IRIS: ALLOCYCLIC SEGMENTS AS CHROMOSOME MARKERS?

M. COLASANTE* and C.G. VOSA°

* *Dipartimento di Biologia Vegetale, Università «La Sapienza», Rome, Italy*
° *Dipartimento di Scienze Botaniche, Università di Pisa, Italy*

ABSTRACT - Besides the linear differentiation of chromosomes, obtained by various methods including C banding, Q-banding and G- banding, a type of differentiation is sometimes visible after normal fixation and Feulgen staining. Such differential segments are found in the chromosomes of many bulbous and rhizomatous *Iris* species and appear to be species and population specific. Chromosome analysis of several Irises has shown that there is some variation in the length and distribution of the AC-segments in different species and occasionally also in different populations of the same homeologous chromosomes. Therefore the AC-segments could be used as markers to elucidate the ancestry of *Iris* natural hybrids.

KEY WORD - *Iris*, cytotaxonomy, allocyclic segments

INTRODUCTION

The genus *Iris* L., which is particularly rich in species and natural and artificial hybrids, has many taxonomic and nomenclatural problems as yet unresolved, including (a) problems of synonymy, (b) population polymorphism, and (c) the hybrid origin of certain populations. Macromorphological analysis alone is often insufficient to resolve such problems, especially in the case of natural hybrids. Other methods of research include cytotaxonomy and in particular the comparative study of chromosome morphology. In some cases the presence of sizeable heterochromatic segments (H-segments) revealed by various banding techniques, may help in chromosome characterisation, but so far these have not been found in the genus *Iris*. Generally, the standard Feulgen method in the absence of any pretreatment does not reveal the H-segments. However, in some organisms simple Feulgen staining shows a linear differentiation consisting of chromosome segments which have a different condensation cycle. Such allocyclic segments (AC-segments) cannot be considered heterochromatin in the classical sense of Heitz (1935). AC-segments have been found in quite a number of organisms and their size and distribution in the chromosomes are usually species-specific (Dyer, 1963; Vosa, 1986). Although clearly visible in the chromosomes of the genus *Iris*, AC-segments have not been noted until quite recently (Vosa and Colasante, 1995). The present study utilises information on the presence of

AC-segments to interpret the origins and phylogeny of some species and populations of the genus *Iris*.

Materials and methods

Material consisted of bulbous and rhizomatous *Iris* species belonging to the subgenus *Scorpiris* (Mathew, 1981) (the genus *Juno* of Rodionenko, 1961) and subgenus *Iris* section *Iris* (Mathew, 1981) collected in the field and cultivated in pots.

Species used in the study, collection localities and collectors names are as follows:

I. palestina Boiss., low hills south of Reliovot (Israel), in overgrown citrus groves (coll. C.G. Vosa); *I. planifolia* (Mill.) Fiori and Paoletti, Comiso, Sicily (coll. I. Ricci); *I. pseudopumila* Tineo, Gravine di Laterza (legit M.A. Colasante); *I. pseudopumila* Campobasso (legit F. Lucchese); *I. pallida* Lam. San Giuliano Terme, Pisa (coll. G. Martinoli); *I. cengialti* A. Kerner Lago di Garda, Riviera orientale, Passo di Spino (m.1125; coll. G. Martinoli); *I. illyrica* Tomm. Monte Nanos (coll. I. Ricci); *I. lutescens* Lam. Torre d' Orlando, Civitavecchia (legit M.A. Colasante); *I. revoluta* Colas. Scoglio Mojuso, near Porto Cesareo, Taranto (legit M.A. Colasante); *I. bicapitata* Colas. Sannicandro Garganico, Gargano (legit M.A. Colasante); *I. marsica* I. Ricci and Colas., Piana della Rocca near Pescasseroli, L'Aquila (legit M.A. Colasante); *I. setina* Colas., Monte Trevi, Sezze, Latina (legit M.A. Colasante); *I. sabina* N. Terracc., Monti Lucretili, Palombara Sabina (legit M.A. Colasante); *I. relicta* Colas., Monte delle Fate, Monti Ausoni, Sonnino (legit M.A. Colasante).

Root-tips at the correct stage of development were pretreated with an aqueous solution of colchicine (0.02 %), fixed and stained following the standard Feulgen method. For *I. palestina*, a particularly sensitive species, the pretreatment was made with an aqueous solution of 0.02 % colchicine mixed *V/V* with 0.02 M of hydroxyquinoline in water. Moreover, a drop of acetic orcein added to the Feulgen method, before squashing the roots apex, did not change the evidence or the distribution of the AC-segments (Fig.1).

