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Biosystematic studies on the Vicia villosa complex in Europe

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RÉSUMÉ

ROTI-MICHELOZZI, G. (1986). Etudes biosystématiques sur le complexe Vicia villosa en Europe. *Candollea* 41: 399-411. En anglais, résumé français.

Une étude morphologique et caryologique des trois entités *Vicia villosa* subsp. *villosa, Vicia villosa* subsp. *varia* et *Vicia villosa* subsp. *pseudocracca,* appartenant au groupement de *Vicia villosa,* en Europe, est présenté. On a étudié neuf populations sauvages et deux cultivées. Une plus grande variabilité de la morphologie des chromosomes et aussi de la morphologie extérieure a été remarquée dans les populations italiennes, dont on a trouvé, pour la première fois, parmi deux d'entre-elles, le nombre chromosomique $4n = 28$. Le polymorphisme morphologique et caryologique relevé confirme le traitement de toutes les entités étudiées au niveau infraspécifique.

ABSTRACT

ROTI-MICHELOZZI, G. (1986). Biosystematic studies on the Vicia villosa complex in Europe. *Candollea* 41: 399-411. In English, French abstract.

A biosystematic study on the three entities of the *Vicia villosa* complex: *Vicia villosa* subsp. *villosa, Vicia villosa* subsp. *varia* and *Vicia villosa* subsp. *pseudocracca* from Italian and other European localities has been carried out. Both morphology and karyology of nine natural and two agricultural populations have been investigated. Greater karyological and morphological polymorphism was noticed in the Italian populations, in two of which, for the first time, tetraploidy was also noticed. The morphological and karyological polymorphism found supports a treatment at infraspecific level of all the investigated entities.

Vicia villosa, first described by ROTH (1793) from Vegesack (Germany), is common in West, Central and Southern Europe, where it occurs in natural populations, as well as a weed of cereal crops, and is also cultivated for fodder or soil improvement via Nitrogen fixation through radical symbiosis with bacteria. Recently it has been introduced in America, where it is similarly cultivated and often naturalized in U.S.A., Canada (GUNN, 1971) and in Central America (GUNN, 1979).

Like in many variable cultivated plants, the taxonomy and nomenclature of *Vicia villosa* sensu lato are very confused. This complex comprises strains which belong to two-five annual-biennial entities, variously treated at specific or infraspecific level during the last centuries. Some authors have considered them separate species and have described them with several names (there are more than 20 synonyms), of which the more frequently used are: *V. villosa* Roth, *V. varia* Host, *V. dasycarpa* Ten., *V. eriocarpa* (Hausskn.) Halàcsy, *V. microphylla* D'Urv. (REICHENBACH & BECK, 1903; ASCHERSON & GRAEBNER, 1910; HESS & al., 1970; CHOOI, 1971; GUNN, 1971; YAMAMOTO, 1973; KUTA, 1980). Ohters (GAMS, 1924; BALL, 1968; PLITMANN, 1970; ZANGHERI, 1976) treat these entities at infraspecific level. PIGNATTI (1982) considers two entities as subspecies (*V. villosa* Roth subsp. *villosa* and *V. villosa* Roth subsp. *varia* (Host) Corb.) and one (*V. pseudocracca* Bert.) as a separate species. Here Ball's taxonomic treatment and nomenclature are accepted, and the following entities have been considered: *V. villosa* Roth subsp. *villosa, V. villosa* Roth subsp. *varia* (Host) Corb., *V. villosa* Roth subsp. *eriocarpa* (Haussk.) P. W. Ball and *V. villosa* Roth subsp. *pseudocracca* (Bert.) P. W. Ball.

This account took its roots from the occasional finding, in Toscana (Central Italy) of a natural tetraploid specimen of *V. villosa* subsp. *varia.* Tetraploids are rare in the whole tribe *Vicieae* (GOLDBLATT, 1981) and particularly in the genus *Vicia,* in which they are recorded in perennials

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(ROUSI, 1961; METTINX & HANELT, 1968; GADELLA & KLIPHUIS, 1970; CHOOI, 1971; CHRTKOVA-ZERTOVA, 1973; HOLLINGS & STACE, 1974; KUTA, 1980; CINCURA, 1981; ROTI-MICHELOZZI, 1984). Chromosome counts of *V. villosa* sensu lato, revealing always $2n = 14$, have often been reported (PODLECH & DIETERLE, 1969; STRID, 1971; KOZUHAROV & al., 1972; FERNANDES & QUEIROS, 1978; NATARAJAN, 1978; VAN LOON & KIEFT, 1980). Study of karyotype morphology and drawing of idiograms, in this complex, has been performed by SRIVASTAVA (1963), CHOOI (1971), YAMAMOTO (1973), D'AMATO & al. (1978) on cultivated plants and by KUTA (1980) on wild plants from Poland. To the best of my knowledge, no karyological study has yet been carried out on wild Italian plants. Since wild specimens may be a source of genetic variation for improving cultivated plants, and karyological differences may be found in different geographic and environmental conditions, I thought it could be interesting to study both morphology and karyology of wild Italian strains of this aggregate, in comparison with several natural and agricultural ones throughout the range of its European distribution area, from Portugal to Crimea.

The main aim of this paper was to determine to what extent karyotype morphology is correlated with the other major taxonomic characteristics in this complex.

Material and methods

The material consisted of wild plants collected by the author, in Toscana and Sicilia (Italy), and of seeds, kindly obtained from Prof. Brilli-Cattarini and collected from the wild in Marche region (Italy), or from Italian and European Botanic Gardens. The seeds from the Italian Botanic Gardens and many of the non-Italian ones were also collected from natural populations. Some seeds of each population were sown in pots in order to obtain plants which could account for their correct taxonomic position and nomenclature. From these plants voucher specimens were made, which are deposited in the Genova herbarium (GE), together with the specimens collected by the author. The source localities, origin of the material studied, voucher specimens and ploïdy of each population are listed in Table 1.

