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Giemsa Banded Karyotypes, Systematics, and Evolution in *Anacyclus* (*Asteraceae-Anthemideae*)[1]

By

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Key Words: *Asteraceae* (*Compositae*), *Anthemideae*, *Anacyclus*.—Karyosystematics, cytotaxonomy, Giemsa C-banding, specific banding patterns, heterochromatin, karyotype evolution.

Abstract: Giemsa C-banding allows the differentiation of six, otherwise very similar karyotypes from the small genus *Anacyclus*. "Banding style"—with stable centromeric and nucleolar bands, and diverse specific banding patterns in distal chromosome segments—contributes significantly to generic demarcation and systematic grouping. The amount of banding corresponds to heterochromatic chromocentres and increases from perennials to annuals. Relationships with other nucleotype parameters and evolutionary mechanisms are discussed.

In recent years a number of staining techniques with fluorochromes or Giemsa became known which differentiate metaphase chromosomes longitudinally (see: HSU 1973, OCKEY & BATEMAN 1974). These "chromosome banding" techniques have brought to cytogenetics refinements which, particularly in mammalian systems, allow the recognition of individual chromosomes and the analysis of chromosomal polymorphism and mutations. They proved most valuable in studies of evolutionary relationships and, in addition, stimulated the analysis of the chemical organisation of the chromosome.

Among the abundant chromosome banding techniques now being routinely used in animal and human cytogenetics not all have proved feasible with plant material. So far mainly fluorochromy and Giemsa procedures for the preferential staining of constitutive heterochromatin ("C-banding") have been successfully applied to plant chromosomes.

[1] First contribution of a series on Giemsa Banded Karyotypes, Systematics, and Evolution in *Anthemideae* (*Asteraceae*).

Considerable differences in the arrangement and amount of Giemsa C-bands have been demonstrated in some plants of related groups (MARKS & SCHWEIZER 1974, GILL & KIMBER 1974, FILION 1974, SHCHAPOVA & BAUTINA 1974, HADLACZKY & BELEA 1975) but apart from this and a parallel study on *Scilla* (GREILHUBER & SPETA 1976) no broader systematic application has been attempted in the plant kingdom yet.

Within the framework of larger research programs on the systematics and evolution of the *Compositae* tribe *Anthemideae* a number of karyosystematic and cytogenetic studies have appeared during the last decades. They partly refer to individual groups (cf. *Achillea*: EHRENDORFER 1959; *Chrysanthemum* s.l.: TANAKA & SHIMOTOMAI 1968; *Leucanthemum*: VILLARD 1971, PAPEŠ 1975, PRZYWARA 1974; *Matricaria* etc.: MITSUOKA & EHRENDORFER 1972, *Argyranthemum*: HUMPHRIES 1975, 1976; and earlier references cited), partly to more broadly orientated aspects (cf. UITZ 1970, EHRENDORFER 1970) or stress nucleotype parameters and evolutionary strategies in related perennials and annuals (NAGL & EHRENDORFER 1974). With such a background a programme on Giemsa-C-banded karyotypes in *Anthemideae* appeared promising.

This first contribution concerns *Anacyclus*, a small and well demarcated genus of about 13 perennial and annual species, centered in the S. W. Mediterranean (HUMPHRIES, pers. comm.). We hope to contribute to the following questions:

1. Do the new banding methods improve the results of classical karyotype analyses in Angiosperms?

2. What correspondence is there between the grouping of taxa according to banded karyotypes and to external morphological characters?

3. How variable is banding and to what an extent does it correlate with other nuclear parameters and evolutionary mechanisms?

Materials and Methods

Provenances, cultivation, and comments on morphology and taxonomy. The *Anacyclus* samples studied are listed in Table 1. Voucher specimens are deposited at the herbarium of the Institute of Botany, University of Vienna (WU). Seedlings were grown in pots (usually more than one plant per pot) in the experimental garden of the Botanical Garden of the University of Vienna in spring and summer 1974 and 1975. In some instances plants were temporarily kept in a growth cabinet.

Because seeds received from botanical gardens are often wrongly determined, and because of the generally confused taxonomic state of *Anacyclus*, a careful systematic study of the plant material used for this karyological study was essential. Furthermore, Dr. C. J. HUMPHRIES has been kind enough as to check our vouchers. In order not to encroach upon

his *Anacyclus* monograph under preparation, we have used informal names between quotation-marks in two instances. The following passages contain some critical comments about our samples.

Anacyclus pyrethrum and *A. depressus*. The perennial members of *Anacyclus* are quite variable and have usually been lumped under *A. pyrethrum* (L.) CASS. Yet, the plants which we have received from various garden sources so obviously fall into two distinct types that we prefer to keep them specifically separate, at least for the moment: In *A. pyrethrum* (L.) CASS. s. str. the inner, but particularly the outer achenes are clearly

Table 1. List of *Anacyclus* species studied

Species	Abbr.	Life form	Provenance	HBV acc. no.
A. depressus BALL	dep	perennial	HB Würzburg GFR (g)	AC-136
			HB Lausanne CH (g)	AC-142
A. pyrethrum (L.) Cass.	pyr	perennial	HB Amsterdam N (g)	AC-138
A. radiatus LOIS.	rad	annual	HB Krefeld GFR (g)	AC-141
A. "purpurascens"	purp	annual	HB Tabor CS (g)	AC-129
A. valentinus L.	val	annual	HB Liège F (n)	AC-147
			Pyr.-Or., Rivesaltes	
A. clavatus (DESF.) PERS.	clav	annual	HB Oeiras P (n)	AC-148
A. "coronatus"	cor	annual	HB Reading GB (n): Morocco, Sous valley[1]	AC-150

HB = Botanical Garden; HBV = Botanical Garden Vienna; g = garden origin; n = natural habitat/wild origin.

[1] ESE of Ait Melloul, 12 km S of Ej-Jorf, on track to Biougra, 70 m; 30°20′ N, 8°25′ W; HUMPHRIES, JURY, MULLIN & RICHARDSON 274, 4/5/74.

winged, the wings consisting of several layers of short and mat cells; the fruit epidermis is whitish-grey without dark brown idioblasts; the receptacular scales are broadly obovate, blunt, and with a soft and ± torn hyaline margin. In contrast, *A. depressus* BALL has non-winged greyish-brown achenes, usually with numerous dark idioblasts in the epidermis; the receptacular scales are ± pointed, rather firm and entire. The former taxon is wide-spread in N.W. Africa and the S. Iberian Peninsula, while the latter appears to be limited to the higher Atlas mountains. Intermediate forms are reported. AC-131, named *A. depressus* by NAGL & EHRENDORFER (1974) belongs to *A. pyrethrum* s. str.

Anacyclus radiatus and *A. "purpurascens"*. Among the annual members of *Anacyclus* these two types are characterized by achenes with two short horn-like appendages which continue downwards into ± narrow wings with several layers of short and mat cells; the inner involucral bracts have distinct, round and fimbriate appendages. Whilst the W. Mediter-

ranean *A. radiatus* LOIS. is characterized by intensively yellow ray flowers and well developed fruit wings, material we have received from several sources (usually as *A. "officinarum"*) has white ray flowers in life (drying to pale yellow), with purple stripes on the lower side, outer achenes only very narrowly winged (ca. 0.2 mm), and inner non-winged. This type may correspond to *A. radiatus* LOIS. var. *purpurascens* (PERS.) DC. (1838) = *A. purpurascens* (PERS.) DC. (1815), but a garden hybrid origin with *A. radiatus* as one of the parents should also be considered. An informal name therefore appears appropriate for the moment.

Anacyclus valentinus, A. clavatus, and *A. "coronatus"*. These annual species have (outer) achenes with auriculate appendages which continue downwards into broader wings and inner involucral bracts without (or with less distinct) appendages. In the two former species fruit wings are thin, marginally 1-layered, and consist of long prosenchymatous and shining cells; there are no involucral appendages. While ligulate flowers lack in the W. Mediterranean *A. valentinus* L., the omni-Mediterranean *A. clavatus* (DESF.) PERS. has white ray flowers. Our sample AC-150 from Morocco deviates from *A. clavatus* particularly by large fruit wings with several layers of shorter and mat cells, by somewhat appendiculate inner involucral bracts, and longer white ray flowers. According to Dr. C. J. HUMPHRIES this corresponds well with the type material of *A. radiatus* var. *coronatus* MURBECK, Lund Univ. Årsskr. Avd. 2, **19** (1): 55 (1923) and may deserve specific status. It is here informally listed as *A. "coronatus"*.

Karyological procedure. As this paper is mainly concerned with interspecific differences several plants of a given sample were usually processed together. Root tips were pretreated with colchicine (0.05% aqueous solution) for 2–4½ hours at room temperature, fixed in 96% ethanol/glacial acetic acid (3 : 1) and stored in the refrigerator overnight. For Giemsa banding the improved procedure of MARKS (1975; and personal communication) was employed.

Only metaphases showing maximum banding response were selected for detailed analysis (usually 5–6 per species). The metaphases were photographed and enlarged 3,250 times. A transparent foil (as is used for overhead projection) was superimposed on the photograph and the chromosomes (outlines) and Giemsa bands were drawn with black ink and simultaneously reexamined in the microscope. Overlapping chromosomes were separately drawn by shifting the foil. Cut-out karyotypes were obtained from Xerox copies and all measurements were made by the aid of a toothed clock weel (BAUMBERGER 1970). An estimate of the amount of banding was obtained from the sum of banded chromosome length expressed as percentage of total chromosome length (MARKS & SCHWEIZER 1974). Karyotype parameters based on mitotic colchicine-metaphases were calculated according to SCHWEIZER (1973a).