Results

Karyotype analysis has established the existence of AC-segments which in *I. palestina*, abulbous species, are found mostly at the distal end of the long arm of the chromosomes and sometimes occupy more than half of the arm, indipendently of the stage of condensation (Figs. 1, 2, 3). In *I. planifolia* (same systematic group), there are some chromosomes characterized by the same type of AC-segments as in *I. palestina* (see, for example, arrows in Figs.1, 2, 4).

In *I. pseudopumila* (Figs. 5, 10, 11), a rhizomatous species, the length of the AC-segments is variable even in chromosomes of the same size, while in *I. pallida* (Figs.10, 13,14 c) there exists some chromosome polymorphism but with AC-segments in proportion to the length of the arm. Analysis of AC-segments in some plants of populations of *I. pseudopumila* from different localities, has shown a certain variability in their length and distribution (Figs. 5,10, 11). Clearly visible AC-segments are also found in the chromosomes of *I. illyrica* (Figs. 6, 10), *I. cengialti* (Figs. 7, 10), *I. revoluta* (Figs. 8, 9) and *I. lutescens* (Fig. 9, 12, 14 b).

Analysis of the chromosome complement of other *Iris* species is in progress and for some we have already established the basic karyograms (Figs. 9, 10).

Discussion

AC-segments are chromosome parts which, in the absence of any pretreatment, show a different cycle of condensation during mitosis. Their condensation cycle is generally somewhat retarded respect to the rest of the chromosome, so that they seem to mantain a kind of spiralization comparable to that seen at certain stages of prophase throughout the mitotic cycle, and are clearly visible at metaphase. They are not classic heterochromatin (*sensu* Heitz, 1935), because they do not maintain an anaphasic condensation during interphase nor do they appear as chromocentres in the resting nucleus. AC-segment distribution and size showed some interspecific variability in species studied here. Other parallel investigations, carried out in collaboration with Prof. W. Sauer of the University of Tubingen Germany (in preparation), on highly variable species such as *I. pseudopumila,* have revealed a seemingly balanced polymorphism of certain AC-segments which are characteristic of the species and apparently valid chromosome markers.