Entity	*Source locality*	*Origin*	*Voucher specimen*	*Ploidy*
Vicia villosa subsp. *villosa*	Italy, Toscana, near Follonica	wild	Roti-Michelozzi 850527 (GE)	$4n = 28$
				$2n = 14$
	Germany, near Berlin	wild	Roti-Michelozzi 840607 (GE)	$2n = 14$
	Hungary, Vacratot area	wild	Roti-Michelozzi 840702 (GE)	$2n = 14$
	Roumenia, Iasi, Podii Iloasei	wild	Tirteo 830504 (GE)	$2n = 14$
Vicia villosa subsp. *varia*	Italy, Toscana, near Certaldo	wild	Roti-Michelozzi 800614 (GE)	$4n = 28$
		wild	Roti-Michelozzi 810814 (GE)	$2n = 14$
		wild	Roti-Michelozzi 830626 (GE)	$2n = 14$
	Italy, Marche, Montecalvo in Foglia	wild	Roti-Michelozzi 850531 (GE)	$2n = 14$
	Italy, Isle of Pantelleria	wild	Roti-Michelozzi 840416 (GE)	$2n = 14$
	Portugal, Oeiras Bot. Gard.	cultiv.	Roti-Michelozzi 850611 (GE)	$2n = 14$
	Great Britain, Southampton Bot. Gard.	cultiv.	Tirteo 830413 (GE)	$2n = 14$
	U.R.S.S., Crimea, near Jalta	wild	Tirteo 830505 (GE)	$2n = 14$
Vicia villosa subsp. *pseudocracca*	Italy, Sicilia, Piano Zucchi, Madonie	wild	Roti-Michelozzi 830608 (GE)	$2n = 14$

Table 1. — Source localities, origin, voucher specimens and ploidy of the studied populations.

For karyotype analysis, root tip meristems of seedlings were pretreated with a 0.2-0.4% aqueous solution of colchicine or a saturated aqueous solution of α-bromonaphtalene for 2-4 h., fixed in aceto-alcohol (1/3) for at least 30', hydrolised with HC1 1N at 60°C for 7-10', stained with 1-1.5% Gomori's hematoxylin (MELANDER & WINGSTRAND, 1953) and squashed on to permanent slides, vouchers of which are kept in the Genova Botanic Institute. About 120 metaphase plates were observed for each population, about 30 of these were suitable for chromosome counts, and about ten were photographed for chromosome study. This study was performed following a 6000 enlargement. Idiograms were drawn from the mean value of the chromosomes of five-seven plates with a similar degree of chromosome spiralization. The total, short and long arm lengths were determined for each chromosome, the length of satellites, when present, being included within the measurements. Since there was variability in presence, sometimes number of visible nucleolar chromosomes in each metaphase plate, these were reported in the idiograms only if they were present in the 60% of the plates studied. The karyotypic formula (according to LEVAN & al., 1964), whole complement length and total karyotype index of symmetry (according to LADIZINSKY, 1978) were provided for each population (Table 3). The best results were obtained during summer.

Results

Morphology of the plants

Vicia villosa sensu lato is described as a climbing annual-biennial with compound tendrilled leaves, each of which with 4-12 pairs of leaflets. It bears axillary racemes of 2-30 violet or blue flowers, sometimes with white wings. The main differences among the subspecies described by BALL (1968), in the regions studied, are pointed out in Figures 1, 2 and in Table 2.

Entity	*Stem and leaves*	*Flowers per raceme*	*Lower calyx teeth*	*Legume*
V. villosa subsp. *villosa*	villous	10-30	longer than tube	glabrous
V. villosa subsp. *varia*	glabrous-pubescent	10-30	shorter than tube	glabrous
V. villosa subsp. *eriocarpa*	glabrous-pubescent	5-20	shorter than tube	pubescent
V. villosa subsp. *pseudocracca* (= *V. pseudocracca* according to PIGNATTI, 1982)	glabrous-pubescent	2-10	shorter than tube (corolla longer and paler than above according to PIGNATTI, 1982)	glabrous

Table 2. — Main morphological differences among the entities of the *V. villosa* complex.

The morphology of this complex may be influenced by varying habitat conditions. Therefore specimens obtained by sowing seeds from different localities in pots, did not always show the characteristics by which the original plants were first determined. Moreover, often the nomenclature first adopted resulted as mistaken. Changes in taxonomic status or in nomenclature resulted as necessary in four populations:

Vicia villosa subsp. *villosa* (seeds from Oeiras bot. Gard., Portugal)	= *Vicia villosa* subsp. *varia* (the specimens obtained were completely glabrous)
Vicia dasycarpa var. *glabrescens* (seeds from Jalta Bot. Gard., U.R.S.S.)	= *Vicia villosa* subsp. *varia* (change in nomenclature only)
Vicia villosa subsp. *eriocarpa* (seeds from Southampton Bot. Gard., Great Britain)	= *Vicia villosa* subsp. *varia* (the specimens obtained had completely glabrous legumes)
Vicia pseudocracca (specimens from Madonie Mts., Sicilia, with 2-6 flowered racemes)	= *Vicia villosa* subsp. *pseudocracca* (the specimens obtained had up to 20-flowered racemes)

Therefore, while the material studied appeared to belong to four entities, only three will be dealt with here: *V. villosa* subsp. *villosa, V. villosa* subsp. *varia* and *V. villosa* subsp. *pseudocracca.*

The karyotypes

Vicia villosa Roth subsp. **villosa**

In the population from Italy (Toscana, near Follonica), two chromosome numbers, $2n = 14$ and $4n = 28$ were found together, in the same specimen (polysomaty) (Fig. 3A and B, 4A and B); the karyotypic formulae (LEVAN & al., 1964), the whole complement lengths and the total indexes of symmetry (LADIZINSKY, 1978), are listed in Table 3.

