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Karyotype Analysis of *Aphanius fasciatus* (Pisces, Cyprinodontiformes): Ag-NORs and C-band Polymorphisms in Four Populations from Sicily

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Zusammenfassung

4 Populationen von *Aphanius fasciatus* aus Sizilien wurden unter Einsatz der Silbernitrat-Technik und drei von ihnen mit C-banding untersucht. Silber-Färbung zeigte einen hohen Grad von Intrapopulations-Variabilität hinsichtlich der Anzahl der aktiven Nukleolus-Organisatoren (NORs). Polymorphismus der NOR-Lokalisation wurde in der Salso-Fluß-Population beobachtet, wahrscheinlich auf paracentromerische Inversionen zurückzuführen. Polymorphe C-Banden wurden in 3 Populationen gefunden und sind offenbar das Ergebnis von telomerischen Heterochromatin-Variationen.

Karyologie – Bandenchromosomen – NOR-Polymorphismus – *Aphanius fasciatus* – Cyprinodontiformis

Abstract

Four populations of the cyprinodont fish *Aphanius fasciatus* from Sicily have been analyzed by silver nitrate and three of them by C-banding. Ag-staining showed a high degree of intrapopulational variability in the number of active nucleolus organizer regions (NORs) in all investigated populations. Polymorphism of NOR location has also been observed in Salso river population, presumably due to paracentromeric inversion. C-bands were found to be polymorphic in three populations due to the presence of telomeric heterochromatin variations.

Karyology – banded chromosomes – NOR polymorphism – *Aphanius fasciatus* – Cyprinodontiformes

Introduction

The cyprinodont genus *Aphanius* includes *A. dispar*, *A. hiberus*, *A. fasciatus* and two endemic anatolian species: *A. anatoliae* and *A. chantrei*. Strains of *A. fasciatus* are commonly found in the coastal brackish waters of estuaries and lagoons (TORTONESE, 1970; MARCONATO, 1982; VILLWOCK, 1982) as well as in the fresh waters of rivers (TIGANO, 1982).

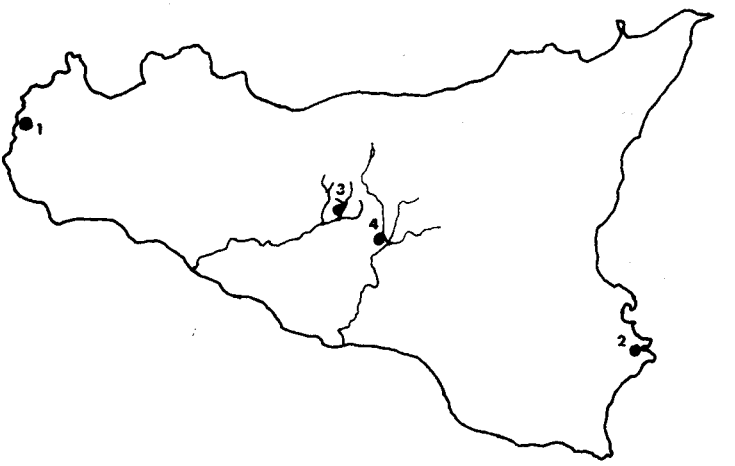

Fig. 1. Map of collection for *Aphanius fasciatus* specimens:

1 = Salt ponds of Trapani (SPT); 2 = Salt ponds of Siracusa (SPS); 3 = Fiumicello river (FRC); 4 = Salso river (SRC)

Morphometric, meristic, and chromatic studies have shown that substantial differences occur among *A. fasciatus* populations from Sicily (TIGANO, 1982). Similarly, different electrophoretic patterns have been found in two strains of the same species (COMPARINI et al. 1984–85) – one from Sardinia and the other from the Northern Adriatic Sea – thus, making these authors hypothesize the existence of an initial inter-populational micro-evolutionary process.

Such results along with the fact that cytogenetics of *A. fasciatus* remains virtually unknown, only the haploid chromosome number of this species has been reported (KARBE, 1961), have encouraged us to start a chromosome analysis of *A. fasciatus* from Sicily.