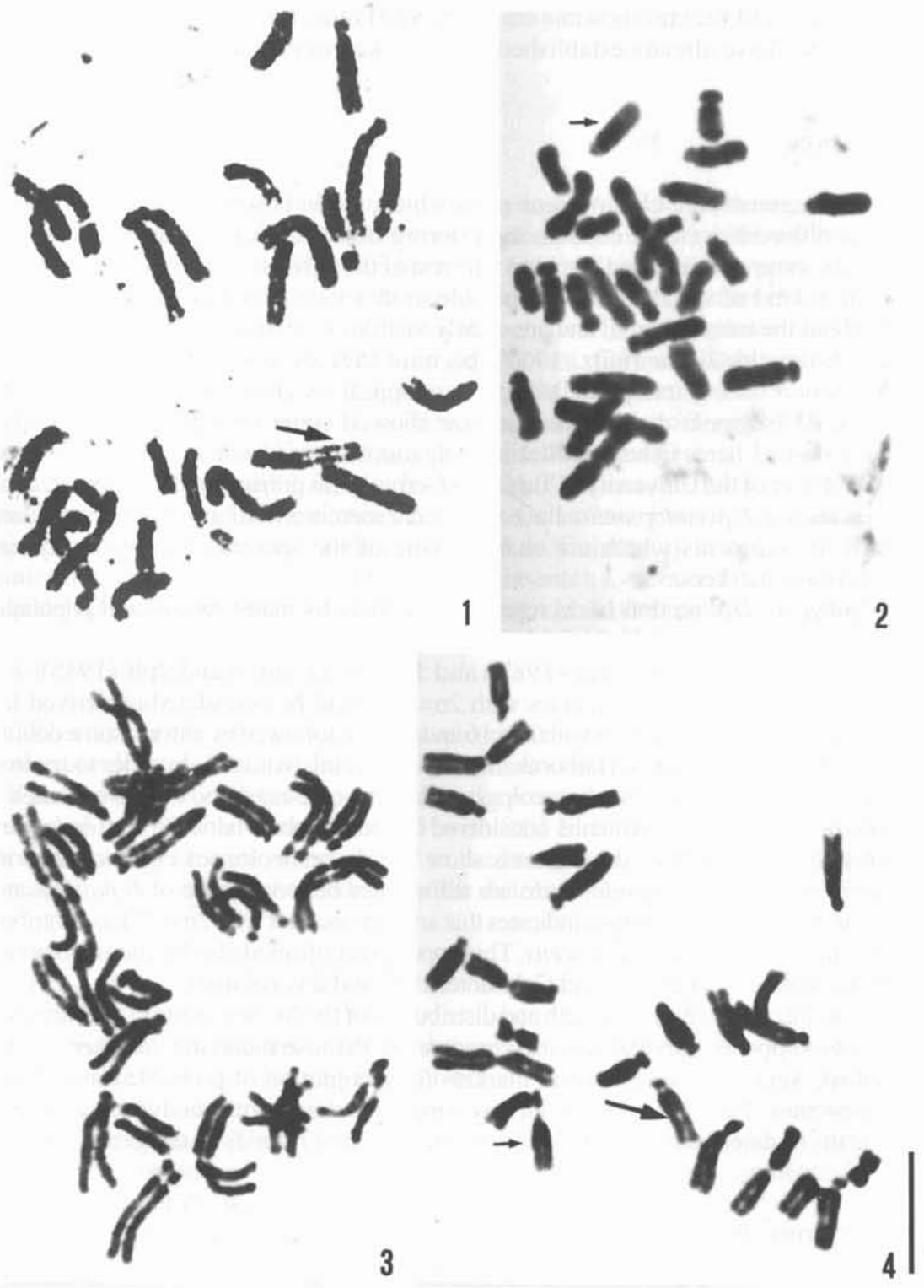
Subgenus *Iris*, section *Iris* is represented in Italy by many species and populations having chromosome numbers of 2n = 16, 24, 40 and 44. The experimental studies of Simonet (1951), Werkmeister (1981) and Sturtevant and Randolph (1945), have conclusively shown that the species with 2n= 40 could be considered as derived from natural hybrids between species with 2n= 16 and 2n= 24, followed by chromosome doubling hybrids (Fig. 2 in Williams, Harborne and Colasante, this volume). In order to try to use the AC- segments as possible homeologous chromosome markers, we have studied the karyotype of *I. lutescens* which is considered a natural hybrid between *I. pseudopumila* and *I. pallida.* While the AC-segments show clearly homeologous chromosomes in *I. lutescens* and *I. pseudopumila*, there are differences between those of *I. pallida* and *I. lutescens* (Fig. 14 b and c), which indicates that another ancestor with 2n = 24 has contributed to form the natural hybrid *I. lutescens*. This appeared confirmed also by chemotaxonomic analysis (Williams, Harborne and Colasante, 1997 and this volume).

Careful observations of length and distribution of the AC-segments in the karyotypes of the two supposed parental species, compared with those found in *I. lutescens,* indicate that AC-segments can be useful markers for recognition of probable homeologous chromosomes. Thus, through a relatively simple cytotaxonomic analysis, we can have the means of discovering the probable origin of natural hybrids in the genus *Iris*.