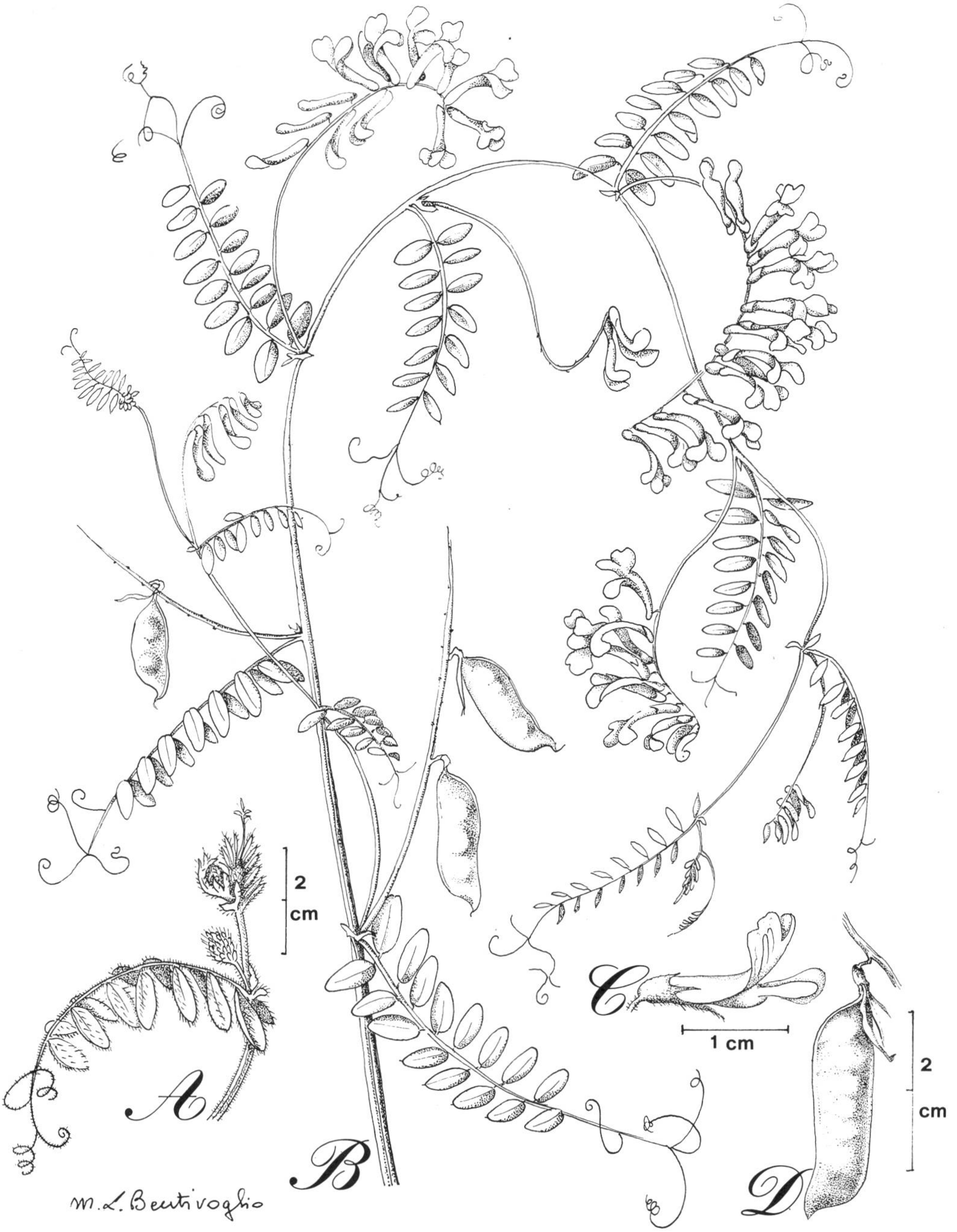

Fig. 1. — *Vicia villosa* subsp. *villosa:* **A,** branch; **C,** flower; **D,** legume. *Vicia villosa* subsp. *varia:* **B,** habit.

