Giemsa C-banded karyotypes in Serapias L. (Orchidaceae)

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Giemsa C-banding is utilized for the first time to characterize eight taxa of the genus Serapias. Heterochromatin distribution indicated that the Serapias species form a very homogeneous group. All the species possess chromosome pairs with similar heterochromatin patterns. C-banding showed conspicuous bands located around the centromeres, with some heterochromatic short arms. There was more heterochromatin in S. apulica and S. nurrica than in the other taxa. Extensive centromeric heterochromatin may indicate recent structural rearrangements in the chromosome complement. Taken altogether, karyomorphology indicates a rather recent origin for the genus Serapias, which might also account for the small amount of interspecific variation observed.

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ADDITIONAL KEY WORDS:—heterochromatin distribution — Italy — karyotype evolution — phyletic relationships.

INTRODUCTION

Serapias L. is a genus of Orchidaceae comprising about twenty entities, ten of which are present in Italy (Quentin, 1995). Previous cytological studies have shown
that in this genus most species possess \(2n = 2x = 36\) chromosomes; with only \(S. \text{lingua}\) having \(2n = 4x = 72\) (Heusser, 1938; Scrugli, De Martis & Mulas, 1976; Del Prete, 1977; Mazzola, Crisafi & Romano, 1982; Cauwet-Marc & Balayer, 1986; Bianco et al., 1987).

In previous reports (D’Emerico et al., 1990; D’Emerico, Bianco & Medagli, 1992; Bianco et al., 1990, 1992) the karyomorphological characteristics of \(S. \text{vomeracea}\) subsp. \(\text{vomeracea}\), \(S. \text{vomeracea}\) subsp. \(\text{laxiflora}\), \(S. \text{parviflora}\), \(S. \text{politisii}\) and \(S. \text{apulica}\) had been investigated using Feulgen analysis of the chromosome complements. All revealed a complex chromosome morphology and the karyotypes were found to be moderately asymmetrical with a predominance of submetacentric chromosomes.

The variation among species of the genus \(S. \text{rapias}\) is rather limited as compared with other Orchidaceae genera, and a recent study based on sequence divergence of the ITS of ribosomal genes showed little differentiation among the species (Pridgeon et al., 1997).

In this contribution we report on chromosomal distribution of heterochromatin as revealed by Giemsa C-banding in eight taxa of \(S. \text{rapias}\), all collected in Italy.

MATERIAL AND METHODS

Preparations of mitotic and meiotic chromosomes were made from immature ovaries. These were pre-treated with 0.3% colchicine at room temperature for 2 h. For Feulgen staining they were fixed for 5 min in 5:1:1:1 (v/v) absolute ethanol, chloroform, glacial acetic acid and formalin (Battaglia, 1957a). Hydrolysis was made at 20°C in 5.5 N HCl for 20 min (Battaglia 1957b). The material was then stained in freshly prepared Feulgen stain.

For C-banding, immature ovaries were fixed in ethanol-glacial acetic acid (3:1 v/v) and stored in the deep-freeze for up to several months. Subsequently they were squashed in 45% acetic acid; coverslips were removed by the dry ice method and the preparations air-dried overnight. Slides were then immersed in 0.2 N HCl at 60°C for 3 min, thoroughly rinsed in distilled water and then treated with 4% \(\text{Ba(OH)}_2\) at 20°C for 4 min. After thorough rinsing they were incubated in 2 × SSC at 60°C for 1 h. The stain used was 3–4% Giemsa (BDH) at pH 7.

Chromosome pairs were identified and arranged on the basis of length. The nomenclature used for describing karyotype composition followed Levan, Fredga & Sandberg (1964).

RESULTS

The provenance and chromosome numbers of each studied taxon are reported in Table 1.