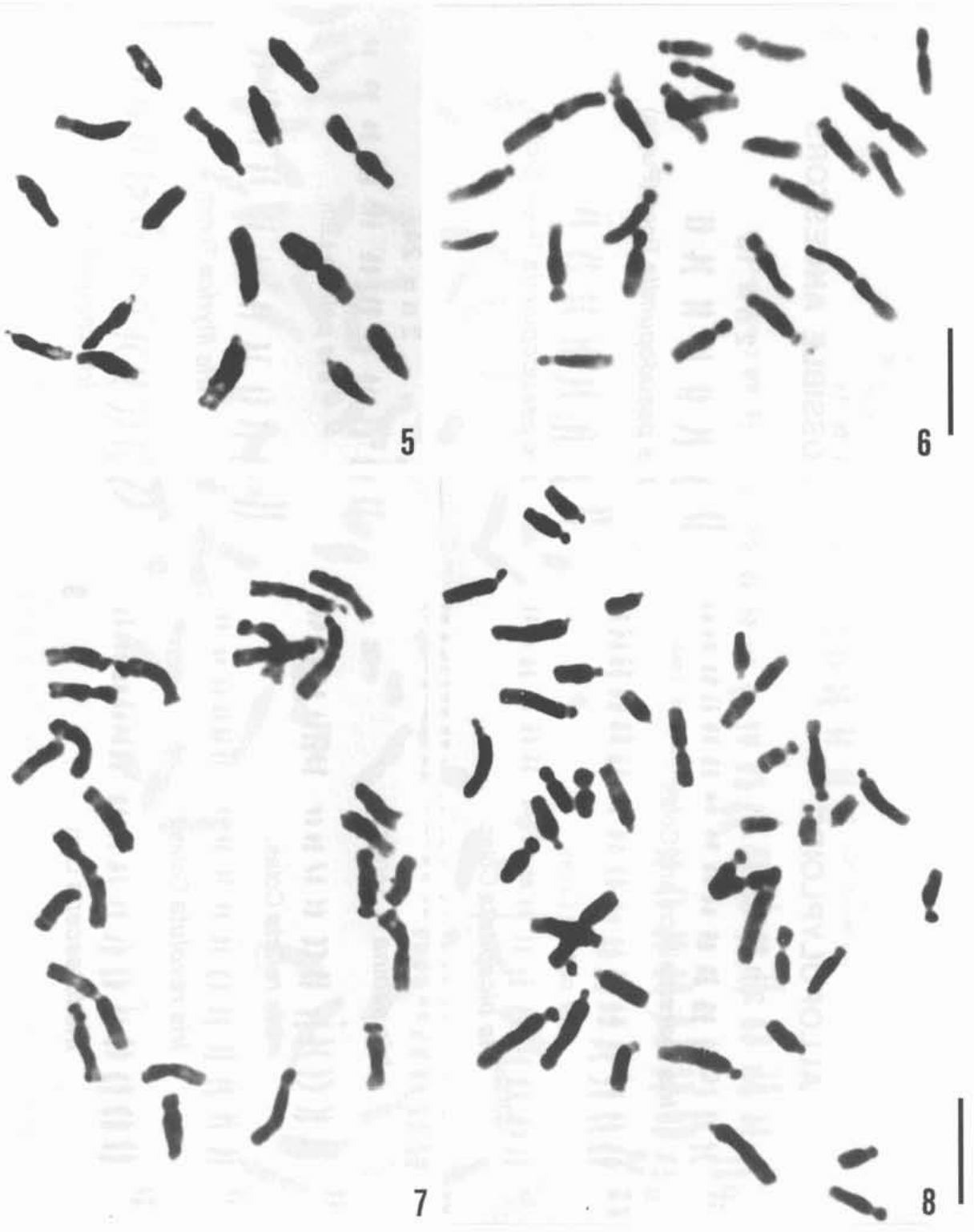
Conclusions

1. Because of their differing condensation cycle we can speculate that the AC-segments, in analogy with the H-segments, may have an effect on the meiotic recombination process.