Karyological terminology and definitions. In this report we use the term "C-banding" in a purely descriptive sense to design darkly staining chromosome segments obtained by a particular Giemsa method. It does not imply that these C-bands are all chemically identical nor does it mean that they are necessarily homologous to Giemsa bands obtained with similar techniques in other systems.—The ratio between chromosome arms (centromeric index) is calculated as: long arm/total length of

the chromosome (BAUMBERGER 1970). Chromosomes with an arm ratio falling into the range of 0.50–0.54 are referred to as "metacentric", whereas chromosomes with an arm ratio of 0.55–0.69 are referred to as "submetacentric". Non-satellite metacentric and submetacentric chromosomes (group A) for the sake of simplicity are subsumed under "metacentrics" in Figs. 11, 13, and Table 3. Satellite chromosomes (group B) are also called "nucleolar chromosomes".

Karyotypes and Specific Banding Patterns

The following detailed karyotype descriptions of all *Anacyclus* samples available show a somatic chromosome complement of 2 n = 18, thus verifying previous karyological studies (listed in the various Chromosome Indices). Two groups of chromosomes can be recognized in all *Anacyclus* species: a main set of 6–7 (sub)metacentric (A), and a group of 2–3 submetacentric (rarely metacentric) satellite chromosome pairs (B). In the perennials (*A. depressus*, *A. pyrethrum*) the chromosome groups (A) + (B) consist of 7 + 2, in the annuals (*A. radiatus*, *A. "purpurascens"*, *A. valentinus*, *A. clavatus*, *A. "coronatus"*) of 6 + 3 pairs. In some of the annuals further chromosomes of group (A) may be nucleolus organising, because it is difficult to decide whether some of the terminal bands are satellites or simply non-nucleolar telomeric bands. In *A. radiatus*, *A. "purpurascens"* and *A. "coronatus"* a terminally banded chromosome pair was discernable (no. 6; cf. Figs. 4, 5, 10) which is a likely candidate for a possible fourth pair of nucleolar chromosomes.

A comparison of our data with reports on *Anacyclus* karyotypes in the literature suffers from certain limitations. Different pretreatment and staining procedures have been used, and the plant material is often doubtful. Critical taxonomic determinations and vouchers appear to back the studies of RODRIGUES (1953), UITZ (1970), FERNANDES & QUEIRÓS (1971) and DELAY & PETIT (1971), but this does not apply to the plant material used by MARTÍNEZ VÁZQUEZ (1960, 1962) for her extensive karyotype studies on *Anacyclus*. All of her seeds were received from botanic gardens (where material offered as *Anacyclus* is usually incorrectly determined) and according to personal informations no taxonomic checking was done and no vouchers were preserved. From the carefully established karyotypes it is obvious that the ones referred to "*Anacyclus pyrethrum* DC.", "*A. officinarum* HAYN.", "*A. clavatus* PERS." and "*A. tomentosus* DC." have not even been made from *Anacyclus* but rather from *Anthemis* species. (*Anthemis altissima* L. is commonly offered as "*Anacyclus officinarum* HAYNE" which seems to have become extinct from cultivation.) The karyotype depicted as "*Anacyclus officinalis* L." may be from this genus, but a taxon of this name does not exist. Only the plants from which the karyotypes "*A. depressus* BALL", "*A. valentinus* L." and "*A. radiatus* LOIS." have been elaborated may have been correctly determined.

***Anacyclus depressus* and *A. pyrethrum*.** These perennials are represented by the karyotype of AC-136 (*A. depressus*) with an average length of 110 μm (Table 2); there are 7 pairs of metacentric chromosomes (the largest being about 1.5× longer than the smallest) and two pairs

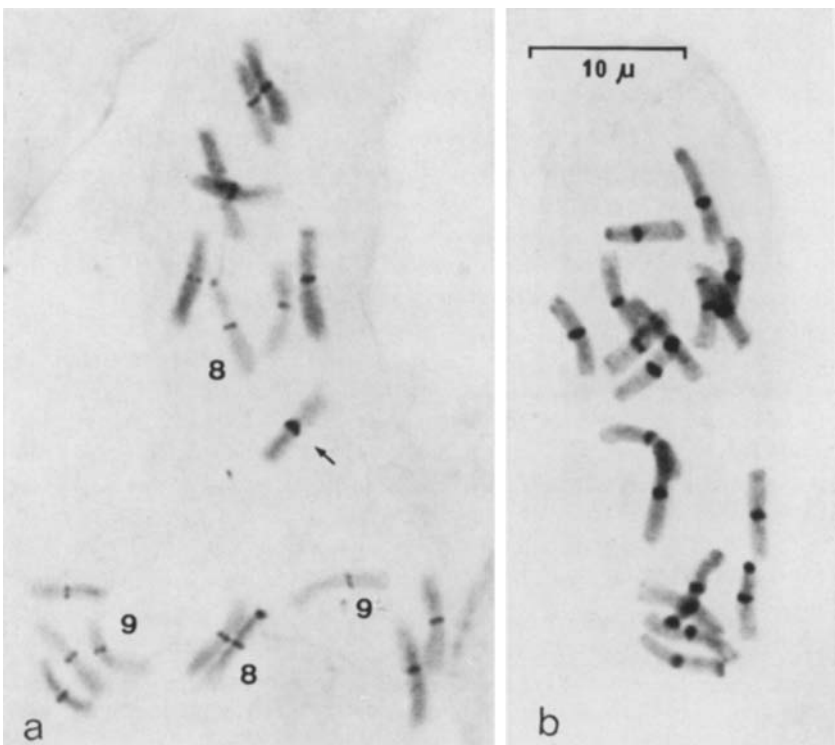

Fig. 1. *Anacyclus*, Giemsa C-banded somatic metaphases. *a* Complement of *A. depressus* showing one metacentric chromosome with a darkly staining band at the centromere (arrow); the centromeric region in remaining chromosomes appears as two darkly staining dots (cf. Fig. 2*c*). *b* Complement of *A. valentinus* showing distinct proximal and some terminal Giemsa bands (cf. Fig. 6*e*)

of submetacentric chromosomes with satellites (Fig. 1 *a*, Fig. 2). This corresponds well with the *A. depressus* karyotype obtained after oxyquinoline pretreatment and aceto-orcein staining by MARTÍNEZ VÁZQUEZ (1962) and the *A. pyrethrum* karyotype from colchicine and aceto-carmine preparation described by UITZ (1970). The 2 C nuclear DNA content has been found to be 12.42 g^{-12} in *A. pyrethrum* (AC-131) by NAGL & EHRENDORFER (1974).

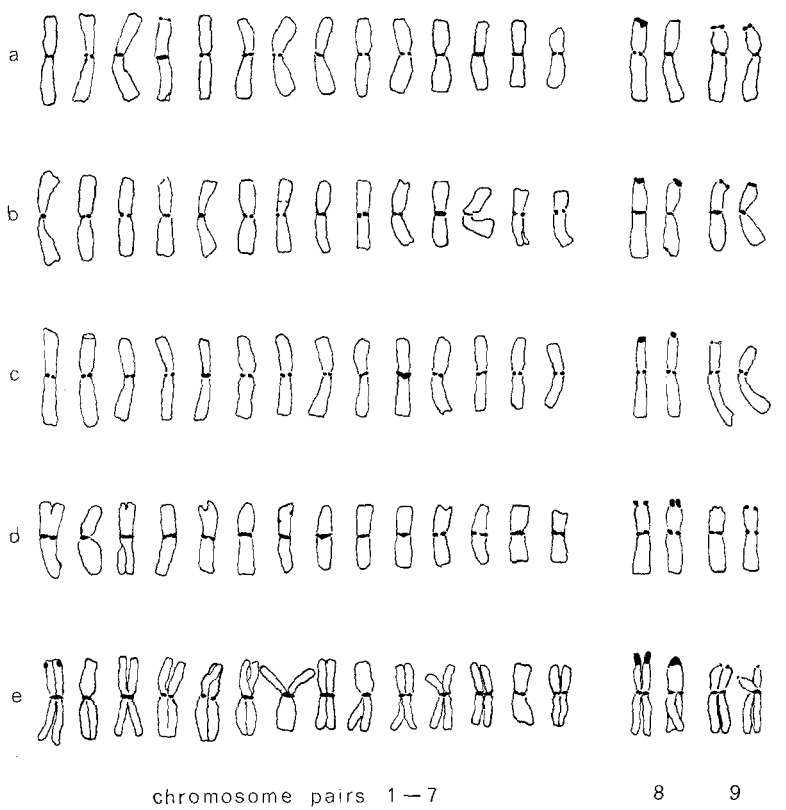

Fig. 2. Diploid karyotypes of *Anacyclus depressus* showing the distribution of Giemsa bands

All chromosomes of the *A. depressus* (AC-136) complement possess centromeric C-bands and these make up the major proportion (4.8%) of total heterochromatin (6.2%) of the genome (Table 2). The centromeric heterochromatin appears either as a single band or as two dots at the primary constriction. The width of the proximal band is about the same in most of the chromosomes, however, in a few cases, a significantly larger proximal band is present in a single chromosome suggesting heteromorphy for this region. The submetacentric chromosome pair (8, arm ratio 0.56) has a well defined terminal band which is present in both homologues in most metaphases (in 11 out of 12 chromosomes). The banded terminal segment obviously is a satellite and may be classified as NOR-associated heterochromatin because Giemsa stained nuclei show an association of 1–2 large chromocenters with nucleoli, in addition

to the well scattered or polarised small chromocentres resulting from the centromeric bands (Fig. 14 *a*). A second pair (9) of smaller submetacentric chromosomes (arm ratio 0.61) is evidently satellited, too. It exhibited in 8 of 12 cases a small terminal band or terminal dots. In a few instances a metacentric chromosome possessed an interstitial or subterminal band but this was not a constant feature.

