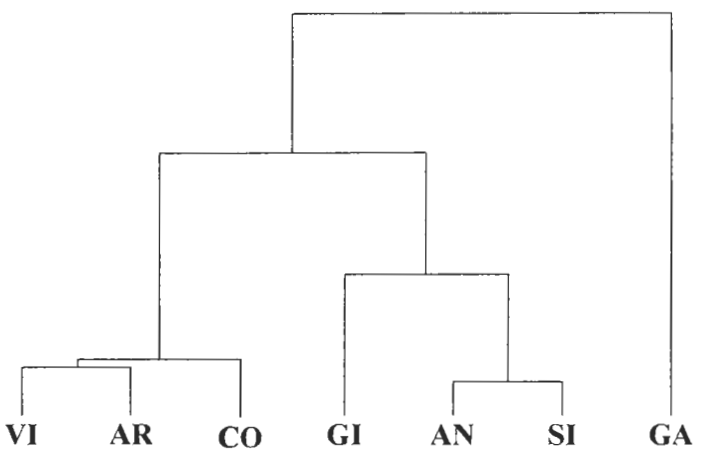

FIG. 5. Hierarchical Clustering Analysis showing the distances between the seven species from the first four axes of the PCA. AN: *Crassostrea angulata*, CO: *Saccostrea commercialis*, GA: *Crassostrea gasar*, GI: *Crassostrea gigas*, AR: *Crassostrea ariakensis*, SI: *Crassostrea sikamea*, VI: *Crassostrea virginica*.

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