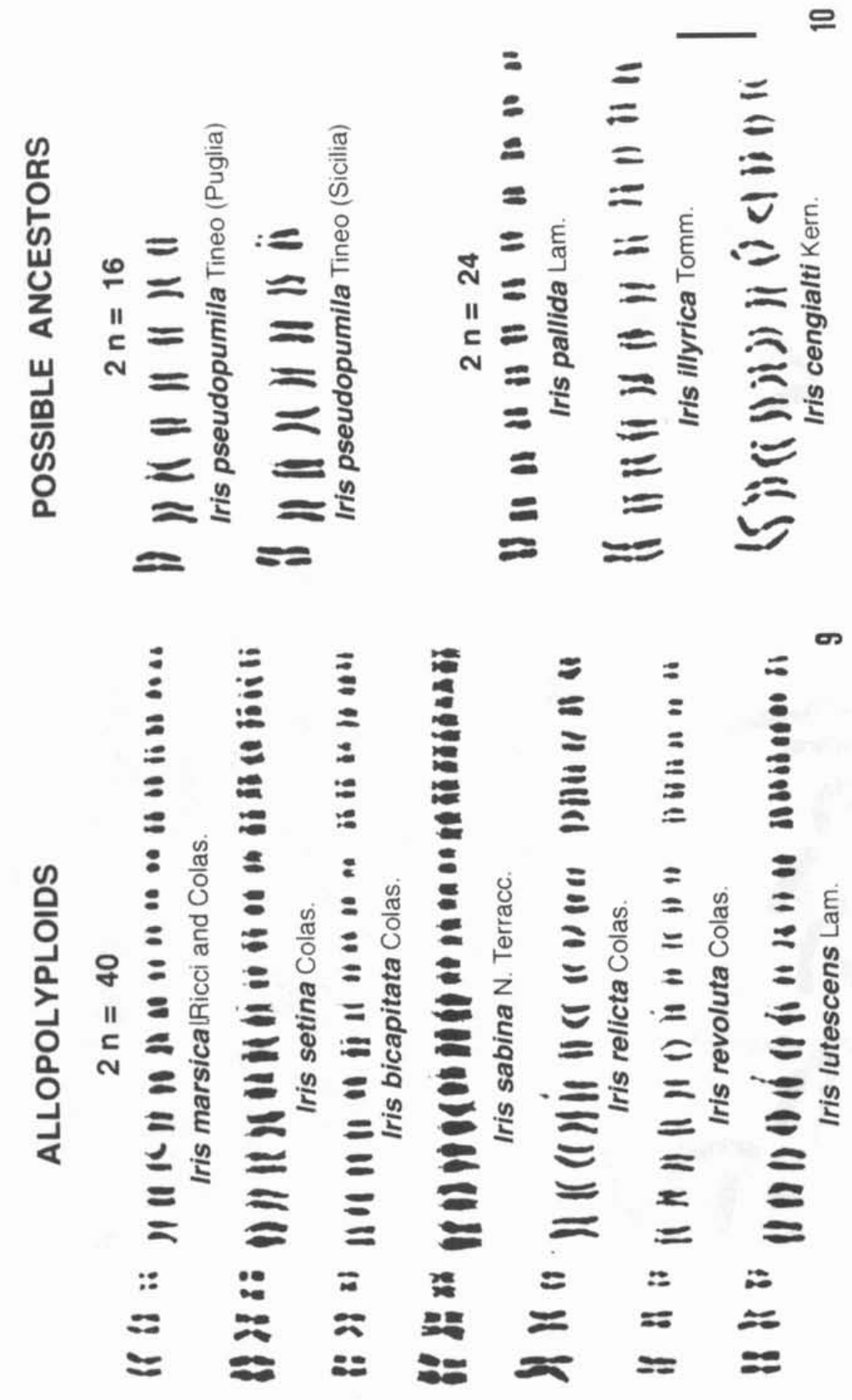
2. *I. palestina* and *I. planifolia* are considered to be very near each other systematically. The presence of AC-segments in supposedly homeologous chromosomes seems to confirm this hypothesis.