In addition, the Giemsa banding responses of an other provenance of *A. depressus* (AC-142) and of the closely related *A. pyrethrum* (AC-138) were checked and banding patterns were essentially similar to AC-136.

A. radiatus. This species has an average karyotype length of 117 μm (Table 2). There are 6 pairs of metacentric or submetacentric chromosomes (A; 1–6) and three pairs of heterobrachial satellite chromosomes (B; 7–9) as in the other annual *Anacyclus* species. These findings verify the aceto-orcein resp. aceto-carmine karyotypes described for *A. radiatus* by MARTÍNEZ VÁZQUEZ (1960) and UITZ (1970); there is also general agreement with chromosomes depicted from natural populations of this species by RODRIGUES (1953), FERNANDES & QUEIRÓS (1971) and DELAY & PETIT (1971). 2 C nuclei of *A. radiatus* contain 16.92 g^{-12} DNA (NAGL & EHRENDORFER 1974).

Giemsa staining revealed a characteristic banding pattern which allowed the identification of at least 4 pairs out of the 18 chromosomes of the diploid *A. radiatus* complement (Figs. 3 *a*, 4). All chromosomes have a distinct centromeric band and some exhibit, in addition, subterminal or terminal banding. The three B-pairs of submetacentric chromosomes (7–9) are readily recognised by their knob-like terminal bands in the short arms. In each case this band evidently corresponds to a satellite or a part of it. Both homologues of the largest pair (7; arm ratio 0.58) of which the satellites are smallest occasionally have subterminal and irregularly expressed terminal bands in their long arms. The other two pairs (8, 9) are indistinguishable by length or arm ratio (both 0.61) and have no regularly occurring marker band in their long arm but one (8) has a larger terminal knob. In one metaphase this pair of nucleolar chromosomes (8) displayed an unusual patterning of faint bands in the long arm (Fig. 3 *a*, 4 *e*).

One slightly heterobrachial pair of group A (6; arm ratio 0.56) has a tiny terminal band and could possibly be nucleolus organizing. Of the remaining metacentrics (1–5) one pair (3) is distinguished by much banding: it usually exhibits a subterminal double band in one arm and a single band near the telomere of the other arm. The remaining four metacentric pairs (1, 2 and 4,5) all may possess subterminal or terminal minor bands but the patterning varied between homologues both within and between metaphases.

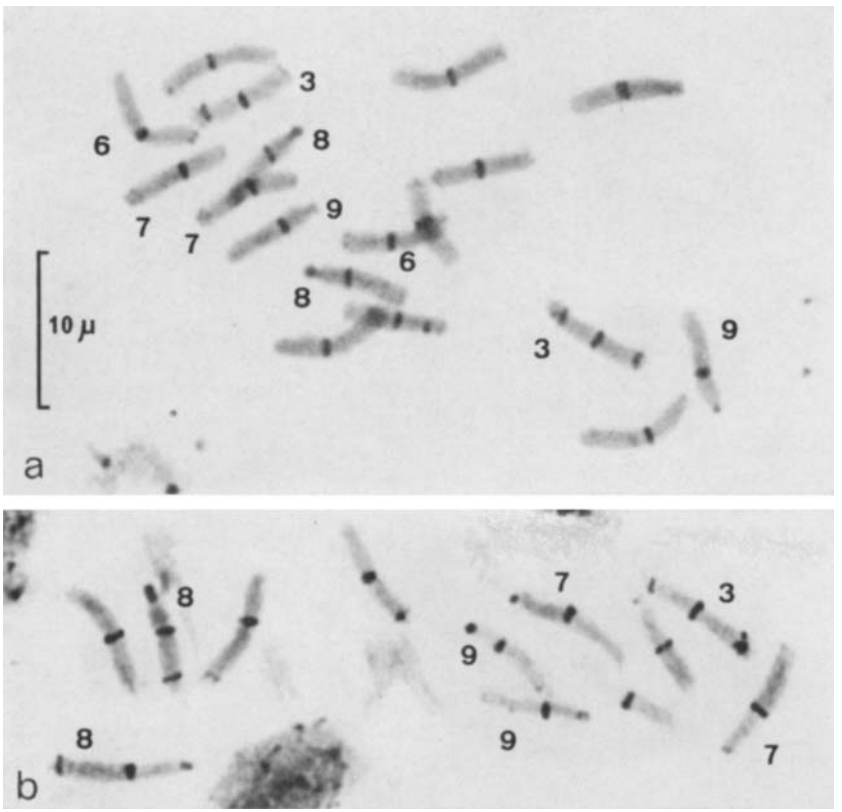

Fig. 3. *a* Metaphase showing full complement of *Anacyclus radiatus* (cf. Fig. 4). Five chromosome pairs are readily identified by their Giemsa banding patterns and morphology. *b* Part complement of *Anacyclus "purpurascens"* (cf. Fig. 5) showing similar patterning as in *a* but long arms of chromosome 8 are banded. Chromosome 8 is also heteromorphic for the darkly staining satellite in the short arm

A. "purpurascens". The diploid complement of this type is the longest among our *Anacyclus* samples: 134 µm (Table 2), and again consists of 6 pairs of metacentric or slightly submetacentric chromosomes (A; 1–6) and three pairs of heterobrachial chromosomes (B; 7–9). About 8.3% of the total chromosome length is banded (Table 2, Figs. 3 *b*, 5). All chromosomes possess distinct centromeric heterochromatin and in one metaphase two of the six heterobrachial chromosomes had proximal double bands (Fig. 5 *c*). All chromosomes of the group B (7–9) normally have knob-like terminal bands in their short arms which evidently are satellites and represent NOR heterochromatin. Within

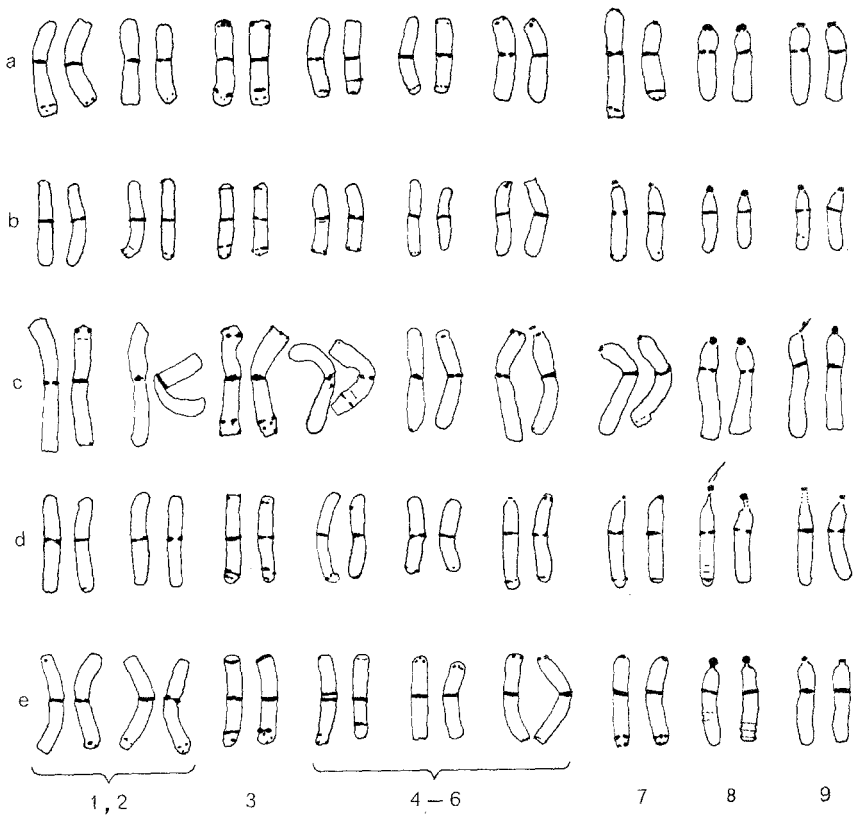

Fig. 4. Drawings of full complements of *Anacyclus radiatus* showing the range of banding observed

group B the homologues could be identified and paired on the basis of chromosome length, arm ratio, and size of the terminal bands. The longest pair (7; arm ratio 0.59) has a relatively small terminal segment. The intermediate pair (8; arm ratio 0.62) displays usually the largest terminal knob whereas the third pair (9) is distinguished by its arm ratio of 0.67. There occurs another chromosome pair belonging to the first group (A, no. 6) with an arm ratio of 0.55, which is possibly also satellited as it has a small terminal band or a tiny Giemsa positive terminal knob at the shorter arm. It was only recognised in 3 of the 6 metaphases analysed in detail.

