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Heterochromatin characterization and distribution in the chromosomes of two populations of *Idotea baltica basteri* Audouin, 1826 (Isopoda, Valvifera)

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Abstract. Two Mediterranean populations of *Idotea baltica basteri* from Messina and Naples showed a set of chromosomes composed by 58 all-biarmed chromosomes. The heterochromatin was located in the pericentromeric region of the chromosomes, and its composition appeared heterogeneous. In fact, not all the homologs showed heterochromatin resistant to digestion with three restriction enzymes (Alu I, Hae III and Sau 3A). Moreover, the two populations showed polymorphism in a band of G + C-rich telomeric heterochromatin, which was present only in the population from Messina. It is hypothesized that chromosomal polymorphism might reflect the geographical isolation of the two populations. It is also suggested that a process of diversification is taking place.
Key words. Chromosomes; crustacea; *Idotea baltica*; isopod.

Idotea baltica (Pallas, 1772) is an isopod widespread in sea and brackish waters of American and European coasts, where it has undergone differentiation, giving rise to several geographical races.

In European waters, four subspecies of *I. baltica* are present: the nominal subspecies and *I. baltica tricuspidata* Desmarest, 1916, distributed in the Baltic Sea, and *I. baltica stagnea* Tinturier-Hamelin, 1960, and *I. baltica basteri* Audouin, 1826, both present in the Mediterranean Sea.

The various populations of the last subspecies, present in Italian coastal waters, are remarkably polymorphic both in colour and sexual size[1,2].

Karyological investigations by conventional staining methods have been performed on three of the four European subspecies (*baltica, tricuspidata, basteri*[3–6]). It has been shown that the subspecies at issue share a karyotype of 58 chromosomes, most of which are metacentric or submetacentric.

This paper reports the data of a chromosomal study carried out by conventional and banding (C-, digestions with restriction enzymes, DNA base-specific fluorochromes) staining methods in two allopatric Italian populations of *I. baltica*, living in very different environments: the Miseno Bay in the Gulf of Naples (seawaters where the alga *Gracilaria* sp. prevails) and Lake Ganzirri near Messina (brackish waters connected via a little canal to the Tyrrenian Sea, where the filamentous algae Chlorophiceae prevail).

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Materials and methods

The *I. baltica basteri* specimens from the Miseno Bay population were collected almost monthly during 1994. The specimens from Lake Ganzirri were collected in the spring of 1994.

The specimens of both populations were kept in a thermostatic chamber at 15 °C and exposed to light for 12 h.

The chromosomes were obtained from gonads or embryos following the scraping plus air-drying method and by short-term culture of embryos and then were stained to define their standard morphology and banding.

Scraping and air-drying. From gonads: At least three male or female specimens were anesthetized by immersion in ice, and their gonads were removed and incubated for 30 min in an Eppendorf test tube with 1 ml of a solution of NaCl 0.9%, 0.042% KCl, and 0.025% $CaCl_2$ containing 10 µl of an 0.5 mg/ml colchicine solution. After centrifugation for 10 min at 1000 rpm, the gonads were incubated for 30 min in a hypotonic solution (0.8% sodium citrate). After further centrifugation, the supernatant was added with 1 ml of fixative (methyl alcohol and acetic acid, 3:1), which was immediately made to flow up and down through the tip of an automatic micropipette in order to obtain an opalescent suspension. After 15 min, the supernatant was collected by centrifugation for 10 min at 1500 rpm, resuspended in 1 ml of fresh fixative and re-centrifuged. Finally, after further centrifugation, the supernatant was resuspended in 100 ml of fresh fixative, and 10-µl aliquots of the suspension were dropped onto a clean slide and air-