The present paper deals with conventional karyotypes of four samples from geographically separated locations. Moreover, since the development of the silver staining technique for detection of the sites of nucleolus organizer regions (NORs) (GOODPASTURE and BLOOM, 1975: HOWELL and BLACK 1980) and the C-banding technique for demonstration of the amount and distribution of constitutive heterochromatin (SUMNER, 1972) have greatly facilitated comparative studies within and between species, silver (Ag)-staining and C-banding patterns of this species are also described and discussed.

Materials and Methods

A. fasciatus specimens were collected by seine from natural populations inhabiting the following localities: salt ponds near Trapani = SPT (20 females and 6 males), salt ponds near Siracusa = SPS (16 females and 6 males), Fiumicello river (Mussomeli, Caltanissetta) = FRC (50 females and 22 males), and Salso river (Caltanissetta) = SRC (5 females and 3 males) (Fig. 1). Specimens were collected during many different field trips to these sites and identified according to the guidelines of TORTONESE (1970).

Mitotic metaphase chromosomes were prepared from pooled gill, spleen, and kidney cells, following the standard air-drying method (VITTURI et al., 1993). Each specimen was injected intraperitoneally

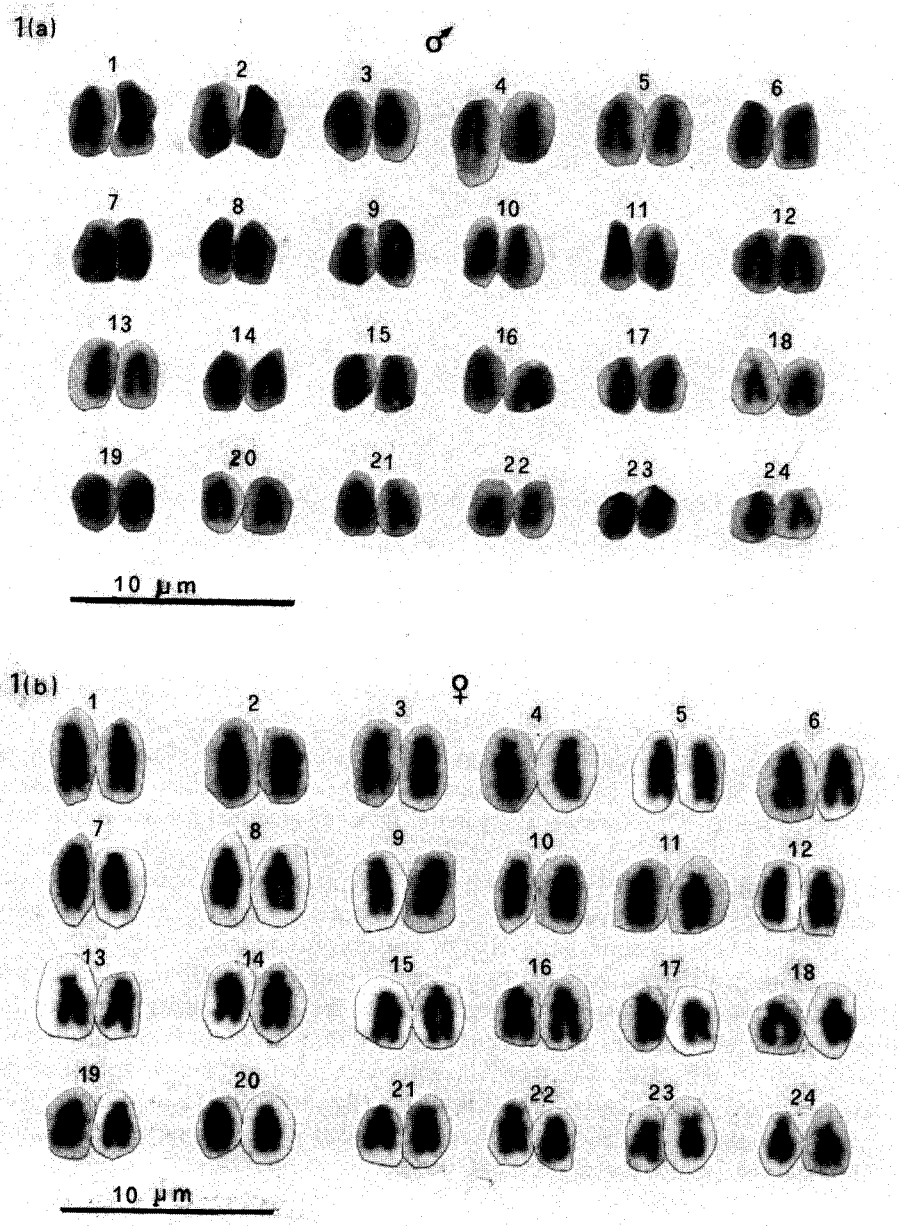

Fig. 1a. Giemsa-stained male karyotype
Fig. 1b. Giemsa-stained female karyotype

with colchicine (0,1 %, 1 ml per 30 g body weight) and sacrificed 2 h later. Kidney, spleen. and gill tissues were minced in 0.075 M KCl. The suspensions were centrifuged, supernatants discarded, and cell sediments fixed in two successive changes of fresh methanol-acetic acid solution (3:1). Drops of cell suspensions were then placed on clean slides at 0 °C. Slides were stained with Giemsa 5 %, pH 6.8, for construction of conventional karyotypes.

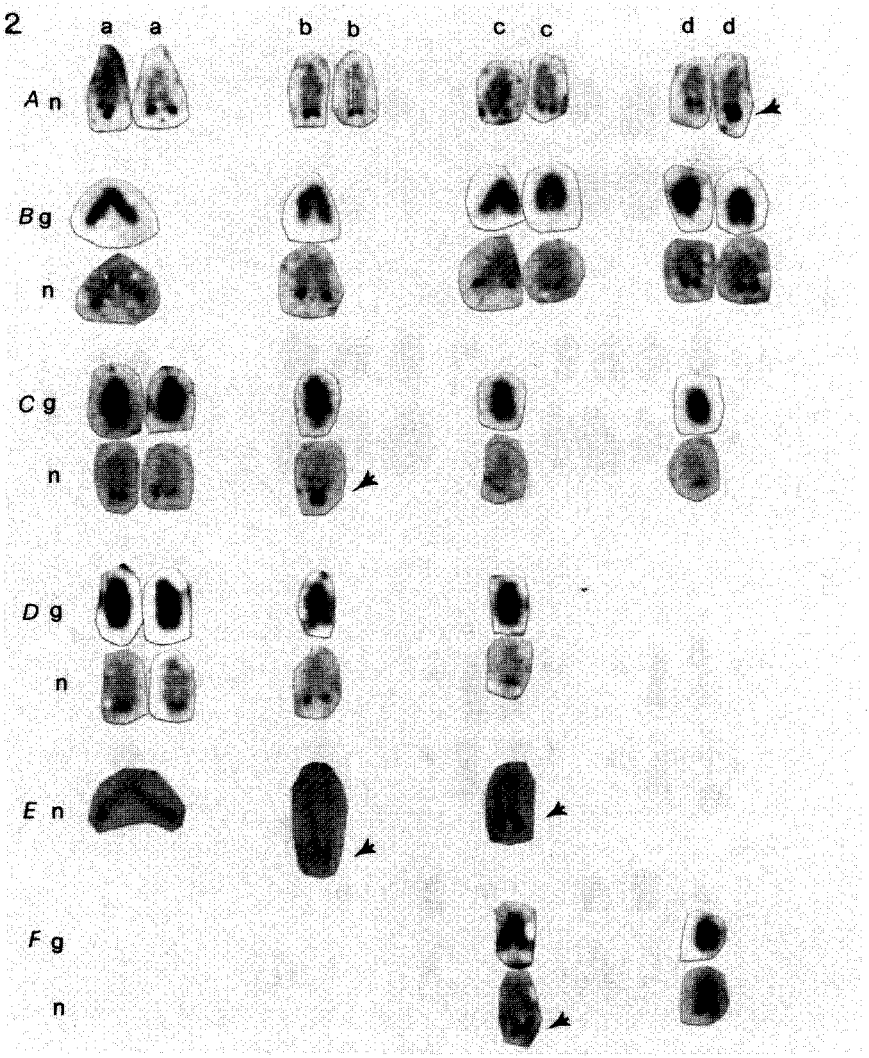

Fig. 2. Silver chromosome phenotypes of Fiumicello river population displaying:
(A) eight chromosomes; (B) six chromosomes; (C) five chromosomes;
(D) four chromosomes; (E) three chromosomes and
(F) two chromosomes, all with terminal NORs