A characteristic feature of the C-banded karyotypes of *A.* "*purpurascens*" are the subterminal bands (rarely double bands) which irregularly occur in almost all chromosome types. Terminal bands,

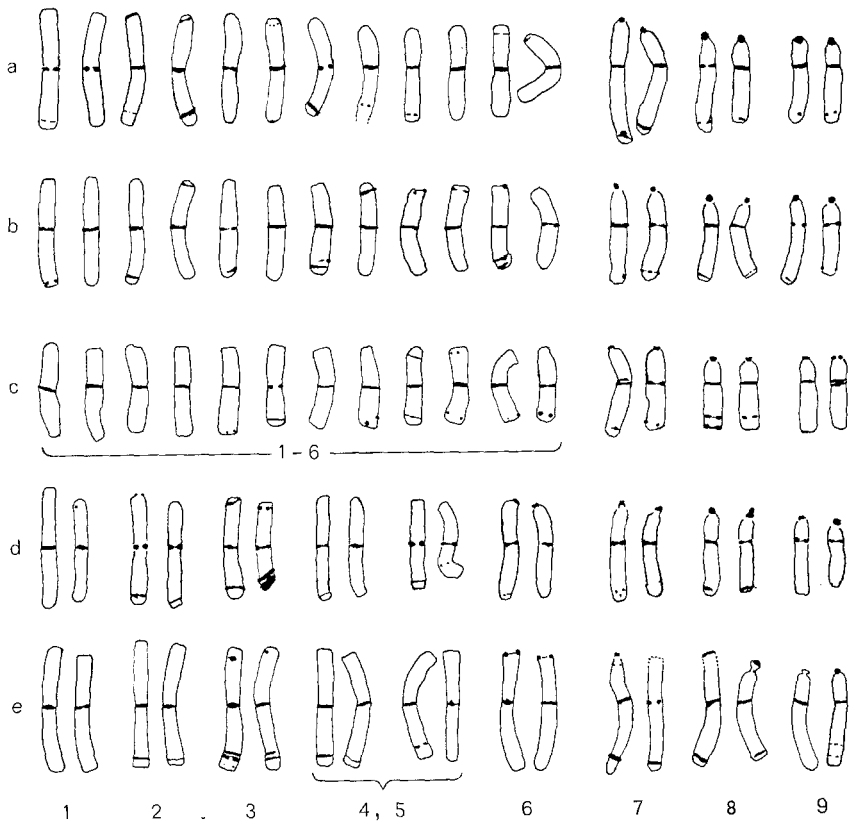

Fig. 5. Examples of the diploid karyotypes of *Anacyclus "purpurascens"* showing the distribution of Giemsa bands. *a–c* Metacentric and submetacentric chromosomes 1–6 are arranged after decreasing length. In *d* and *e* chromosomes 1–6 were paired so that banding heteromorphism was minimal. Note similarity of C-banded karyotypes *d* and *e* with banded karyotypes of *A. radiatus* (Fig. 4)

except banded satellites, are rare; e.g. chromosomes 1–6 had the lowest proportion of terminal banding of all annuals tested (0.6%, Table 3). There was some tendency of banding heteromorphism in the plants investigated, and among the chromosomes 1–6, except in two cases (Fig. 5 *d, e*), it was not possible to base the recognition of the homologues on their banding patterns. The patterning of chromosome 3 in Figs. 5 *d* and 5*e* is similar to that of chromosome 3 in *A. radiatus* (Figs. 3, 4) and if both Giemsa banded karyotypes are compared some similarity is apparent.

A. valentinus. The karyotype of this species has an average length of 98 μm (Table 2) and consists of a group of 6 pairs of metacentric chromosomes (A; 1–6); in group B there are one pair of satellited metacentric chromosomes (number 7) and two similar pairs of heterobrachial chromosomes (8, 9) which also have satellites on their short arms (Figs. 1 *b*, 6). An aceto-orcein karyotype shown by MARTÍNEZ VÁZQUEZ (1962) seems to correspond with this, but only two satellite chromosome pairs are reported. The 2 C nuclear DNA content has been determined to be 11.40 g^{-12} for *A. valentinus* (NAGL & EHRENDORFER 1974).

Apart from the distinct Giemsa bands at the centromeres, some chromosomes of *A. valentinus* have additional small intercalary bands or terminally located banded segments. In the three B-pairs the satellites usually stain as dark terminal knobs or bands; their ratios are 0.52, 0.55, and 0.58, and the third pair (9) usually has the smallest satellites. All three types of nucleolar chromosomes show occasionally subterminal or terminal minor bands in their long arms.

Unlike the other annual *Anacyclus* species studied *A. valentinus* has only few intercalary and subterminal bands but there are more bands located very close to or at the telomeres (all classified as "terminal bands"). In 2 of the 5 metaphases analysed up to 4 pairs of metacentrics had small terminal bands. As in some other annual *Anacyclus* species some of the terminally banded metacentric chromosomes of group A (1–6) may be nucleolus organising.

A. clavatus. The diploid complement of this species on an average is 102 μm long (Table 2) and consists of 6 pairs of metacentric chromosomes (group A, 1–6), one pair of submetacentric satellited (nucleolar) chromosomes (7) and two shorter pairs of submetacentric nucleolar chromosomes (8, 9; Figs. 7, 8) (group B). In his otherwise corresponding karyotype of *A. clavatus* UITZ (1970) has depicted only one pair of satellite chromosomes. 2 n = 18 has been verified for a natural population of this species from S. France (LOON et al. 1971). 2 C nuclei contain 10.48 g^{-12} DNA in *A. clavatus* according to NAGL & EHRENDORFER (1974).

Each chromosome of *A. clavatus* has a distinct Giemsa C-band at the centromere. Among the 12 metacentrics rather few have additional C-bands in non-centromeric positions and it is, therefore, difficult to pair the homologues of this group. There were in the metaphases analysed only 1–4 of the 12 metacentrics with a subterminal C-band and only 2–4 chromosomes had a small terminal band. In three favourable cases it seemed possible to pair the metacentric chromosomes (Figs. 8 *a–c*). Usually 2 pairs are devoid of noncentromeric bands, 1–2 pairs have

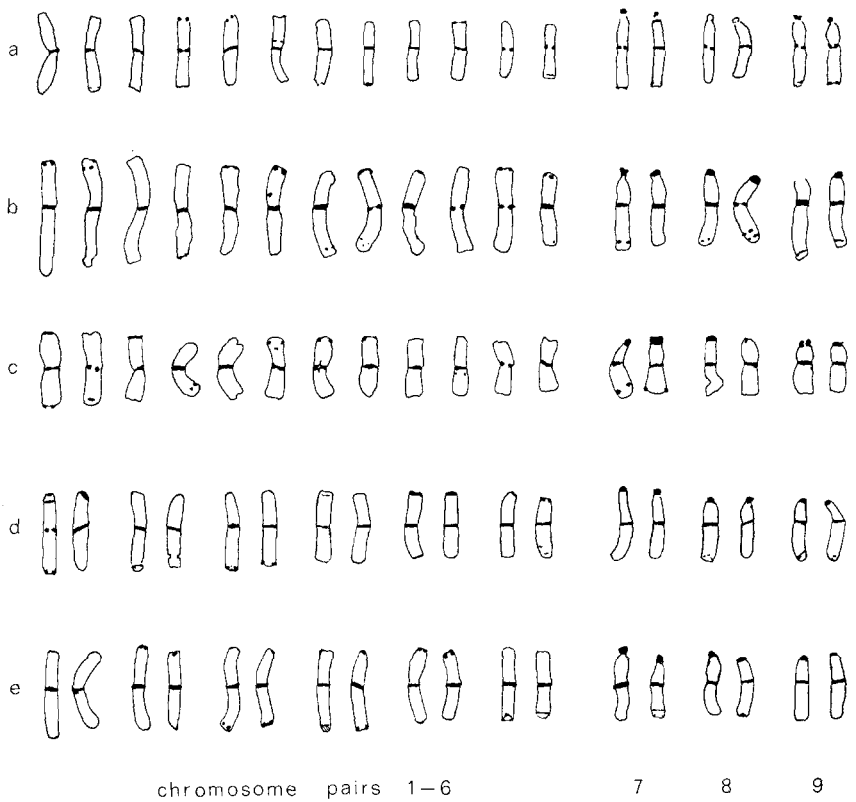

Fig. 6. Drawings of 5 diploid karyotypes of *Anacyclus valentinus* showing the range of Giemsa banding. In *d* and *e* an attempt was made to pair similar chromosomes, in *a–c* metacentrics have been arranged in order of decreasing length

intercalary bands but are frequently heteromorphic, and 2–3 pairs have terminal bands, also often in heteromorphic combinations.

The three pairs of nucleolar chromosomes all have apart from centromeric heterochromatin a very densely staining segment in a terminal or subterminal position of their short arm and frequently a small subterminal band in their long arm. In the first pair (no. 7, arm ratio 0.55) the terminal C-banded segment, which is interpreted as being a satellite, was heteromorphic in size in 6 of 8 metaphases observed. Of the two smaller pairs of nucleolar chromosomes (8, 9) one pair (8) with an arm ratio of 0.60 is distinguished by the large knob-like terminal band at the short arm. The C-banding pattern of the other pair (9) was quite unusual among the six *Anacyclus* species studied (Figs. 7, 8, 12).