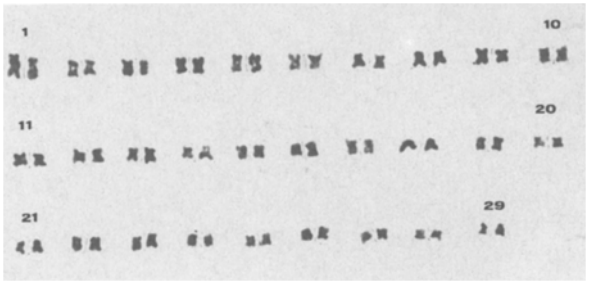

Figure 1. Karyotype of *I. baltica basteri* from Lake Ganzirri (Messina). (The specimens from Miseno (Naples) show a similar karyotype). The bar is 10 μm.

dried. The suspension which is not utilized can be kept for months at −20 °C and re-used after renewal of the fixative.

From embryos: Ovigerous females were anesthetized by immersion in ice, and after lifting the osteogytes, at least 10 embryos at stage I and as many at stages II, III and IV [stages identified on the basis of Stromberg's[4] tables] were taken. The embryos were transferred into an Eppendorf test tube containing an aliquot (about 100 μl) of a solution of NaCl 0.9%. During this step, females are not damaged, and can therefore be re-used. Embryos were manually and gently scraped with the aid of a pestle. After centrifugation for 10 min at 1000 rpm, the supernatant was removed, and the embryos were processed as described for the gonads.

Short-term culture from embryos. Embryos at various stages of development were collected from ovigerous females, as described above. After gentle scraping, they were incubated for 4 h in 1 ml calf serum (GIBCO) containing 100 U penicillin and 100 mg streptomycin. After 30 min, 100 ml of a cholchicine solution (0.5 mg/ml) were added. After centrifugation for 10 min at 1500 rpm, the supernatant was suspended in 1 ml of a hypotonic solution (0.8% sodium citrate) at room temperature. After 30 min and further centrifugation for 10 min at 1500 rpm, the supernatant was resuspended with 1 ml of fixative, and the slides were processed as described for the gonads.

Chromosomes were stained with 5% Giemsa (pH 7) when both methods were used.

Staining. Staining with 5% Giemsa (pH 7) was utilized to define standard morphology of the chromosomes.

The following banding staining methods were performed: C-banding according to Sumner[7], using $Ba(OH)_2$ at 45 °C for 5 min; DAPI according to Schweizer[8]; dystamicin plus chromomycin (DA/Chromomicin) following Schmid and Guttenback[9]; Quinacrine mustard following Schmid[10]; digestions with the restriction enzymes (Alu I, Hae III, Rsa I, Sau 3A, Taq I) according to Mezzanotte et al.[11], using the buffer concentrations recommended by the supplier.

Results

Both the *I. baltica basteri* populations showed a chromosome set of 58 biarmed chromosomes, gradually decreasing in length (fig. 1). Since many chromosome pairs had the same morphology and length, pairing was uncertain for almost all the homologs; thus, the numbers indicated in the karyotypes of the two populations are widely arbitrary.

In the Neapolitan population both the two DNA A + T-specific fluorochromes, quinacrine and DAPI, and the DNA G + C-base specific fluorochrome, chromomycin, uniformly stained the chromosomes (figs 2a, b).

In the population from Messina, however, chromomycin stained a pair of submetacentric chromosomes (tentatively the second pair) showed a strongly highlighted band on the short arm (fig. 2c).

In both populations, constitutive heterochromatin was localized on the centromere of all the pairs of homologs