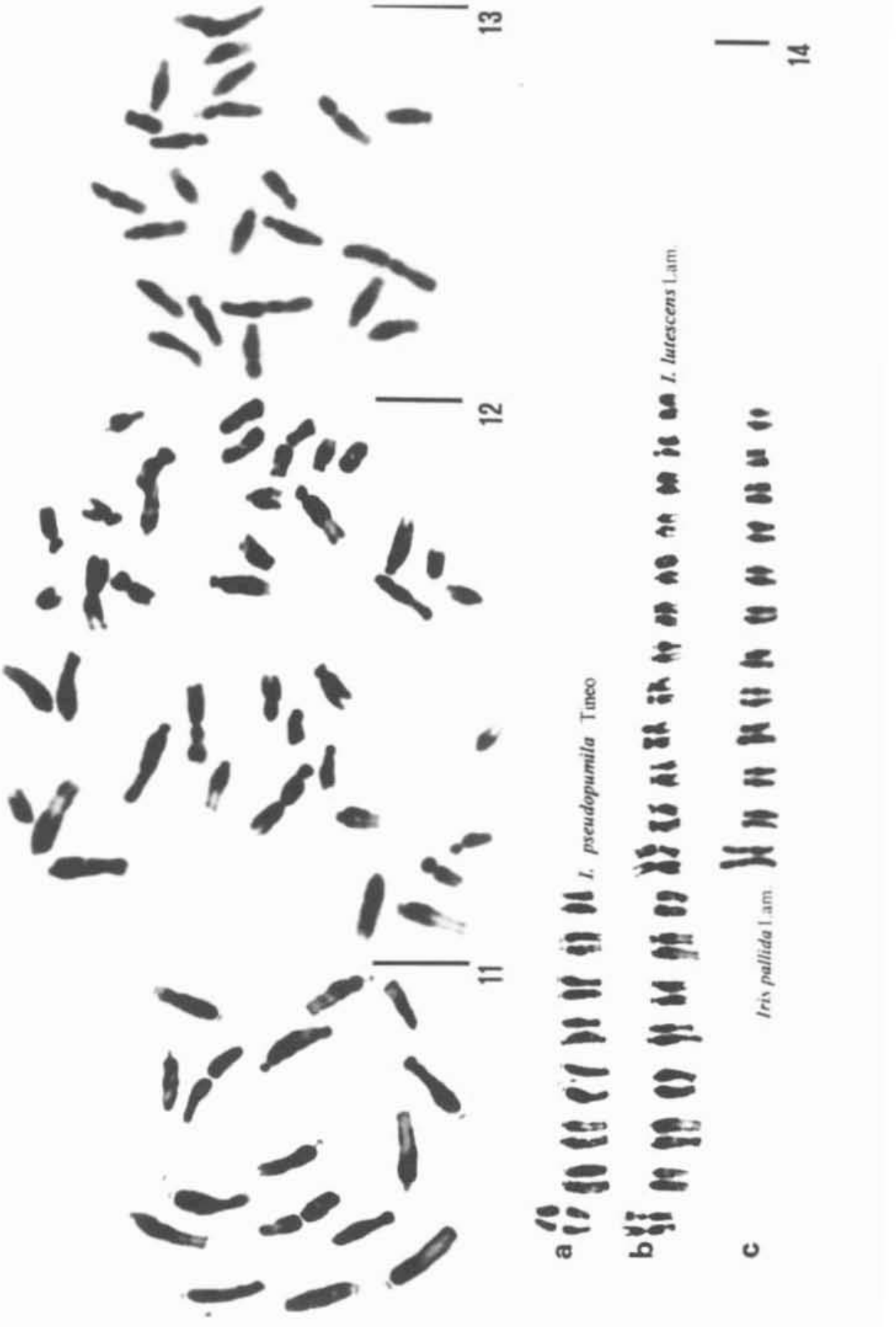
Figures 1, 2, 3 - *I. palestina.* Three karyotypes at different stage of chromosome condensation: prometaphase (Fig. 1), metaphase (Fig. 2), meta-anaphase (Fig. 3). Figure 4 - *I. planifolia* karyotype. (Scale bars=10 µm).



Figures 5, 6 - *I. pseudopumila* (Fig. 5), *I. illyrica* (Fig. 6) karyotypes (Scale bar=5.8 µm).
Figures 7, 8 - *I. cengialti* (Fig. 7), *I. revoluta* (Fig. 8) karyotypes (Scale bar=6.2 µm).



Figures 9, 10 - Karyograms of: *I. marsica*, *I. setina*, *I. bicapitata*, *I. sabina*, *I. relicta*, *I. revoluta*, *I. lutescens* (Fig. 9); *I. pseudopumila* (from Puglia and Sicily), *I. pallida*, *I. illyrica*, *I. cengialti* (Fig. 10). (Scale bar=10 µm).



Figures 11, 12, 13 - *I. pseudopumila* (Fig. 11), *I.lutescens* (Fig. 12), *I. pallida* (Fig. 13) karyotypes with AC-segments. Figure 14 - Comparison of three karyograms with AC-segments: a) *I. pseudopumila*, b) *I. lutescens*, c) *I. pallida.* Scale bars=5.5 µm.

3. *I. pseudopumila* shows intraspecific variability.

4. *I. pallida*, *I. cengialti* and *I. illyrica*, considered closely related but often given different taxonomic rank (Kerner, 1871; Ambrosi, 1854; Foster, 1886; Tommasini, 1875; Pampanini, 1909; Lausi, 1964; Mathew, 1981; Colasante, 1995: Terpin *et al.*, 1996), present significant differences in the distribution and in the size of the AC-segments. More details are required because this may have taxonomic and nomenclatural consequences.

5. The hypothesis that *I. lutescens* may be derived from an amphidiploid hybrid between *I. pseudopumila* and *I. pallida* seems to be amply confirmed by the analysis of the AC-segments present in the chromosomes of *I. pseudopumila*, but not in the chromosomes belonging to *I. pallida*.

6. An extended analysis of the supposedly natural hybrid populations of *Iris* could contribute to our knowledge of the phylogeny and evolution of the species of subgenus *Iris*, section *Iris*, with consequent changes in the systematics of some *Iris* species.

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