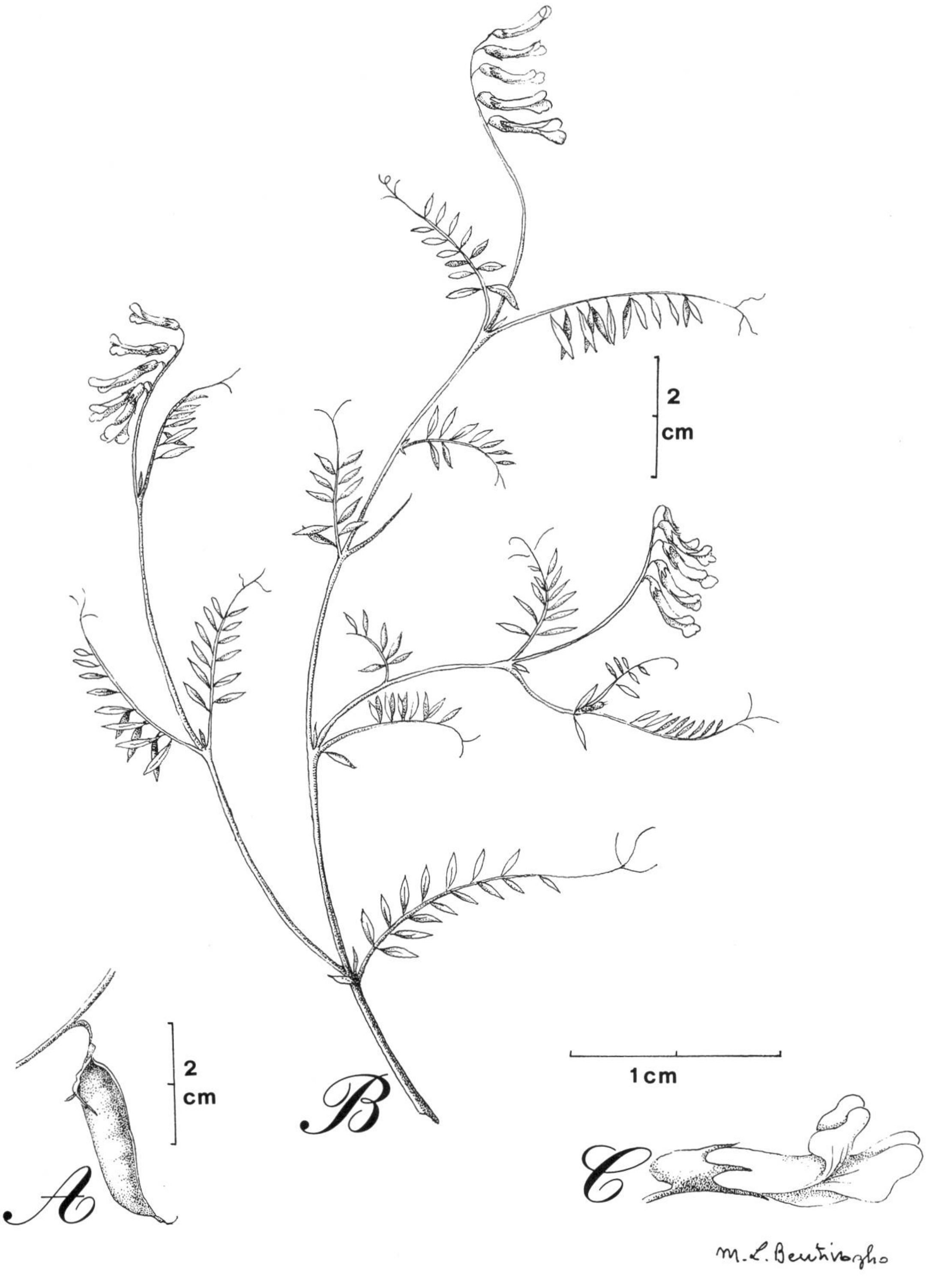

Fig. 2. — *Vicia villosa* subsp. *pseudocracca*.
A, legume; **B,** habit; **C,** flower.

The three non-Italian populations, all diploid and all natural, showed a slight variability in length and morphology of the chromosomes, therefore their karyotypic formulae were variable too (Table 3, Fig. 6A, B, C). Previous chromosome counts, in this entity (SRIVASTAVA, 1963; PODLECH & DIETERLE, 1969; CHOOI, 1971; KOZUHAROV & al., 1972; YAMAMOTO, 1973; KUTA, 1980) always recorded $2n = 14$.

From the morphological point of view, these differences could be noticed among the karyotypes of the Italian and the non-Italian populations: in these last ones satellited chromosomes could be seen very rarely, and therefore they have not been reported in the idiograms (Fig. 6A, B, C); in the Italian populations the number of the nucleolar chromosomes visible per metaphase plate varied, even within the individuals studied.

Vicia villosa Roth subsp. **varia** (Host) Corb.

The populations of this entity showed variable karyotypes, different karyotypic formulae, whole complement lengths and total indexes of symmetry (Table 3). In the natural population from central Italy (Toscana, near Certaldo), two different chromosome numbers ($4n = 28$ and $2n = 14$) were found, in specimens collected in subsequent years. Moreover the tetraploid cytotype had much longer chromosomes than the diploid one (Fig. 3C and D, 4C and D) and its karyotype was more asymmetric (Table 3). The other two Italian populations and all the non-Italian ones were diploid (Table 1). Previous counts made by STRID (1971), FERNANDES & QUEIROS (1978), NATARAJAN (1978), D'AMATO & al. (1978), VANLOON & KIEFT (1980), reported always $2n = 14$.

Also in this entity there was variability in presence-absence of nucleolar chromosomes; these were found only in the karyotypes of eastern or southern Italian populations (Table 3, Fig. 3E, 5A), and their position in the karyotypes was not always the same (fig. 4E and F).

The two agricultural populations from the Portuguese and British botanic Gardens had similar karyotype indexes of symmetry and the same number of submetacentric and subtelocentric chromosomes, though not in the same position (Table 3 and Fig. 6E, F). The karyotype of the natural population from Crimea (U.R.S.S.) was the more symmetric among all those studied in this entity (Table 3 and Fig. 6D).

Vicia villosa Roth subsp. **pseudrocracca** (Bert.) P. W. Ball

The karyotype of the natural diploid population ($2n = 14$) from Piano Zucchi (Madonie Mts., Sicilia, Italy) differed from the complements of the above mentioned entities, mostly for the morphology of the nucleolar chromosomes. These last ones were found in third position instead of sixth or seventh position, and the satellite was attached to the long arm. The chromosome lengths were, on the contrary, about the same as those previously noticed in the diploid populations both of *V. villosa* subsp. *villosa* and *V. villosa* subsp. *varia* (Table 3, Fig. 4G and 5B). To the best of my knowledge, chromosome counts have been made in this entity only by BALL (1968).