Silver staining followed the controlled technique of HOWELL and BLACK (1980), while C-banding was performed according to SUMNER (1972).
Observations were made with a Jenamed 2 light microscope and chromosomes were photographed using an Agfa Gevaert AG 25 film.
Chromosomes were classified on the basis of the arm-length ratio, as suggested by LEVAN et al. (1964).

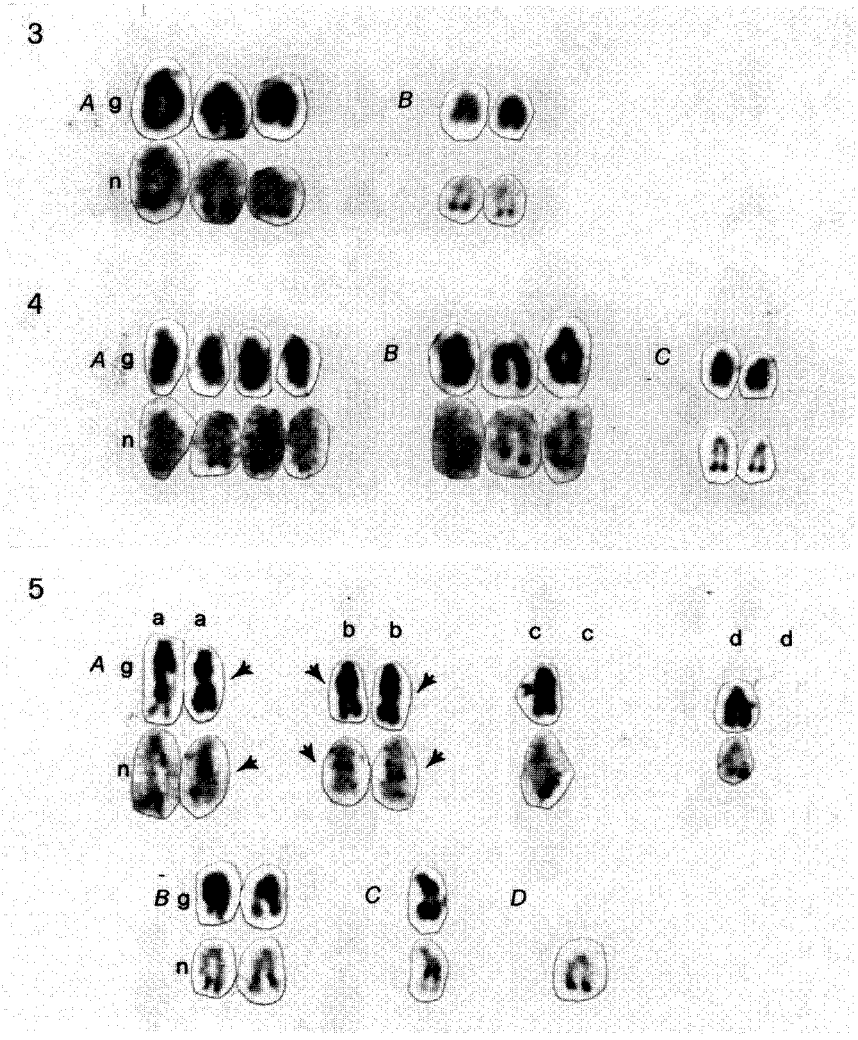

Fig. 3. Silver chromosome phenotypes of a Siracusa salt pond population displaying:
(A) three chromosomes and (B) two chromosomes, all with terminal NORs

Fig. 4. Silver chromosome phenotypes of a Trapani salt pond population displaying:
(A) four chromosomes; (B) three chromosomes and (C) two chromosomes, all with terminal NORs

Fig. 5. Silver chromosome phenotypes of Salso river population displaying:
(A) six chromosomes, three with interstitial NORs (arrows) and three with terminal NORs;
(B) two chromosomes, one with terminal NORs and one with interstitial NORs;
(C) one chromosome with interstitial NORs and (D) one chromosome with terminal NORs