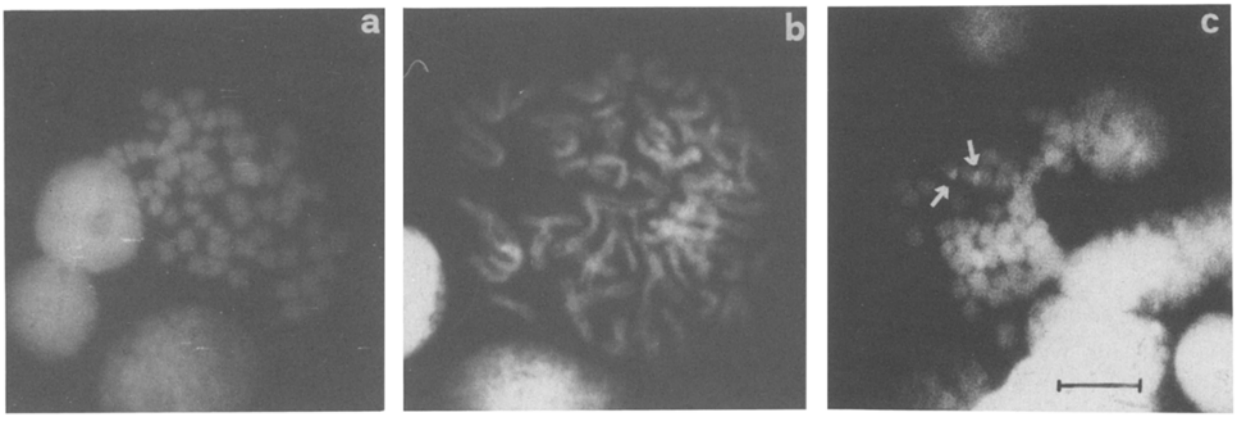

Figure 2. Metaphase plates of *I. baltica basteri* from Miseno (Naples) stained with DA/DAPI (*a*) and DA/chromomycin (*b*). Metaphase plates of *I. baltica basteri* from Lake Ganzirri (Messina) stained with DA/chromomycin (*c*). The bar is 10 μm.