Entity	*Populations*	*Karyotypic formula (LEVAN & al., 1964)*	*Total complement length*	*IS*
Vicia villosa subsp. *villosa*	Follonica	20sm + 2m^s + 4m + 2sm	83.42 μm	0.46
		10sm + 2m^s + 2sm	41.92 μm	0.49
	Berlin	6sm + 2m + 2sm + 4m	32.76 μm	0.56
	Vacratot	2sm + 2st + 10sm	37.34 μm	0.45
	Iasi	14sm	42.56 μm	0.49
Vicia villosa subsp. *varia*	Certaldo	2st + 2sm + 2st + 22sm	104.60 μm	0.37
		12sm + 2m	38.10 μm	0.49
	Montecalvo	2st + 10sm + 2m^s	38.82 μm	0.46
	Pantelleria	12sm + 2sm^s	44.04 μm	0.48
	Oeiras	2st + 12sm	42.34 μm	0.43
	Southampton	2sm + 2st + 10sm	34.42 μm	0.44
	Jalta	6sm + 2m + 2sm + 4m	32.18 μm	0.55
Vicia villosa subsp. *pseudocracca*	Madonie Mts.	4sm + 2sm^s + 2m + 4sm + 2m	40.86 μm	0.50

Table 3. — Karyotypic formulae, whole complement lengths and total karyotype indexes of symmetry of the studied populations.

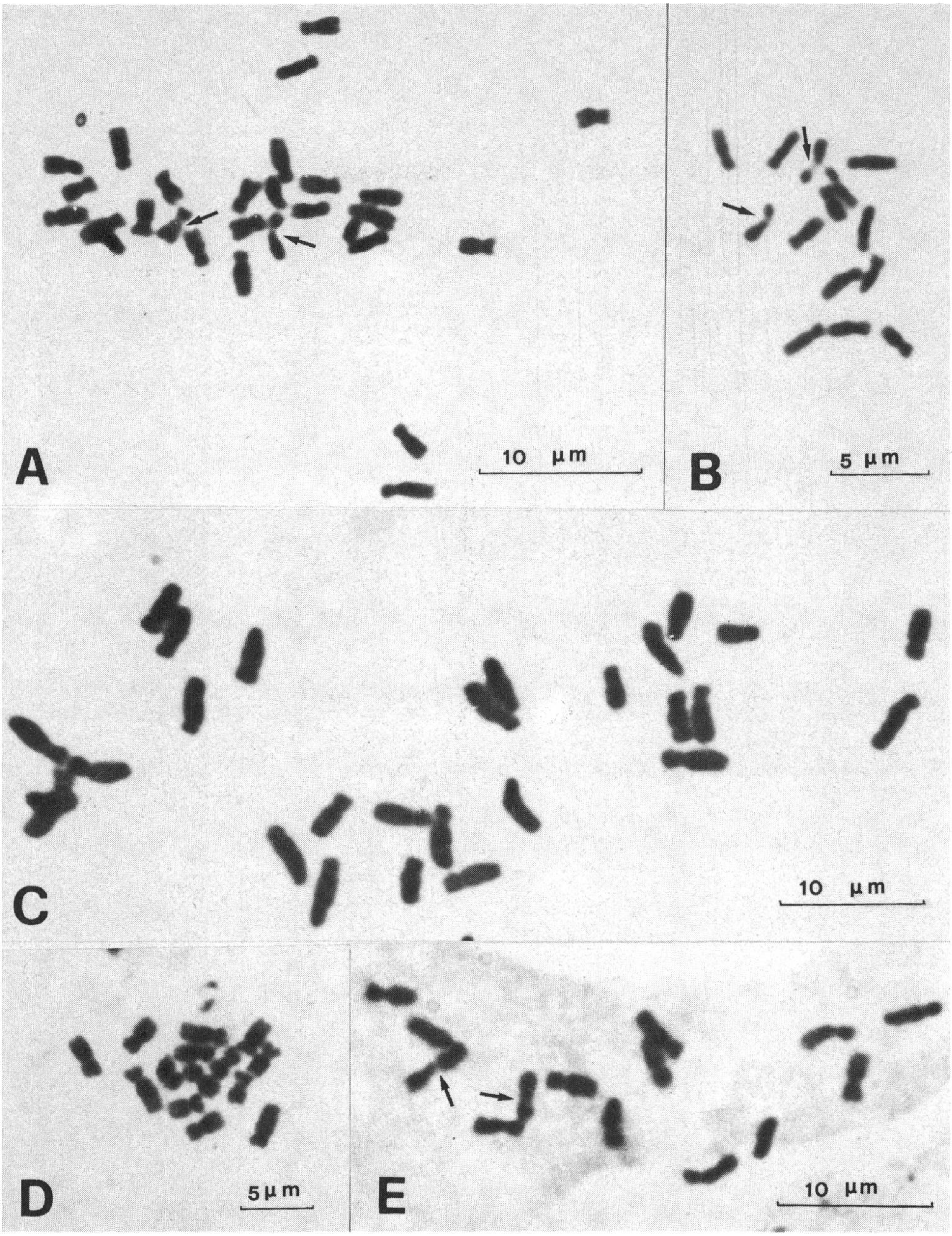

Fig. 3. — Metaphase plates.
A, B, *Vicia villosa* subsp. *villosa,* population from Follonica (Toscana); *Vicia villosa* subsp. *varia:* **C, D,** population from Certaldo (Toscana); **E,** population from Pantelleria Island (Sicilia). Arrows indicate nucleolar constrictions.