Results

From counts of 10 metaphase spreads per specimen, a modal diploid number of $2n = 48$ was determined in all four populations. Karyotypes were obtained by tentatively arranging homologous chromosomes of two spreads per individual in order of decreasing size and centromere location. Twenty four homomorphic pairs consistently occurred in both male (Fig. 1a) and

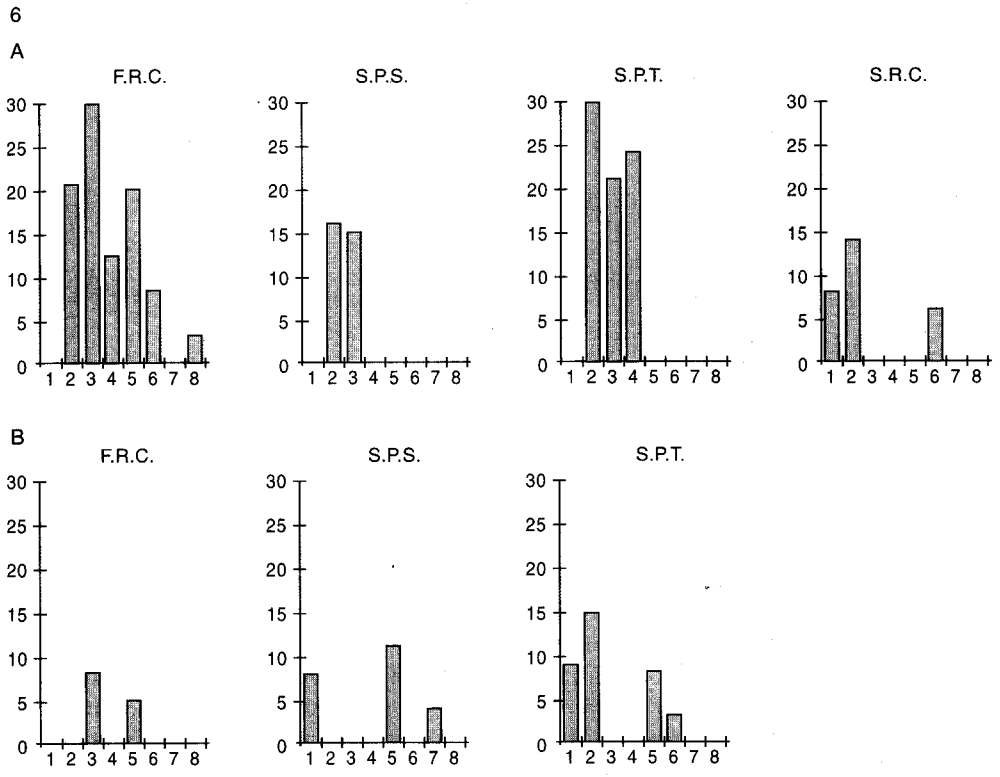

Fig. 6. (A) Distribution of Ag-stained NOR-bearing chromosomes from Fiumicello river (FRC), Siracusa salt pond (SPS), Trapani salt pond (SPT) and Salso river (SRC) populations and (B) distribution of telomeric C-banded chromosomes in (FRC), (SPS), and (SPT) populations

female (Fig. 1b) specimens. Since all pairs in the complement were mono-armed (ST+A), the fundamental number (FN) was 48.

Silver staining carried out on a total of 43 specimens showed that a constant number of NORs occurred in each specimen of the four populations, while a high degree of intra-populational NOR variation could be detected: i.e., 2-8 in Fiumicello river (Figs. 2 and 6A), 2-3 in salt ponds of Siracusa (Figs. 3 and 6A), 2-4 in salt ponds of Trapani (Figs. 4 and 6A), and 1-6 in Salso river (Figs. 5 and 6A).

A maximum number of 8 NORs per cell, which were consistently located terminally on the long arms and looked like black dots, was observed in one specimen collected in Fiumicello river (Fig. 2A). NOR-bearing chromosomes were tentatively paired as „aa", „bb", „cc", and „dd", but, because of the similarity of these chromosomes with other chromosomes in both morphology and size, we were not able to conclusively identify their position in the karyotype. This implies that the number of observed NOR phenotypes might have been underestimated. Occasionally, Ag-staining showed variability as to the dimensions of the corresponding NORs in different individuals of the same population (Fig. 2, see arrows).