\(S. \text{vomeracea}\) (N.L. Burm.) Briq. subsp. \(\text{vomeracea}\)

The observed chromosome number \(2n = 2x = 36\) in all the investigated populations agrees with previous reports (Heusser, 1938; Del Prete, 1977; Mazzola et al., 1982;
GIEMSA C-BANDING IN SERAPIAS

Table 1. Provenance and chromosome numbers of the species studied

<table>
<thead>
<tr>
<th>Taxon</th>
<th>Provenance</th>
<th>Chromosome number 2n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serapias vomeracea (N.L. Burm.) Briq. subsp. vomeracea</td>
<td>BASILICATA: Potenza, Matera. APULIA: Gargano Peninsula (Foggia), Cassano Murge (Bari), Martina Franca (Taranto)</td>
<td>36</td>
</tr>
<tr>
<td>S. vomeracea (N.L. Burm.) Briq. subsp. laxiflora (Soò) Götz &amp; Reinh.</td>
<td>APULIA: Gargano Peninsula (Foggia)</td>
<td>36</td>
</tr>
<tr>
<td>S. cordigera L.</td>
<td>BASILICATA: Potenza, Matera. APULIA: Gargano Peninsula (Foggia), Cassano Murge (Bari), Martina Franca (Taranto) SARDINIA: Muravera</td>
<td>36</td>
</tr>
<tr>
<td>S. parasiflora Parl.</td>
<td>BASILICATA: Potenza, Matera. APULIA: Gargano Peninsula (Foggia), Cassano Murge (Bari), Martina Franca (Taranto) SARDINIA: Portoscuso, Castiadas</td>
<td>36</td>
</tr>
<tr>
<td>S. politisi Renz</td>
<td>APULIA: Lecce</td>
<td>36</td>
</tr>
<tr>
<td>S. apulica (Baumann &amp; Künkele) P. Delforge</td>
<td>APULIA: Torre Canne (Brindisi), Mattinata (Foggia)</td>
<td>36</td>
</tr>
<tr>
<td>S. nurrica Corrias</td>
<td>SARDINIA: Cortoghiana</td>
<td>36</td>
</tr>
<tr>
<td>S. lingua L.</td>
<td>BASILICATA: Potenza, Matera. APULIA: Gargano Peninsula (Foggia), Cassano Murge (Bari), Martina Franca (Taranto) SARDINIA: Portoscuso, Muravera</td>
<td>72</td>
</tr>
</tbody>
</table>

Bianco et al., 1987). This species possesses a moderately asymmetrical karyotype, consisting of mainly submetacentric chromosomes (D’Emerico et al., 1992). Giemsa C-banding analysis showed very conspicuous bands located at centromeric positions on many chromosomes, together with euchromatic telomeric regions. Only pair 12 has a completely heterochromatic short arm. Pairs 15 to 18 show small amounts of centromeric heterochromatin (Figs 1, 8A).

The karyomorphology and banding pattern in S. vomeracea (N.L. Burm.) Briq. subsp. laxiflora (Soò) Götz & Reinhard were identical to those of S. vomeracea subsp. vomeracea.

Serapias cordigera L.

In this species somatic chromosome counts showed 2n=36 chromosomes in agreement with previous reports (Scrugli et al., 1976; Mazzola et al., 1982; Cauwet-Marc & Balayer, 1986). The karyotype morphology, consisting of 16(18)m + 18(16)sm + 2st chromosomes, is reported here for the first time.

The banding pattern of S. cordigera appears to be similar to that of S. vomeracea in having large centromeric bands on many chromosomes. Pairs 10 and 12 showed large centromeric bands and heterochromatic short arms. Pairs 13, 14, 16, and 18 showed small centromeric bands (Figs 2, 8B).
Somatic cells showed $2n=36$ chromosomes as reported by D'Emerico et al., (1990). With respect to the previously-described taxa, this species showed a more asymmetrical karyotype, comprising mainly submetacentric and subtelocentric chromosomes (D'Emerico et al., 1990). *S. apulica* showed stronger heterochromatin bands than in the other taxa. Pairs 13, 14 and 18 displayed large centromeric bands and heterochromatic short arms (Figs 4, 8C).
Figures 5-7. Fig. 5. *Serapis nurrica*, somatic C-banded metaphase. Fig. 6. *S. lingua*: somatic C-banded metaphase. Fig. 7. *S. lingua*: Feulgen stained metaphase I in EMC showing 36 bivalents. Scale bar: 5 μm.

*Serapis nurrica* Corrias

The number $2n = 36$ confirms a previous finding (Scrugli, 1982) for the species. The banding pattern in *S. nurrica* appears to be similar to that of *S. apulica* in heterochromatin distribution (Fig. 5).