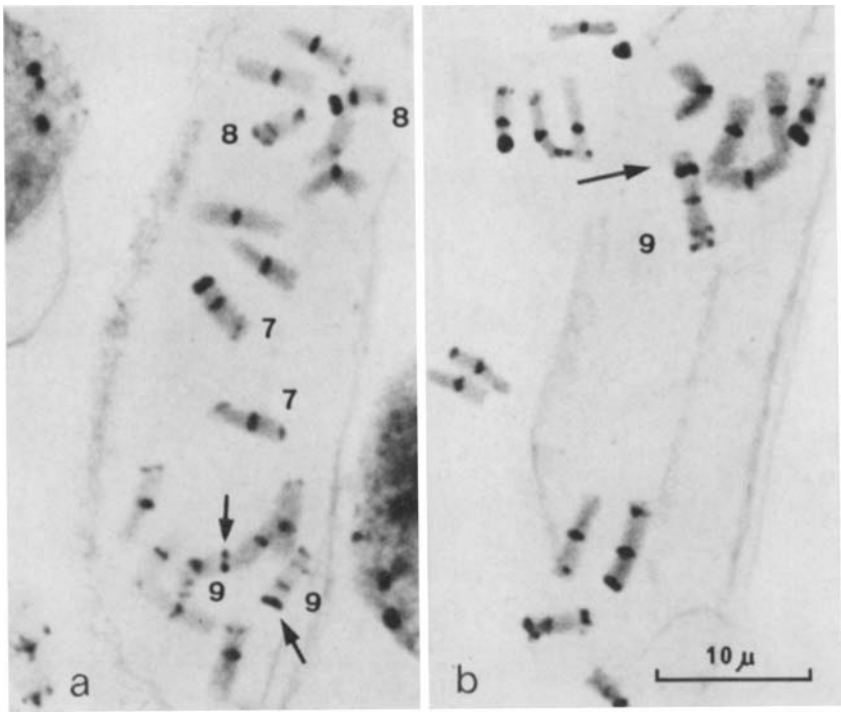

Fig. 7. Metaphase chromosomes of *Anacyclus clavatus*. *a* Complement with 18 chromosomes, also showing homomorphy for chromosome 9; arrows point to darkly staining terminal bands (satellites), compare with Fig. 8*a*. Note also heteromorphy for nucleolar chromosomes 7 and 8. *b* Part complements showing heteromorphic variant of chromosome 9: a pale (euchromatic) terminal segment appears adjacent to the large and heavily staining band (arrow; cf. Figs. 8*c* and *e*)

There is a relatively large and densely staining segment in the short arm which consists of at least two subunits. This band which most likely is NOR-associated heterochromatin as judged by its intense staining occupies a subterminal position. This is best seen in relatively long, undercontracted metaphases in which the short arm adjacent to the dark "NOR-band" has a euchromatic terminal segment (Fig. 7 *b*). The long arm has a terminal (often slightly subterminal) band, and, next to this, in a subterminal position another band which probably coincides with a constriction as the chromosome is frequently narrowed or even bent at this point. This pattern was found to occur in both homomorphic and heteromorphic combinations in different individuals (Figs. 8 and 12). In the latter one of the homologues had one or both bands of the long

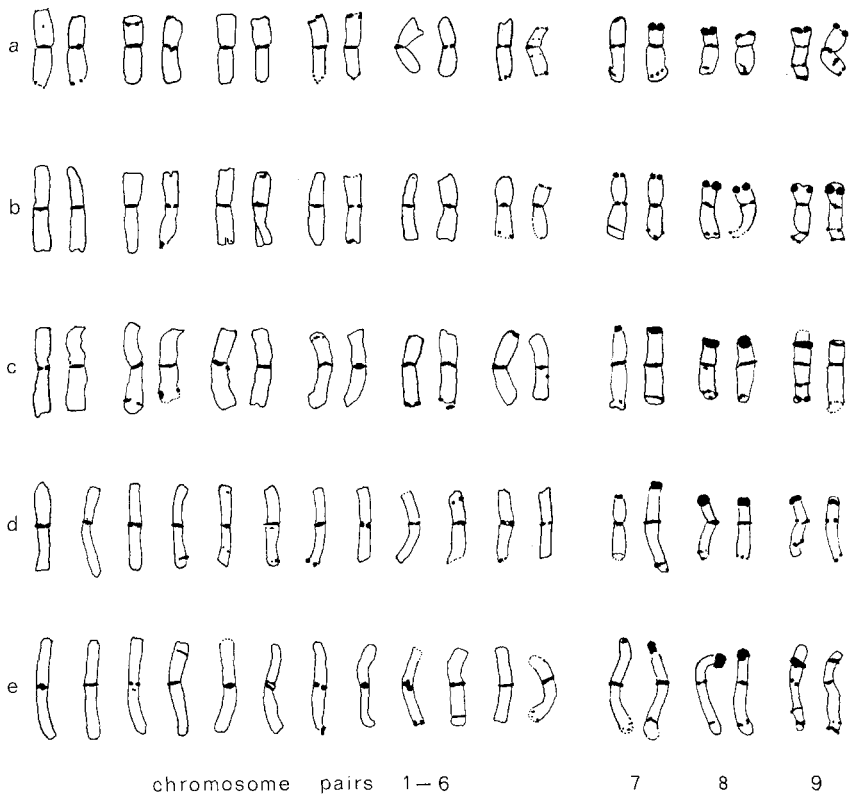

Fig. 8. Examples of diploid karyotypes of *Anacyclus clavatus* showing in some cases heteromorphy for chromosomes 7, 8, and 9, and also for some metacentrics. In *d* and *e* chromosomes 1–6 are arranged in order of decreasing length because pairing of homologues was uncertain

arm missing. In yet another individual both shorter pairs of nucleolar chromosomes were heteromorphic, both with respect to banding patterns and arm ratio.

A. "coronatus". The average karyotype length in this local Moroccan type is 108 μm (Table 2). As in all other annual *Anacyclus* species studied the complement can be subdivided into two main groups (Figs. 9 and 10): 6 pairs of metacentric or submetracentric chromosomes (A, 1–6), and three pairs of satellited submetacentric chromosomes (B, 7–9). Variation was found regarding the centromeric position among those chromosomes attributable to the group (A) for in one case a heterobrachial chromosome 6 was observed with an arm ratio of 0.65 and in

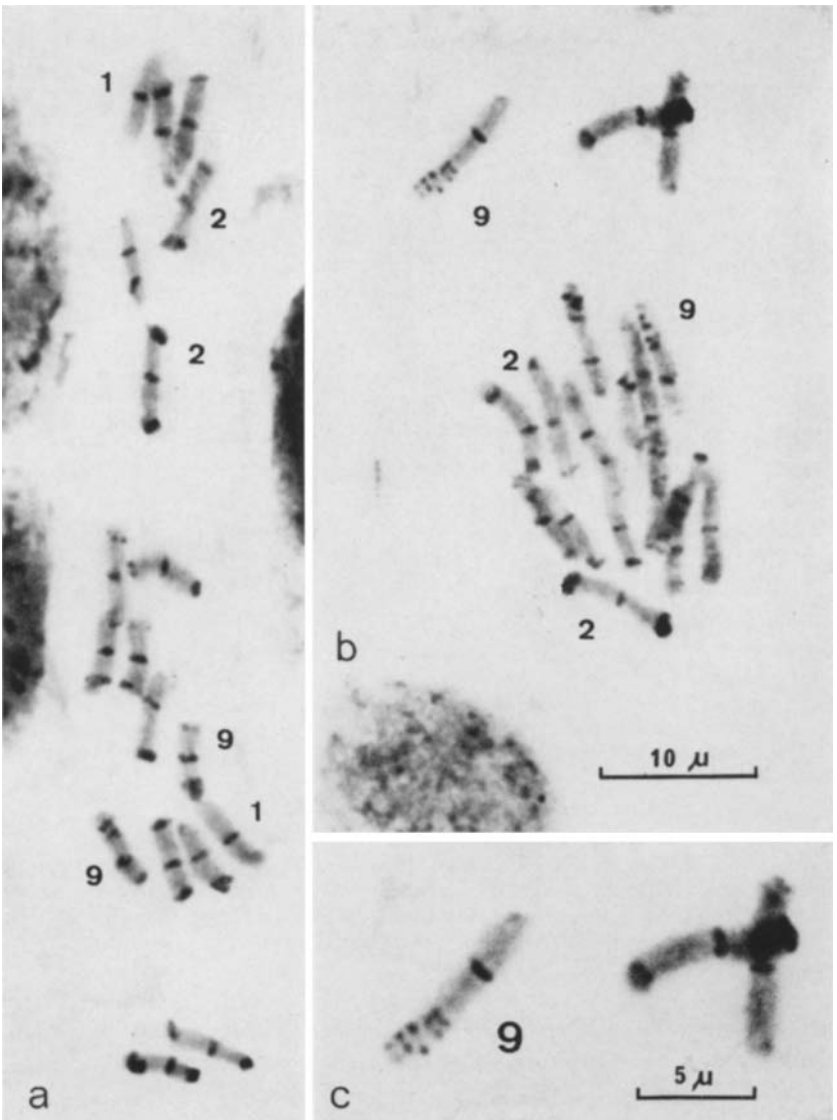

Fig. 9. Metaphase chromosomes of *Anacyclus "coronatus"*. *a* Complement of 18 chromosomes: all chromosomes have additional bands in their arms except chromosome 1. Note heteromorphy for chromosome 2 (cf. Fig. 10*c*). *b* Full complement of the same species showing again heteromorphy for chromosome 2 and multiple bands in distal segment of long arm of chromosome 9 (×2,000). *c* Part of Fig. 9*b* (×3,000) showing multiple bands in nucleolar chromosome 9. The 5 bands readily discernable in the long arm of chromosome 9 are partly fused to form larger bands or blocks in the homologous chromosomes in overcontracted metaphase *a*