Besides a silver pattern involving 8 chromosomes, additional NOR phenotypes including 6 (Fig. 2B), 5 (Fig. 2C) 4 (Fig. 2D), 3 (Fig. 2E), and 2 (Fig. 2F) chromosomes were found in the Fiumicello river population.

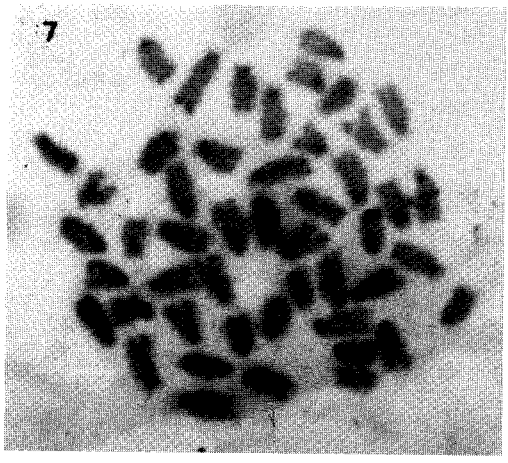

Fig. 7. C-banded metaphase chromosomes of *A. fasciatus*

Similarly, two different NOR phenotypes including 3 and 2 chromosomes have been found in the Siracusa salt pond population (Fig. 3A and B), and three different NOR phenotypes including 4, 3 and 2 chromosomes in Trapani salt pond population (Fig. 4A, B and C).
Four NOR phenotypes have been identified in the 8 specimens from Salso river (Fig. 5). One consisted of 6 silver positive chromosomes three of which displayed interstitial NORs and three terminal NORs (Fig. 5A, arrows indicate interstitial NORs). Another one showed the NORs in two non homologous chromosomes. One NOR was terminal, the other interstitial (Fig. 5B). The remaining two phenotypes each had one silver-stained chromosome with the NOR located interstitially (Fig. 5C) or terminally (Fig. 5D).
The C-banding carried out on a total of 16 specimens from the Fiumicello river, salt ponds of Siracusa and salt ponds of Trapani revealed that all three populations shared more-or-less minute centromeric bands (Fig. 7). Moreover, inter-individual polymorphism of the telomeric C-bands could be detected. Observed variabilities fell in the ranges 5-3, 7-1, 6-1 in the populations from Fiumicello river (Figs. 8A and B), salt ponds of Siracusa (Figs. 9A, B and C), and salt ponds of Trapani (Fig. 10A, B, C and D), respectively.
Application of C-banding to specimens from the Salso river proved to be unsuccesful due to the low abundance of individuals in this sample.

Discussion

Identical modal numbers of chromosomes (2n = 48) were found in the four *Aphanius fasciatus* strains studied. Hence, the existence of inter-populational numerical polymorphisms, previously observed in other fish species (BLACK and HOWELL 1978; OJIMA and KASHIWAGI, 1981), can be excluded in *A. fasciatus* from Sicily. Similarly, since karyotypic analyses revealed that 24 homomorphic pairs occurred in both sexes of this species, it is possible to conclude that differentiated sex chromosomes do not presumably exist in the *A. fasciatus* populations here investigated. Alternatively, as reported for other teleosts (GOLD, 1979; EWULONU et al., 1985), *A. fasciatus* might possess sex chromosomes of a very low grade of morphological differentiation.
Results obtained after silver staining deserve two main considerations. The first is that an intra-populational numerical polymorphism of active NORs (Fig. 6A) occurs in *A. fasciatus*