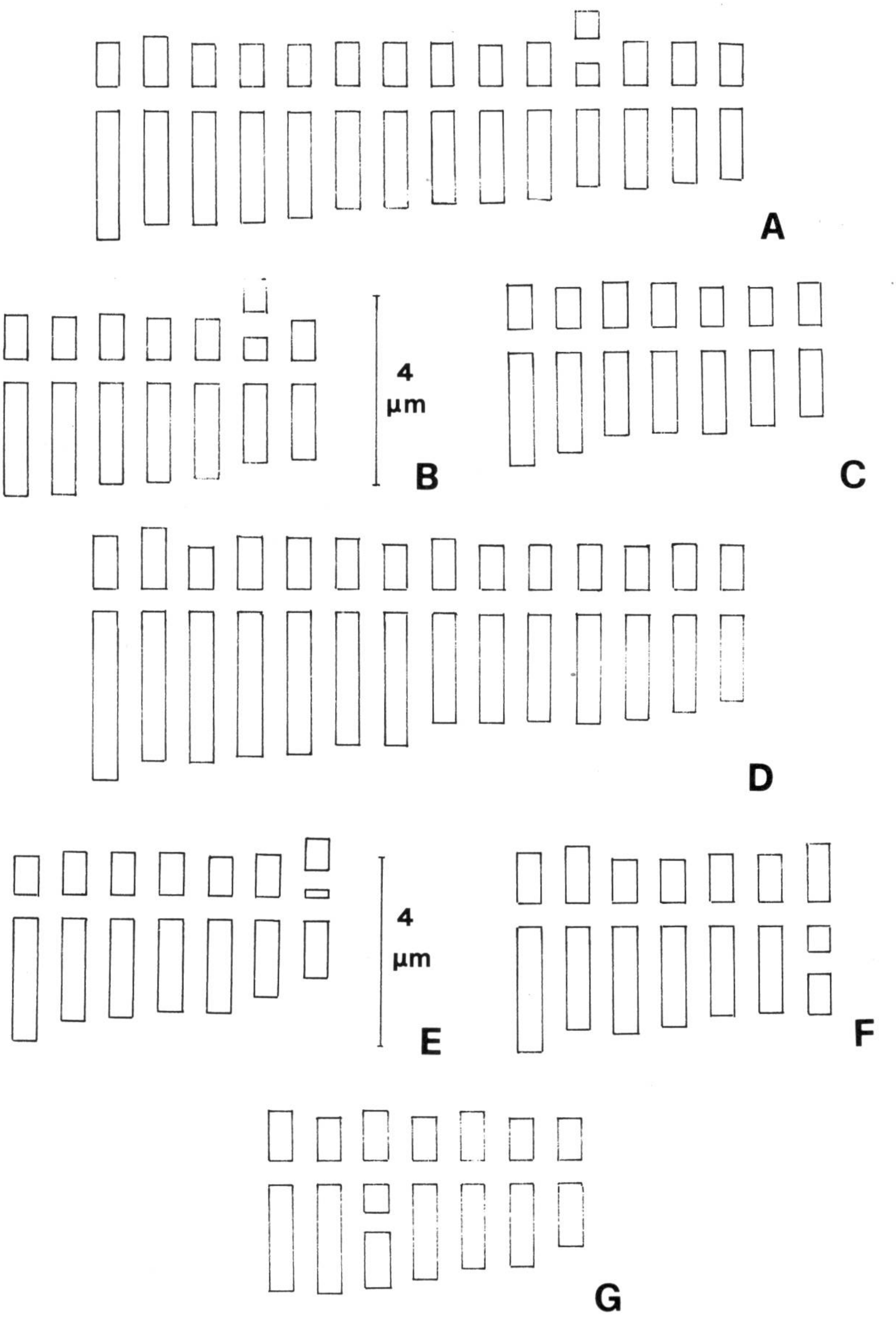

Fig. 4. — Idiograms.
A, B, *Vicia villosa* subsp. *villosa,* population from Follonica (Toscana); *Vicia villosa* subsp. *varia:* **C, D,** population from Certaldo (Toscana); **E,** population from Montecalvo (Marche); **F,** population from Pantelleria Island (Sicilia); **G,** *Vicia villosa* subsp. *pseudocracca,* population from Madonie Mts. (Sicilia).

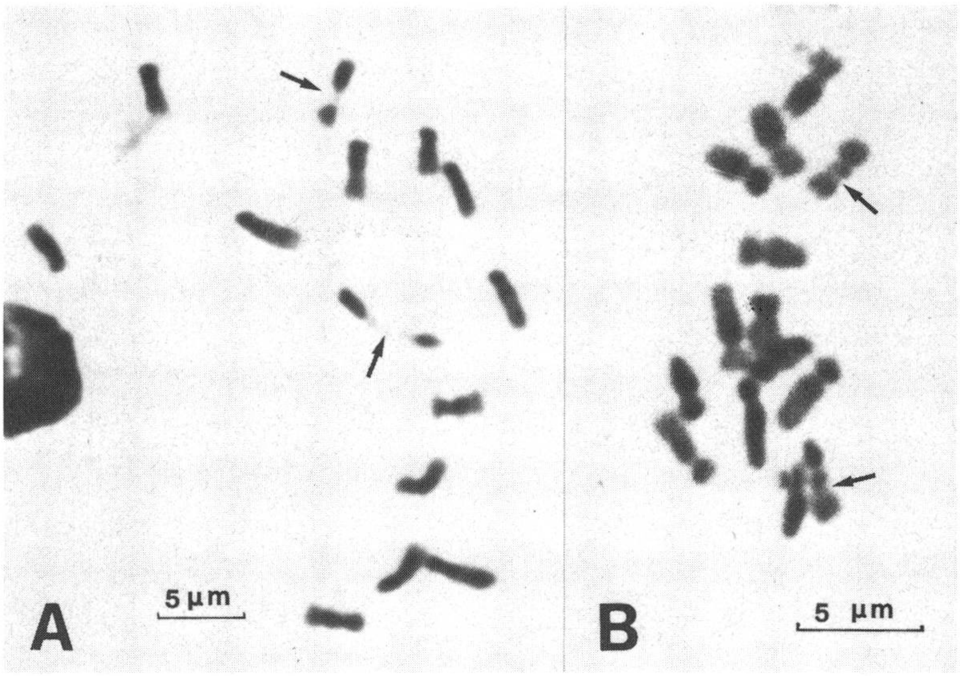

Fig. 5. — Metaphase plates.
A, *Vicia villosa* subsp. *varia* (Montecalvo population); **B,** *Vicia villosa* subsp. *pseudocracca* (Madonie Mts. population).
Arrows indicate nucleolar constrictions.