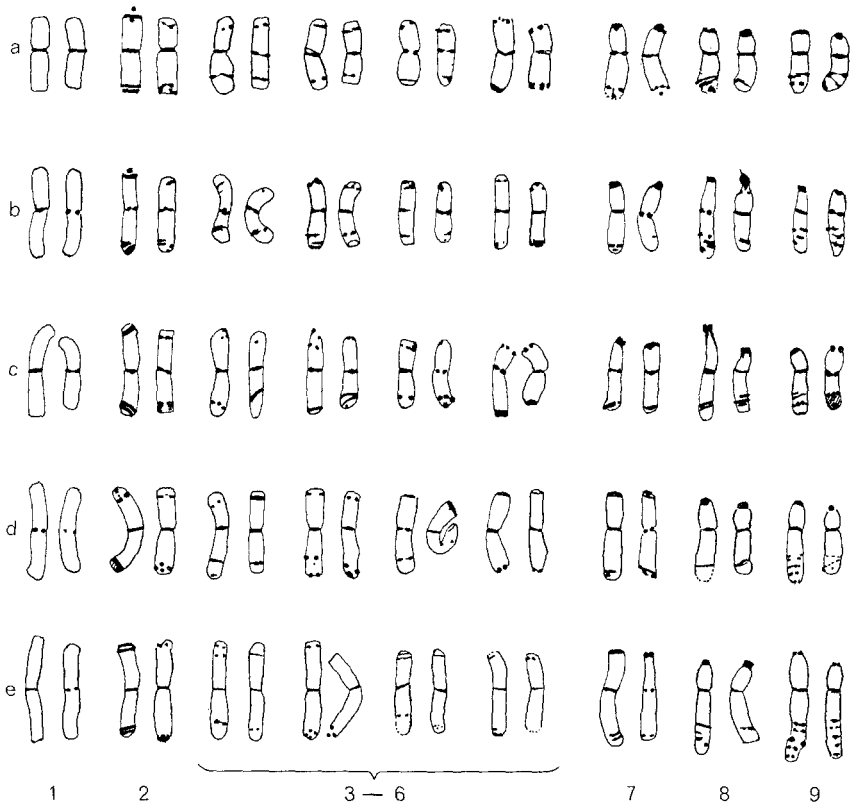

Fig. 10. Diploid karyotypes of *Anacyclus "coronatus"* showing the Giemsa C-banding pattern and demonstrating the range of variation. Only one chromosome pair (1) is devoid of intercalary or terminal bands

three other cases one or more chromosomes were definitely submetacentric.

The C-banding pattern in this species is particularly extensive, about 15% of total chromosome length being banded. In group (A) only one relatively large pair (1) is devoid of non-centromeric bands, all others have additional bands usually in both arms located mainly in subterminal or terminal segments. In most cases it was possible to pair the chromosomes so that banding heteromorphism was minimal. One large metacentric "marker chromosome" (type 2) is extensively but asymmetrically banded in both arms. It occurs only once per diploid complement and its likely homologue has fewer bands in one arm. Chromosome 3–6 are not readily distinguishable but for chromosomes 3 and 6 some characteristic features were apparent in most metaphases.

Table 2. Length of 2 n complement, proportion of Giemsa C-bands (percentage and number of C-chromocentres in annual and perennial

HBV acc. no.	species	life form	cells (N)	length of 2n complement: μm $\bar{x}$	range
AC-136	*A. depressus*	p	6	110	88–163
AC-141	*A. radiatus*	a	6	117	99–149
AC-129	*A. "purpurascens"*	a	6	134	110–157
AC-147	*A. valentinus*	a	5	98	84–119
AC-148	*A. clavatus*	a	6	102	83–122
AC-150	*A. "coronatus"*	a	5	108	96–127

Chromosome pair 3 was heteromorphic with respect to the position of intercalary bands. Chromosome 6 had the least amount of intercalary bands but possessed distinct terminal bands. In the second group (B) all chromosomes have a terminal band in their short arm which evidently is a satellite, and are also banded in a subterminal segment of the long arm, chromosome 8 showing heteromorphy for this region (Fig. 10). Chromosome 9 (arm ratio 0.65) with a relatively small satellite has multiple discrete bands in the distal segment of the long arm and in prometaphasic or stretched chromosomes up to 5 small bands could be discerned (Figs. 9 *b*, *c*).

General Karyological Observations

Giemsa C-banding. All species of *Anacyclus* have a distinct single C-band at the centromere (= proximal or "centromeric" heterochromatin) in every one of their chromosomes. In a few instances proximal double bands were observed in metacentrics as well as in nucleolar chromosomes, but this seems to be an exception (2 in 420 and 2 in 192 respectively).

The main nucleolar chromosomes usually have a very intensely staining terminal segment (satellite or part of it) on their short arms (Fig. 12). Only in one species (*A. clavatus*) such a segment was found to be subterminally inserted as a "NOR-band" (Fig. 7 *b*). Additional intercalary and terminal C-bands occur in the annual *Anacyclus* species studied. In metacentric or submetacentric chromosomes belonging to group (A) one or both arms may have additional bands whereas in nucleolar chromosomes (B) additional bands are only found in the longer, non-satellited arm. If a metacentric chromosome has a double band in one arm then the other arm usually is also banded. Intercalary bands, as a rule, occupy a subterminal region of the chromosome arm

of total chromosome length), number of C-bands per 2 n complement, species of *Anacyclus*. $\bar{x}$ = mean, s = standard deviation

% Giemsa bands								C-bands per 2 n complement		C-chromocentres in interphase nuclei	
proximal		intercalary		terminal		total					
$\bar{x}$	s	$\bar{x}$	s	$\bar{x}$	s	$\bar{x}$	s	$\bar{x}$	s	$\bar{x}$	s
4.8	0.7	0.1	—	1.3	0.5	6.2	1.3	21.8	1.0	20.0	1.5
4.6	0.6	1.9	0.6	3.3	0.5	9.8	1.2	41.5	3.1	27.5	4.5
3.9	0.3	2.2	0.2	2.2	0.5	8.3	0.8	40.3	0.8	28.5	3.0
5.0	0.5	1.1	0.6	4.3	1.0	10.4	1.3	36.6	3.7	27.0	5.5
4.8	0.4	2.0	0.4	3.5	1.0	10.3	1.4	36.3	1.7	26.0	3.0
4.5	0.8	5.7	1.1	4.8	1.2	15.0	2.7	61.4	3.0	45.5	5.5

and there was no case of distinct intercalary banding within the proximal half of the chromosome arm. Intercalary and terminal bands in chromosomes 1–6 (A) and in the long arm of nucleolar chromosomes 7–9 (B) were in most instances of about the same width as centromeric bands. In contrast, the terminal band at the short arm (satellite or part of it) of chromosomes 7–9 (B) is more variable in size.

In all species the proportion of banded chromosome length (including satellites) is higher in nucleolar chromosomes (7–9) than in metacentric chromosomes (Fig. 11; "asymmetrically banded karyotype").

Chromosome length and C-banding. Absolute length values (μm) for total karyotypes and banded zones from all metaphase plates analysed, and the means for all 6 samples studied, are shown in Fig. 16. Variation due to preparation imperfections, developmental heterogeneity and (a probably very limited amount) of genetic differentiation is clearly demonstrated (see also Table 2). Between total karyotype length and DNA content of 2 C nuclei there is good correlation (cf. NAGL & EHRENDORFER 1974). It is obvious that changes of total karyotype length, relative to *A. depressus*, are mainly due to band material in *A. valentinus*, *A. clavatus* and *A. "coronatus"*, while they are more closely related to non-banding material in *A. radiatus* and *A. "purpurascens"*.

The overall proportion of Giemsa C-banding per diploid complement does not correlate with total chromosome length in *Anacyclus* (Table 2). The possibility that the C-banding differences could be correlated with total chromosome length at least for a particular group of chromosomes was examined in annuals by calculating the values separately for chromosomes of group (A) and for nucleolar chromosomes (B) (Table 3). Although the proportions of C-banding are different in the

Table 3. Proportion of Giemsa C-bands (percentage of total chromosome in annual and perennial

HBV acc. no.	species	life form	% Giemsa bands in "metacentrics" (A) proximal $\bar{x}$	s	intercalary $\bar{x}$	s	terminal $\bar{x}$	s	total $\bar{x}$	s
AC-136	*A. depressus*	p	4.8	0.7	0.1	—	0.2	—	5.1	0.8
AC-141	*A. radiatus*	a	4.6	0.6	2.4	0.9	1.8	0.4	8.8	1.4
AC-129	*A. "purpurascens"*	a	3.8	0.2	2.0	0.4	0.6	0.3	6.4	0.6
AC-147	*A. valentinus*	a	5.0	0.5	1.0	0.5	2.5	0.8	8.5	1.0
AC-148	*A. clavatus*	a	4.7	0.4	0.9	0.4	1.0	0.7	6.6	1.0
AC-150	*A. "coronatus"*	a	4.5	0.8	5.0	1.2	2.9	0.7	12.4	2.5

(A) chromosomes 1–7 in *A. depressus*, chromosomes 1–6 in annual species; $\bar{x}$ = mean; s = standard deviation.

two groups, again no correlation with total chromosome length per group is detected. Furthermore, within karyotypes the number of bands per chromosome or the proportion of banded chromosome length is not linked with relative chromosome length. As an example, in *A. "coronatus"* one of the largest metacentric pairs completely lacks non-centromeric bands whilst another pair of similar size has the highest proportion of Giemsa banding of all metacentrics (Fig. 10).

Chromocentres and C-banding. The dark chromocentres seen in interphase nuclei of Giemsa C-stained preparations correspond to the C-bands in mitotic or meiotic chromosomes (MARKS & SCHWEIZER 1974, MARKS 1974, 1975). Fig. 14 shows examples of Giemsa C-stained nuclei of 5 *Anacyclus* species and Fig. 15 demonstrates the relationship between the number of C-bands per diploid complement (Table 2) and the number of chromocentres per nucleus. The plot deviates from the expected 1 : 1 correlation, especially in the higher range. Most likely, this is due to the difficulty to detect minute chromocentres formed by minor bands and due to intra- and interchromosomal fusion of bands giving rise to composite chromocentres.