*Serapis parviflora* Parl.

Specimens from southern Italy and Sardinia showed $2n = 36$, in accordance with Del Prete (1977), Scrugli (1978) and Mazzola et al. (1981). Among the species considered here, *S. parviflora* shows the least asymmetrical karyotype (Bianco et al., 1990). C-banding analysis revealed that this is the species of this complex having
the smallest amount of heterochromatin, with centromeric bands present on fewer chromosomes than in the other taxa. Pairs 6, 7, 10, 12 and 16 showed large centromeric bands and heterochromatic short arms. Pairs 11, 15, 17 and 18 showed thin centromeric bands (Figs 3, 8D).

S. politisii Renz, recently reported from Apulia (Bianco et al., 1992), shows a banding pattern identical to that of S. parviflora.

**Serapis lingua** L.

In this species somatic metaphases showed $2n = 4x = 72$ chromosomes, as already reported (Scrugli, 1978; Mazzola et al., 1982; Cauwet-Marc & Balayer, 1986). Meiotic studies revealed 36 bivalents at metaphase I in EMCs (Fig. 7). The banding pattern of *S. lingua* appears to be similar to the other taxa in having chromosomes with large blocks of centromeric heterochromatin (Fig. 6).

**DISCUSSION**

The genus *Serapis* encompasses a group of taxa of difficult taxonomy due to their highly complex morphology and similarity. Some features of the karyotypes have been described in previous reports (D’Emerico et al., 1990, 1992; Bianco et al., 1992) and the general overall resemblance is in line with the small morphological variation.

In this paper, the study of the chromosomal distribution of Giemsa C-bands in the different taxa of this genus has revealed some interesting aspects of their phyletic relationships.

C-banding showed conspicuous bands in numerous chromosome pairs, located at centromeric positions, with some pairs also characterized by C-bands at the telomeric position on the short arms or heterochromatic short arms. The heterochromatin occupied the entire extent of the chromosomes, with euchromatin being limited to the extremities. This heterochromatin distribution has been observed.
only in a few other cases, e.g. Cicer, Melipona (Galasso et al., 1996; Rocha & Pompolo, 1998).

The investigated taxa were characterized by different amounts of constitutive heterochromatin. In spite of the large number and particular position of C-bands, it is still difficult to recognize homologies between chromosome pairs of different taxa. Nevertheless, the analysis of C-band distribution, along with variation of morphological characters, suggests that the taxa of Serapias form a rather homogeneous group.

In some cases the apparent karyomorphological homology reaches a high degree, as in the case of S. vomeracea subsp. vomeracea and S. vomeracea subsp. laxiflora. In consideration of this high level of karyomorphological and heterochromatin resemblance, it is conceivable that these two taxa are better considered as different morphotypes of the same species. Similar conclusions, based on the remarkable resemblance of their C-banded karyotypes, may be drawn for S. parviflora and S. politisi.

It is interesting to note that S. apulica displayed a more asymmetrical karyotype and a higher amount of heterochromatin bands than the other taxa. In this connection, S. nurraca shows a close similarity to S. apulica in heterochromatin distribution.

Karyological analysis in the tetraploid S. lingua failed to identify all chromosomes precisely, due to the complexity of its karyotype. Nevertheless, based on heterochromatin amount and distribution, it was possible to show that the complement of this species shows some chromosome pairs with smaller amounts of heterochromatin. This feature is a characteristic of the chromosomes of S. parviflora, which indeed resemble many of those of S. lingua. It is therefore possible to suggest that S. parviflora has played a role in the evolution of S. lingua. Whether this latter species is to be considered an autotetraploid or not is still an open question.

Bernard & Miklos (1979) suggested that an increase in centromeric heterochromatin is produced as a consequence of structural chromosome rearrangements. On these grounds, the presence of large centromeric heterochromatin blocks in Serapias might indicate recent structural rearrangements in the chromosomal complement, as also suggested by the asymmetry of the karyotype as a whole (Stebbins, 1971). Taken altogether, these indications might indicate a rather recent origin for Serapias, and the reduced time scale might account for the small interspecific variation of nuclear and morphological characters that are observed in the genus.

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REFERENCES