Discussion

Chromosome numbers

In genus *Vicia* the most common basic chromosome number is $x = 7$, though also other basic numbers have been reported: $x = 6$ (in the sections *Cracca, Vicia* and *Faba*), and $x = 5$ (in the Sections *Cracca* and *Vicia*); $x = 7$ is the case for the taxa of the *V. villosa* complex studied here. To the best of my knowledge, polyploidy, here noticed in two populations, has not yet been found in this aggregate. Contrary to what is generally stated, that cultivated material is more variable than wild material (JONES, 1955 in HOLLINGS & STACE, 1974), this variation was found in two natural populations of both subspecies *villosa* and *varia,* the specimens of which have been collected in Central Italy; in the first population mixoploidy was noticed and in the second specimens first collected resulted tetraploid, and those collected subsequently were diploid. Specimens coming from eastern and southern Italy or belonging to other natural or agricultural European material were diploids.

Chromosome size

The karyotypes of the *V. villosa* aggregate studied were rather uniform in length, there being little difference between the longest and shortest chromosome (average size difference between longest and shortest smaller than 1.7 µm). The mean chromosome length varied between 2-3.7 µm. In the tetraploid cytotypes of *V. villosa* subsp. *varia* from central Italy, contrary to what has been noticed by various authors, such as CHOOI (1971) for *V. cracca* and *V. tenuifolia,* that in tetraploids the chromosomes tend to be shorter than usual, the whole complement length was longer than the double of the longest diploid complement studied in the whole aggregate, and the mean length of the longest chromosomes was 4.65 µm.

Chromosome morphology

The data of the present investigation are more or less in agreement with earlier reports on the chromosome morphology of the taxa within this complex (SRIVASTAVA, 1963; CHOOI, 1971; YAMAMOTO, 1973; FERNANDES & QUEIROS, 1978; D'AMATO & al., 1978; KUTA, 1980). In the complements of the specimens studied here most of the chromosomes were submetacentric (sm), but metacentric (m) and, more rarely, subtelocentric (st) chromosomes also occurred. The marker chromosomes were therefore these last ones and, when present, the satellited