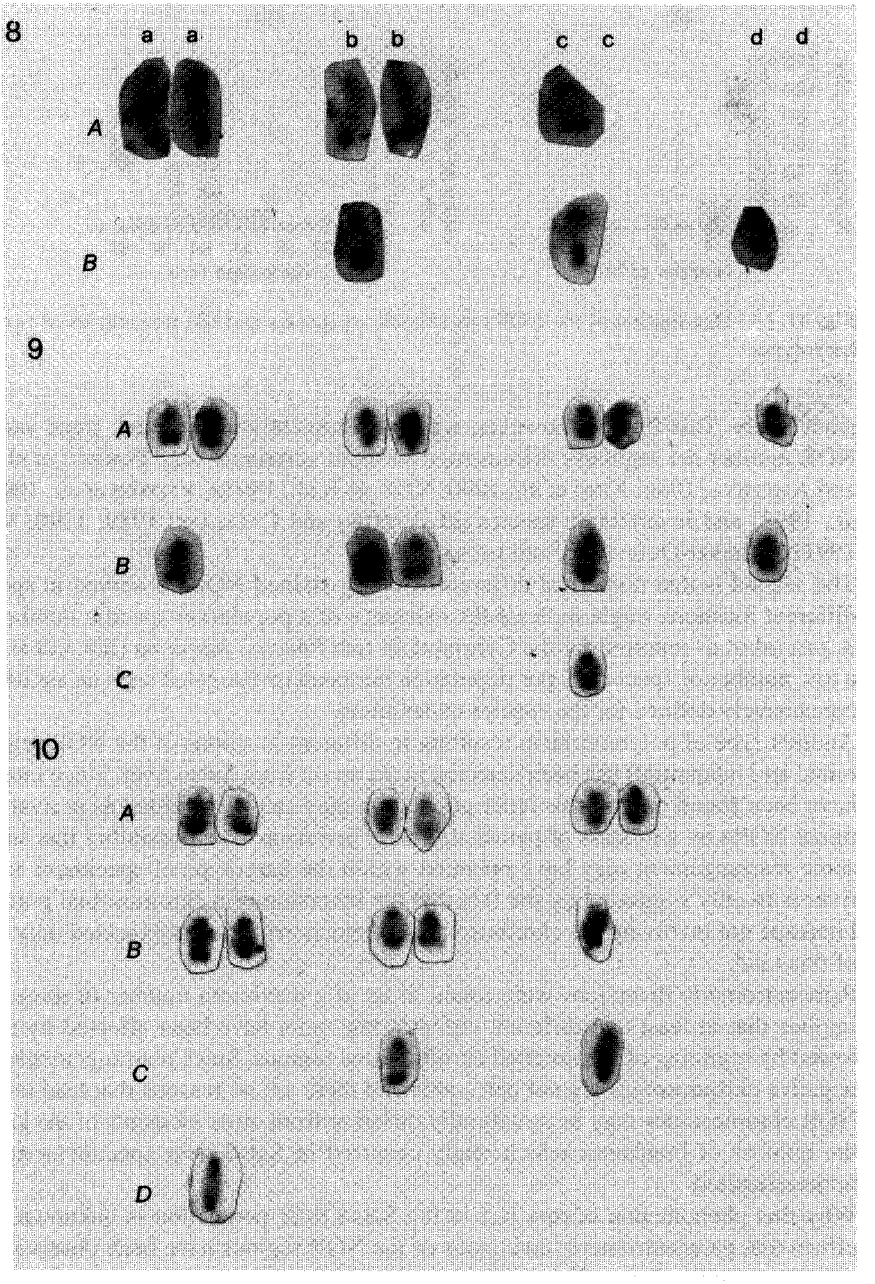

Fig. 8. Telomeric C-band phenotypes of Fiumicello river population displaying:
(A) five chromosomes and (B) three chromosomes

Fig. 9. Telomeric C-band phenotypes of the Siracusa salt pond population displaying:
(A) seven chromosomes; (B) five chromosomes and (C) one chromosome

Fig. 10. Telomeric C-band phenotypes of the Trapani salt pond population displaying:
(A) six chromosomes; (B) five chromosomes; (C) two chromosomes and (D) one chromosome