Comparison between species. A comparative survey of the karyotypes and Giemsa C-banding patterns established for our various *Anacyclus* samples reveals both overall similarities and a certain hierarchy of differences. We propose to use these karyological parameters to characterize the genus and to group the taxa studied.

length) in "metacentrics" (A) and in nucleolar chromosomes (B) species of *Anacyclus*

total length of "metacentrics" (A)	% Giemsa bands in nucleolar chromosomes (B)								total length of nucleolar chr. (B)
	proximal		intercalary		terminal		total		
µm	$\bar{x}$	s	$\bar{x}$	s	$\bar{x}$	s	$\bar{x}$	s	µm
85	4.7	0.7	0	—	5.6	2.1	10.3	2.8	25
79	4.5	0.6	0.8	0.5	6.5	1.0	11.8	1.3	38
92	4.1	0.5	2.5	0.6	5.8	1.5	12.4	2.1	42
67	5.3	0.6	1.4	1.2	8.1	1.9	14.8	2.3	31
70	5.1	0.7	4.5	1.5	9.2	1.9	18.8	2.7	32
73	4.4	0.7	7.3	1.6	8.7	2.6	20.4	3.6	35

(B) chromosomes 8, 9 in *A. depressus*, chromosomes 7–9 in annual species;

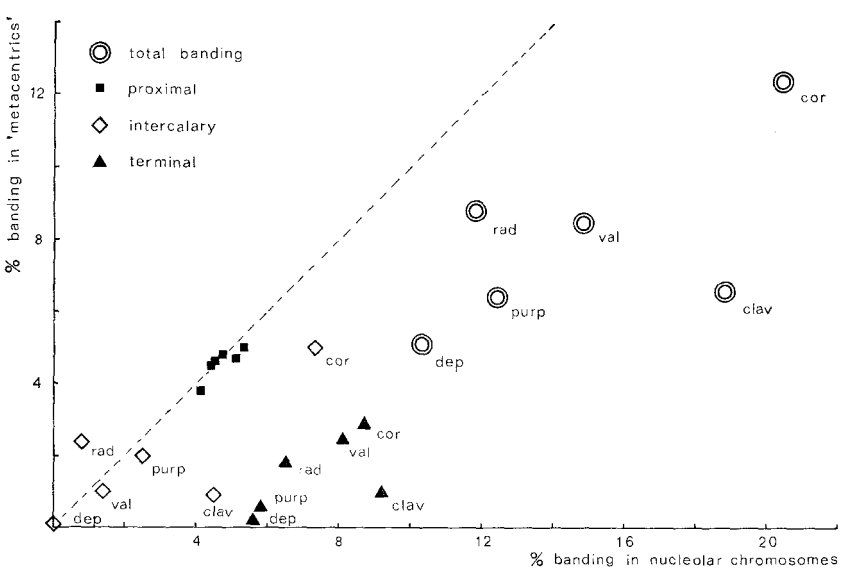

Fig. 11. Asymmetry of banding patterns: Proportion of banding in "metacentrics" (chromosome 1–7 in *Anacyclus depressus*, and chromosomes 1–6 in annuals) in relation to the proportion of banding in nucleolar chromosomes. All chromosomes have proximal (= centromeric) bands, but the amount of terminal bands and total banding is higher in nucleolar chromosomes (group B) than in (sub)metacentric chromosomes of group A

Anacyclus as a genus stands out from other *Anthemideae* by medium to large total length of the chromosome complement (UITZ 1970) and correspondingly by medium to large absolute 2 C-nuclear DNA-values: 10.48–16.92 g^{-12} per 2 C nucleus (NAGL & EHRENDORFER 1974). There is a remarkable chromosome uniformity, i.e. little differences between largest and smallest chromosomes within the total set [$\bar{x} = 1.49$ ($s = 0.14$) for *A. depressus* and $\bar{x} = 1.40$ ($s = 0.12$) for *A. "coronatus"*] or within the groups of non-satellite (A) and satellite (B) chromosomes. Chromosomes are metacentric or submetacentric; subtelocentrics and acrocentrics appear to be absent from *Anacyclus*. Therefore chromosome symmetry is high, and arm ratios (long arm/total length) approach 0.5, both within the two chromosome groups [$\bar{x} = 0.53$ ($s = 0.01$) for A and $\bar{x} = 0.58$ ($s = 0.01$) for B in *A. depressus*, and $\bar{x} = 0.53$ ($s = 0.01$) for A and $\bar{x} = 0.60$ ($s = 0.02$) for B in *A. "coronatus"*] and within the total set [$\bar{x} = 0.54$ ($s = 0.0$) in *A. depressus*, and $\bar{x} = 0.56$ ($s = 0.01$) in *A. "coronatus"*]. These findings substantiate provisional studies by UITZ (1970) and illustrate the remarkable karyotype similarities within *Anacyclus*, even between morphologically very remote species.

With regard to Giemsa C-banding style (cf. p. 139 ff.) the genus *Anacyclus* can be characterized by the omnipresence of well marked proximal bands and by the lack of intercalary bands in the proximal half of the chromosomes. There are always more bands in the satellite (B) as compared to the non-satellite (A) chromosomes. Whenever there are non-proximal bands in the (sub)metacentric (A) chromosomes they are limited to the distal half. There is somewhat of a balance of subterminal-intercalary and terminal banding in these (sub)metacentrics. In chromosomes of group (B) satellites are always banded; otherwise bands develop only in their long, non-satellite arm. On the whole, banding in *Anacyclus* is remarkably symmetrically and evenly distributed over the two arms of the (sub)metacentrics (A) and over all the pairs of the chromosome set in general.

Within these generic limits of karyological differentiation several subgroups can be easily distinguished within *Anacyclus*. First, there is the contrast between the perennials (*A. pyrethrum*, *A. depressus*) and the annuals (all others). The perennials are characterized by a karyotype of 7 pairs of metacentrics (group A, 1–7) and 2 pairs of satellite-submetacentrics (group B, 8–9). In the annuals we have only 6 pairs of metacentrics or submetacentrics (A 1–6) and 3 pairs of satellite-submetacentrics (B, 7–9). These satellite-chromosome pairs of group (B) are evidently homologous among all annuals and may be compared individually (Fig. 12). The largest pair, number 7, is the most symmetrical (arm ratios 0.52–0.59) and the two smaller pairs are usually more

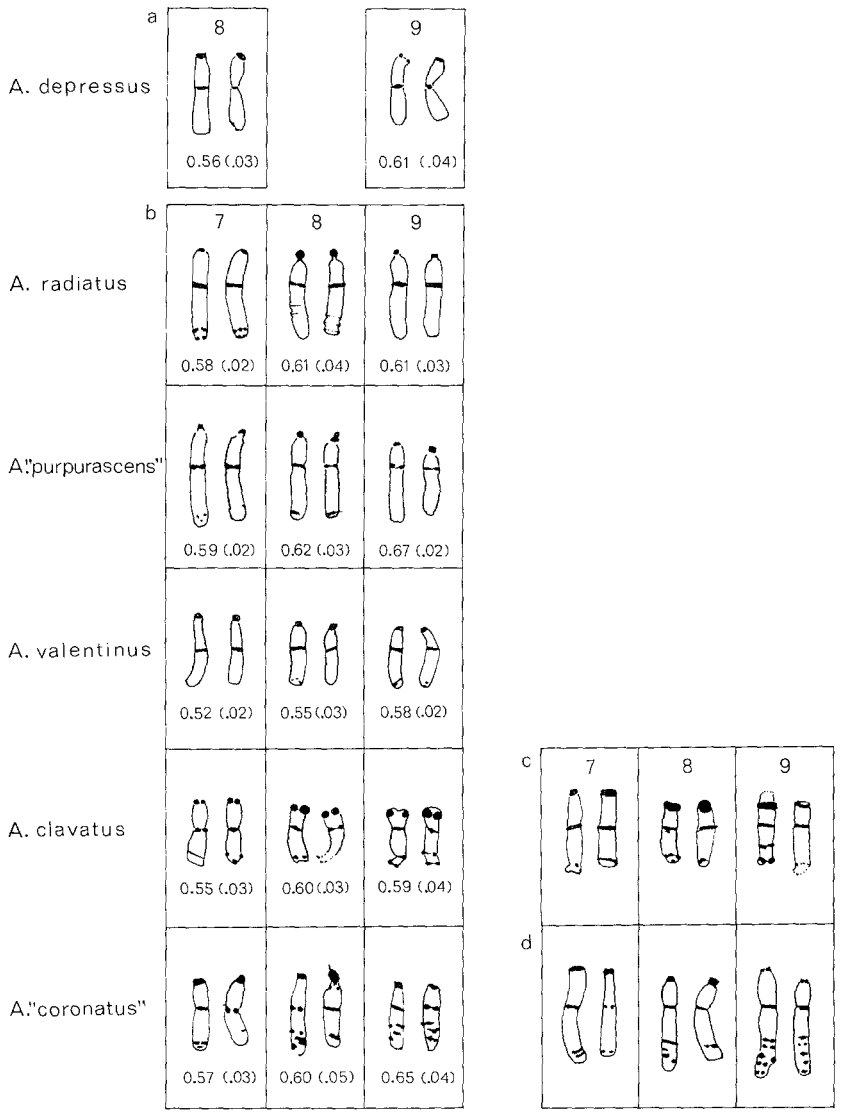

Fig. 12. Examples of Giemsa banding in nucleolar (satellite) chromosomes of *Anacyclus* species, also showing arm ratio (mean of 9–12 chromosomes and standard deviation (in brackets). Corresponding pairs are arranged in columns. *a* The perennial *A. depressus* (2 n = 18) has two pairs of main nucleolar chromosomes (no. 8 and 9). *b* The three pairs of major nucleolar chromosomes (no. 7, 8, and 9) of the annuals studied. *c* Heteromorphic variants for chromosomes 7 and 9 of *A. clavatus*. *d* Undercontracted metaphase chromosomes of *A. "coronatus"* showing multiple discrete bands in long arm of chromosome 9

asymmetrical (arm ratios up to 0.67). The intermediate pair number 8 always has a larger satellite.—Apart from the universal proximal and satellite bands the perennials are practically free of intercalary or terminal bands. *A. depressus* has the lowest proportion of banded chromosome length (6.2%) of all samples studied. Correspondingly, heterochromatic chromocentres in interphase nuclei are weakly represented. By contrast, the annuals develop supplementary intercalary bands in the distal half of their chromosome arms and terminal C-bands, with proportions of banded chromosome lenght ranging from 8.3% to 15%, and their interphase nuclei have more heterochromatic chromocentres.