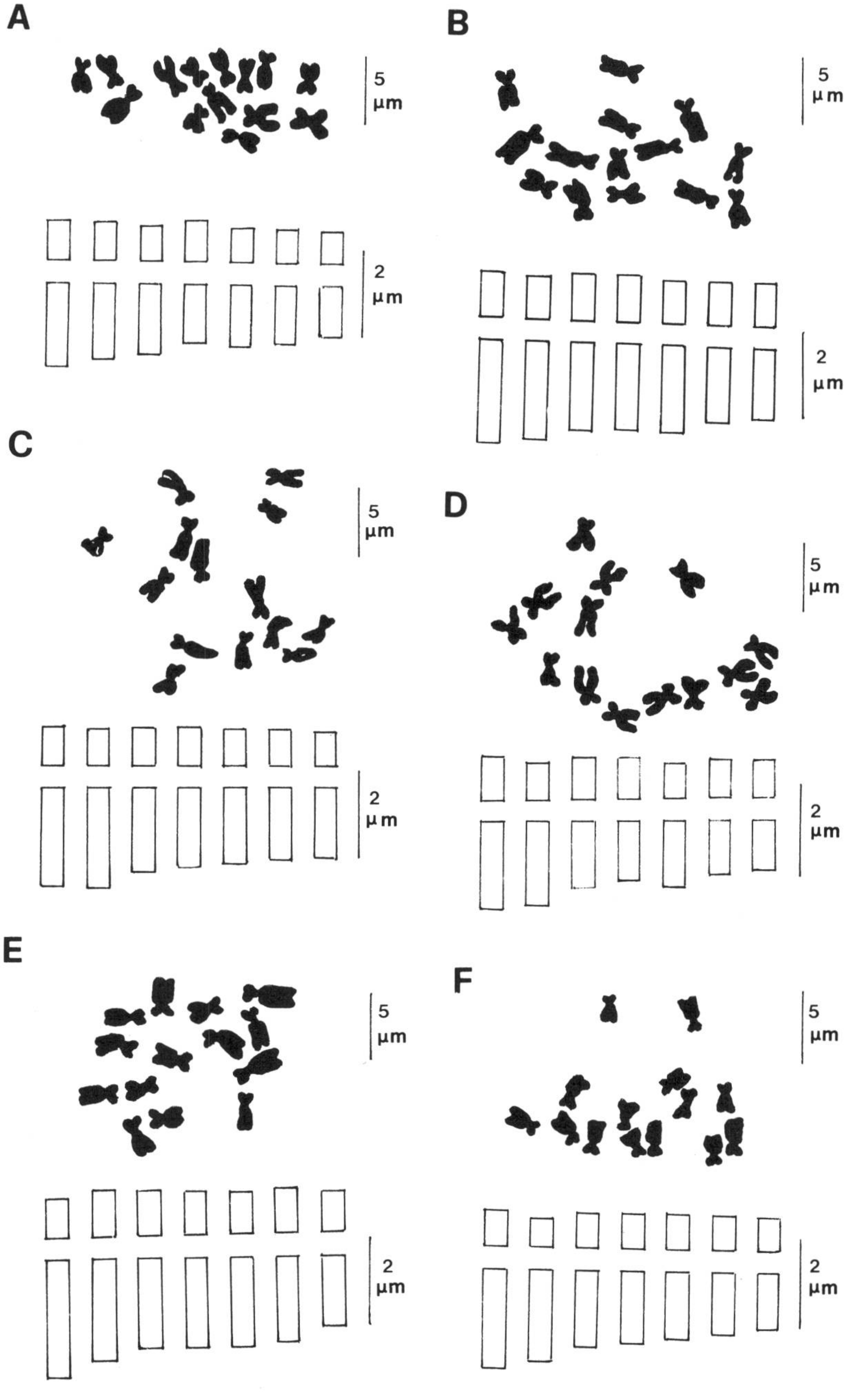

Fig. 6. — Metaphase plates and idiograms.
Vicia villosa subsp. *villosa* populations: **A,** near Berlin; **B,** near Iasi; **C,** Vacratot area. *Vicia villosa* subsp. *varia* populations: **D,** near Jalta; **E,** Oeiras Botanic Garden; **F,** Southampton Botanic Garden.

chromosomes. As was found in the *Vicia cracca* aggregate (ROTI-MICHELOZZI, 1984) not all the karyotypes studied showed satellited chromosomes; moreover sometimes they occurred only in some metaphase plates of the same population. A variability in presence-absence of nucleolar chromosomes has been previously reported, in other species, by various authors, such as FERNANDES & SANTOS (1971) in *Vicia benghalensis,* FERNANDES & QUEIROS (1971, 1978) in *Pulicaria odora, Anthemis mixta, Scorzonera humilis, Trifolium diffusum* etc.

Difference in number of satellited chromosomes has often been pointed out in *Allium* (SATO, 1981; TZANOUDAKIS, 1983, etc.).

A part of this variation probably could be due to technical reasons, i.e. a different degree of contraction in the corresponding couples of chromosomes in the metaphase plates observed (DYER, 1979; SATO & KAWAMURA, 1981) but may be that a natural variation due to biological reasons could exist. According to HADJIOLOV (1985) the site of the nucleolar organizer and the secondary constriction usually coincide, but the correlation is not absolute, therefore other chromosome regions could contain RNA genes. This fact could account for the absence of secondary constrictions in the chromosomes of some populations.

In the specimens of the *V. villosa* complex studied here the nucleolar chromosomes could vary from 0 to two in the diploid complements or from 0 to two, sometimes four in the tetraploid complements. Moreover, the satellites could be attached to the short arm of the sixth or the 11th pair of chromosomes (in the Italian mixoploid population of *V. villosa* subsp. *villosa*), or (in the Italian diploid populations of *V. villosa* subsp. *varia*) to the long or the short arm of the seventh pair of chromosomes (Fig. 3A, B and E, 4A, B, E and F, 5A). In the population of *V. villosa* subsp. *pseudocracca* these were on the long arm of the third pair of chromosomes (Fig. 4G and 5B).

A variability in morphology of the nucleolar chromosomes in this complex has been noticed before by YAMAMOTO (1973), who investigated specimens of four taxa, of which two, *V. varia,* and *V. dasycarpa,* are both synonyms of the same entity, *V. villosa* subsp. *varia.* The karyotypes drawn from these entities differed mostly in the position of the satellited chromosomes (in sixth and fourth position respectively). Moreover, karyotypes drawn from specimens of *V. villosa* subsp. *varia* by D'AMATO & al. (1978) and by KUTA (1980) differed mostly for the position of the satellited chromosomes.

Variability in morphology of satellited chromosomes has been, besides, found in other genera, particularly in the *Liliaceae.* TZANOUDAKIS (1983) noted great variability in these marker chromosomes in specimens of *Allium cupani* and *Allium hirtovaginatum* collected in different Greek localities, and KAMARI (1984) found variability in the satellited chromosomes of *Fritillaria obliqua.* The former author assumed that, in some cases, karyotype variation is correlated with a wide geographical distribution.

According to MATÉRN & SIMAK (1968) who studied the karyotype of *Larix,* the variation in position of the secondary constriction, in different metaphase plates of the same population, could be due to a "reversal of order" of chromosomes. In *Larix,* as in the *V. villosa* complex, there is little difference in length among chromosomes, and therefore, since the contraction of each of them in a plate may not always be wholly synchronized, nucleolar chromosomes may appear in different positions. Similarly the variation in location of NORs on the short or long arm of the same couple of chromosomes in different metaphase plates could be caused by a "reversal of arms" especially when (BENTZER & al., 1971), in a very contracted plate, the long arm tends to shorten more than the short one. On the other hand NICOLOFF & al. (1977) suggested that, by spontaneous chromosome structural changes, NORs may become translocated from their original sites, and this fact could account for the position of satellites both on different arms or different couples of chromosomes. According to these authors transposition of NORs from their standard sites to others, on the same or other chromosomes, by means of interchanges, is without influence on NOR activity.

It must be noticed, however, that greatest variability in the karyotypes of the *V. villosa* complex was found in natural populations from central and southern Italy. This result confirms the statement of HANELT & METTINX (1970), according to whom the Mediterranean Area is a diversification centre of genus *Vicia.*

There seems to be, anyway, on the whole, a close correlation between external polymorphism and karyological polymorphism in the studied specimens of this complex, and this is the reason for which BALL's taxonomic treatment, at infraspecific level, has been accepted in this account.

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