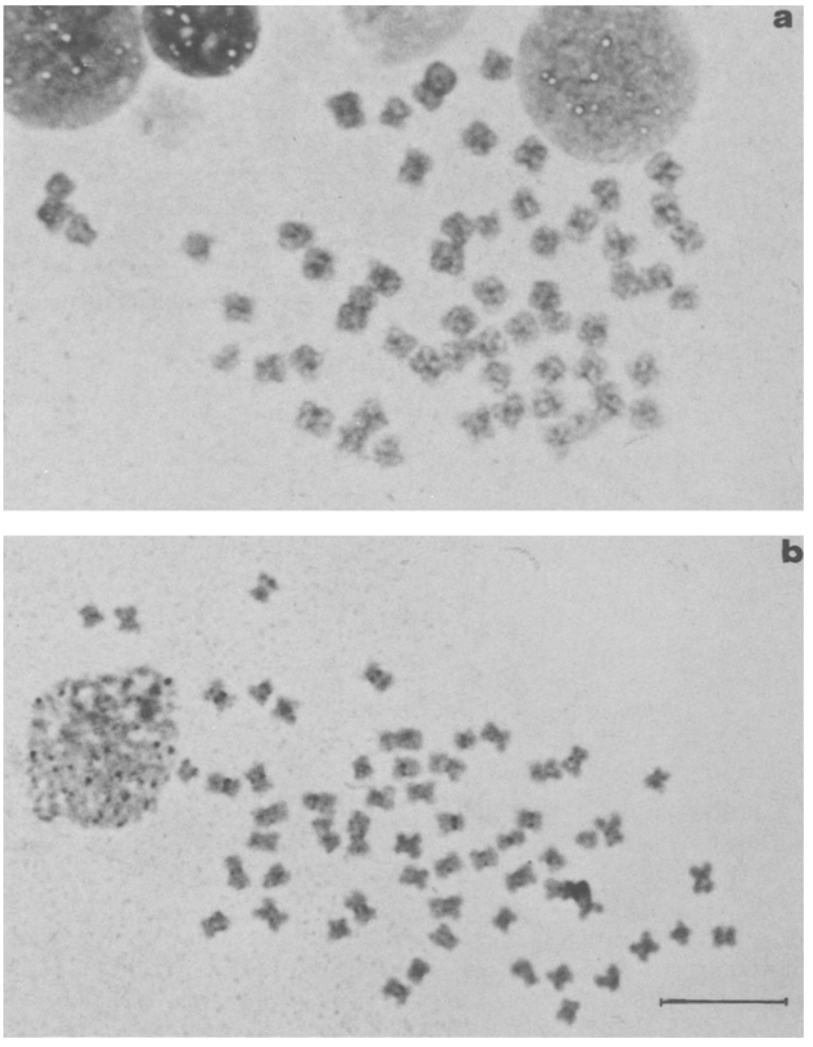

Figure 3. C-banded metaphase plates of *I. baltica basteri* from (*a*) Miseno (Naples) and (*b*) Lake Ganzirri (Messina). Note that the telomeric band of the latter population is resistant to C-banding at higher temperatures. The bar is 10 μm.

(figs. 3a, b). However, in the population from Lake Ganzirri, C-banding also showed a conspicuous block of telomeric heterochromatin on the short arm of a pair of homologs (tentatively the 2nd) (fig. 3b).

Two of the five restriction enzymes used in this study, Rsa I and Taq I, appeared unsuitable for the study of *I. baltica basteri* chromosomes. In fact, after incubation with the two enzymes, the chromosomes acquired a fuzzy aspect not different from that in the control slides for which only the incubation buffer of the enzyme was used (data not shown).

Vice versa, in both the populations, the enzymes Alu I, Hae III and Sau III A extensively digested the chromosomes, leaving the pericentromeric regions of all the chromosome pairs undigested, except at least three of them (fig. 4). Besides the centromeric regions, Hae III also failed to digest the telomeric regions of several pairs of homologs (figs. 4d, e).

Discussion

The study of the karyotype of different species and subspecies of *Idotea* led Salemaa[5] to conclude that although the geographically isolated *I. baltica basteri* populations show differentiation in their external morphology[12,13] and isozyme patterns[1], the karyological features have generally remained unchanged.

This is further supported by the present study, only as regards gross chromosomal morphology. In fact, the *I. baltica* specimens from Messina and Naples share the number ($2n = 58$) and morphology of chromosomes, already described for specimens belonging to both the same subspecies in the Adriatic Sea, coast of Cesenatico[6], and to the *baltica* and *tricuspidata* races[5].

Moreover, in the present study, banding stainings (C-, restriction endonucleases and base-specific fluorochromes) have provided useful information on heterochro-