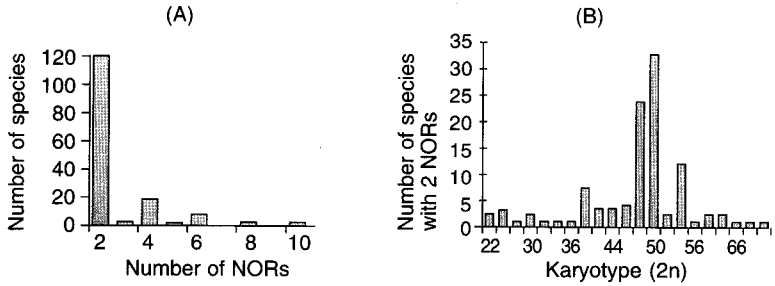

Fig. 11. (A) Distribution of the NORs in 153 teleost species and (B) frequencies of two NORs in fish karyotypes

from Sicily. This finding, however, was not completely unexpected, since variations in the NOR number are regularly documented for both vertebrate (e.g. FORESTI et al. 1981: GOLD and AMEMIYA, 1986; KING et al., 1990; VITTURI et al., 1991a; VITTURI et al., 1992; VITTURI et al., 1993) and invertebrate species (cf. VITTURI and CATALANO 1989; 1990; VITTURI et al., 1991b; THIRIOT-QUIEVREUX and INUSA, 1992).

The second is that numerical differences of Ag-stained NORs observed in specimens from different locations might indicate the existence of a population-specific number of NORs in *A. fasciatus*, as reported for the Cyprinodont fish *Poecilia latipinna* (SOLA et al. 1990). Since a low number of specimens per population has been investigated such an assertion cannot be conclusively defined for the species under study.

Another type of polymorphism resulting in different locations of the NOR regions (i.e., terminal and interstitial) has been detected in the strain from Salso river. Since interstitial NORs have been found only in this latter population they can be regarded as derivatives from terminal NORs by a process of paracentromeric inversion. The possibility that such a chromosome rearrangement may have operated within the karyotype of specimens from the Salso river is mainly suggested by the fact that the inversion of a chromosomal portion following breakage not involving the chromosome's kinetochore region is expected only after an event of this kind.

Paracentromeric inversions were found to be of a maximum number of three per cell. This implies that at least two different chromosome pairs have been affected by breakages followed by rejoining of the inverted chromosomal regions. Since rearrangements within NOR-negative chromosomes are not detectable and there are no reasons that lead to conclude that NOR chromosomes may be structurally different from other elements of the karyotype, then the number of breakages which really occurred in Salso river specimens may have been underestimated.

Why this phenomenon occurs just in the Salso river population is unknown. We can only affirm that paracentromeric inversions of the NOR regions have been observed in *A. fasciatus* specimens from Fiumicello river following exposure to toxicants such as diorganotin(IV)chloro and triorganotin(IV)chloro derivatives of penicillin G (VITTURI et al., 1994). Hence, it is not unreasonable to think that the chromosome rearrangements occurring in Salso river strain can find their origin in the particular environmental conditions such as high variations of both O_2 concentration and water temperature to which individuals of this population are subjected during summer time.

Another interesting point is that, in comparison with other fish species, *A. fasciatus* possesses a high number of chromosomes involved in nucleolus organization. Eight, or a number very

close to this, represents one of the highest numbers of NORs till now reported for teleostean fish. Such an assertion is supported by a graphic representation (Fig. 11 A) constructed on the basis of the NOR data of 153 teleost species. Moreover, available karyological data suggest that 2 NORs mainly occur in complements displaying 48 chromosomes (Fig. 11 B).

Since karyotypes composed of 48 acrocentric chromosomes are widely accepted as primitive within teleosts (OHNO, 1974; SOLA et al., 1990; VITTURI et al., 1991a), the occurrence of 2 NORs should also be regarded as ancestral within this animal group, a higher number as a derivative. This conclusion agrees with results reported by GOLD and AMEMIYA (1986) for Cypriniformes. In consequence, if this notion is correct, the number of chromosomes of the species here investigated would have been retained during evolution, whereas, an increase of chromosomes involved in nucleolus organization would have occurred.

An evolutionary trend of this type is not new within the order Cyprinodontiformes. In fact, the karyotype evolution in the family Poecilidae seems to be numerically and morphologically conservative (SOLA et al., 1981) while a high degree ot NOR variation involving up to seven pairs of NOR-bearing chromosomes has been observed in *Poecilia latipinna* (SOLA et al., 1990).

Acknowledgements

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