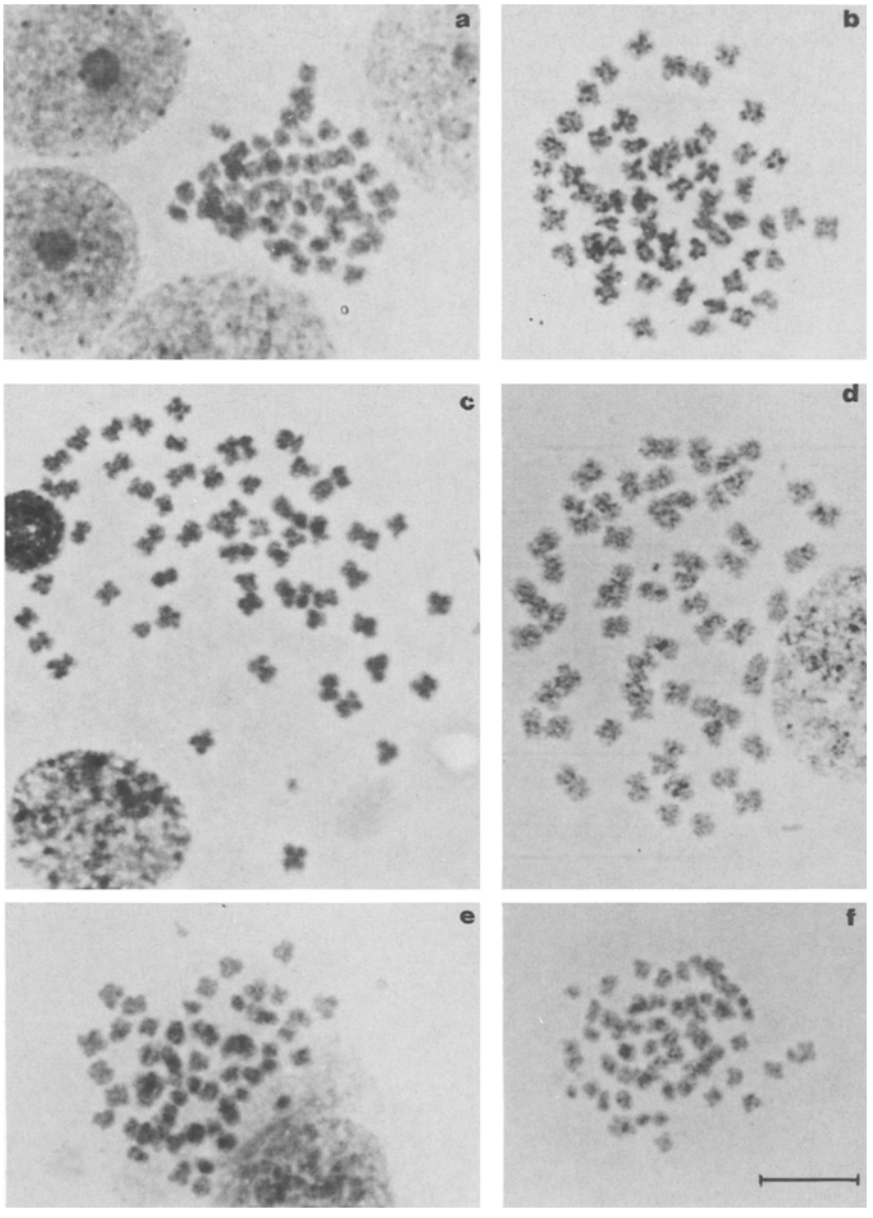

Figure 4. Metaphase plates of *I. baltica basteri* from Lake Ganzirri (Messina) digested with (*a*) Alu I, (*c*) Hae III and (*e*) Sau 3A. Metaphase plates of *I. baltica basteri* from Miseno (Naples) digested with (*b*) Alu I, (*d*) Hae III and (*f*) Sau 3A. The bar is 10 μm.

matin composition and distribution in this isopod species.

In fact, *I. baltica basteri* shows at least two types of heterochromatin, differing both in chromosome location and DNA base content.

One type of heterochromatin includes the heterochromatic DAPI-, quinacrine- and chromomycin-negative blocks present along the pericentromeric regions of all the pairs of homologs. However, it is noteworthy that the pericentromeric heterochromatin of at least three chromosome pairs would be different from that present in the other pairs, being the heterochromatin contained in those three pairs digested by Alu I, Hae III and Sau 3A.

The other type of heterochromatin is represented by the telomeric band, chromomycin-positive and hence G + C-rich, present on the short arm of the second pair of homologs (our preliminary data would indicate that this heterochromatin may be NOR-associated). It is to be pointed out that it is present only in the *I. baltica basteri* specimens of the population from Messina. In this population, it might either have originated de novo, or also be present in the Neapolitan population in amounts undetectable by conventional banding methods.

Karyological differentiation due to the different location of heterochromatin is quite a widespread phenomenon, and has been well documented in several species of invertebrates[14]. The role played by this variation of

heterochromatin in creating effective reproductive barriers is controversial. In fact, several authors[14–16] suggest that this variation would have no role, in contrast with the hypotheses advanced by other investigators[17].

However, it is to be pointed out that the two populations are geographically isolated, and the larvae are incubated in the marsupium of ovigerous females, which, like juveniles, remain in the distribution area of the population. Therefore, the karyological differences in the two populations may suggest that their geographical isolation occurred quite remote in time, and hence their diversification took place early. In this regard it is interesting to stress that the two populations also exhibit morphological and enzymatic differences[18].

Further information can be provided by expanding the study to specimens belonging to different populations of the same subspecies, as well as to other subspecies of *I. baltica*.

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