The annual *Anacyclus* species can be further subdivided by karyological parameters. First, there is the subgroup of *A. radiatus* and *A. "purpurascens"*; they have mean karyotype lengths (and correspondingly 2 C-nuclear DNA-values) exceeding the perennials, while the other annuals are comparable or below the perennials in this respect. In addition *A. radiatus* and *A. "purpurascens"* are set aside by: a) nucleolar chromosomes being more asymmetrical, b) less total banding and less terminal banding in nucleolar chromosomes (i.e. they have smaller satellites; Fig. 11), c) no intercalary banding in nucleolar chromosome number 9 (Fig. 12) and d) presence of the characteristically banded metacentric pair number 3 (Figs. 4, 5). In the remaining subgroup of annuals (*A. valentinus, A. clavatus, A. "coronatus"*) a) the nucleolar chromosomes are more symmetrical, b) total banding and terminal banding in nucleolar chromosomes is higher (i.e. they have larger satellites), and c) the nucleolar chromosome number 9 has intercalary banding.

In spite of their great similarity (cf. proximity in the diagrams Figs. 11 and 13) the karyotypes of *A. radiatus* and *A. "purpurascens"* differ in some respects, e.g. in their mean length, more banding of metacentrics in *A. radiatus*, more banding of nucleolar chromosomes and less terminal and total banding in *A. "purpurascens"* (Tables 2, 3), and different band distribution in chromosome no. 8 (Fig. 12), etc.

Shape and total length of the chromosome complements in *A. valentinus, A. clavatus* and *A. "coronatus"* are virtually indistinguishable (cf. the slight differences in symmetry of nucleolar chromosomes), but "banding style" indicates that the two former species are closer to each other than to *A. "coronatus"*. *A. valentinus* and *A. clavatus* have much less total and intercalary banding, particularly in metacentrics, but also in nucleolar chromosomes (compare e.g. chromosome pair no. 9 with only 0–2 bands in the long arm) than *A. "coronatus"* with the highest banding values in the genus (and with 2–5 bands in the long arm of chromosome no. 9; cf. Figs. 11, 12, 13). But *A. valentinus* and *A. clavatus* also have quite distinct banding patterns: While total

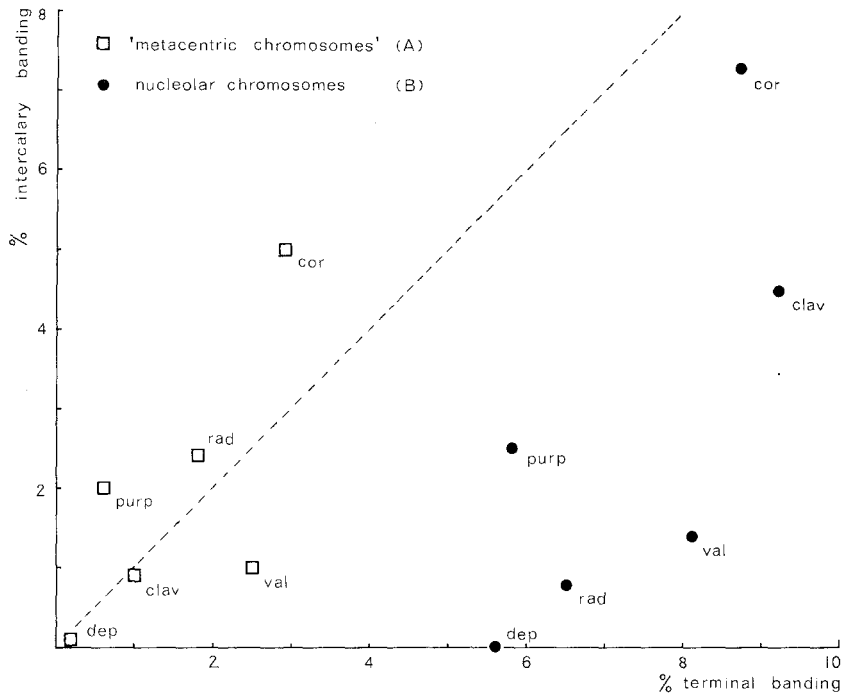

Fig. 13. "Banding style" in *Anacyclus*: Relationships between intercalary banding and terminal banding in "metacentrics" (group A; chromosomes 1–7 in *A. depressus*, or chromosomes 1–6 in annuals) and in nucleolar chromosomes (B). Compare spatial relationships of *A. depressus* (dep), *A. radiatus* (rad) and *A. purpurascens* (purp), and *A. valentinus* (val), *A. clavatus* (clav) and *A. "coronatus"* (cor). Dashed line indicates same proportion of intercalary and terminal banding

intercalary and nucleolar chromosome banding is clearly higher in *A. clavatus*, terminal banding of metacentrics is more than twice as high in *A. valentinus* (Tables 2, 3). Furtheron, the satellite chromosome no. 9 of *A. clavatus* is unique in the genus (Fig. 12).

Discussion

Variation in C-banding within and between individuals. Variation of Giemsa C-banding patterns as illustrated in Figs. 2, 4, 5, 6, 8, 10, and 16 is partly within-individual and due to methodological imperfections: "as with all chromosome banding techniques the quality of staining is somewhat erratic" (Bobrow & Cross 1974; see also Schwei-

ZER 1974). The set of bands revealed by C-banding procedures usually comprises several types of constitutive heterochromatin (JALAL et al. 1974, UTSUMI & TAKEHISA 1974, GREILHUBER 1974, 1975, SCHWEIZER & NAGL 1976), and intra-plant variation in banding patterns must be largely due to threshold differences of different amounts and types of heterochromatin coupled with variation in the amount of chromosome contraction (compare nucleolar chromosomes in *A.* "*coronatus*", Fig. 10). We have attempted to overcome these effects by selecting only those metaphase cells showing maximum banding response.

Another component of variation in C-banding patterns between homologous chromosome sets of one individual and between individuals is certainly constitutional and genetically determined, i.e. not due to developmental or modification differences (for discussion see: CASPERSSON et al. 1972, BURKHOLDER & COMINGS 1972). C-bands have been shown to be characteristic for a particular genotype (e.g. HSU 1973). In *Anemone blanda* and in *Nigella damascena* similar banding patterns were found in mitotic and meiotic chromosomes (MARKS 1974, 1975) and in *Phaseolus* suspensor polytene chromosomes the same principal arrangement of bands was found as in root tip metaphases, although the former offered better resolution (SCHWEIZER 1976). In *Vicia faba* the 14 marker C-bands of the standard karyotype were also observed in mutated karyotypes (DÖBEL et al. 1973). C-bands are inherited as simple polymorphic traits which can be used as chromosome markers both in plants, e.g. *Zea* (HADLACZKY & KÁLMÁN 1975), and in animals, e.g. *Mus* (DEV et al. 1973).

For several plant species C-banding polymorphism both between homologous chromosomes and between plants has been demonstrated. In *Scilla siberica* a detailed analysis of chromosome variation in a garden population revealed that each of the 20 plants studied had its own characteristic band endowment (VOSA 1973). In a population of *A. blanda* it was possible to attribute the many different patterns observed to two basic cytotypes (MARKS 1976). C-band polymorphism has also been demonstrated in population plants and inbred lines of rye (WEIMARCK 1975). Large variation in banding patterns of a marker chromosome in *Leopoldia comosa* both within and between populations has been reported (BENTZER & LANDSTRÖM 1975).

In the present pilot-study obviously hereditary banding polymorphism between homologues within individuals and in some instances between individuals of one sample has been demonstrated not only for the perennial *Anacyclus depressus* (p. 113), but also for most of the annuals, e.g. *A. radiatus* (Fig. 4: pairs 3, 4), *A. purpurascens* (Fig. 5: 3, 8), *A. clavatus* (Figs. 8 and 12: 7, 8, 9) and *A.* "*coronatus*" (Figs. 10 and 12: 2–5, 8–9). From these findings a relativ high incidence of struc-

tural heterozygosity is evident, even for annual *Anacyclus* species, for which UITZ (1970) has shown self-fertility and facultative autogamy (cf. p. 142).

Nuclear parameters and C-banding. The well known relationships between karyotype length, nuclear volumes and nuclear DNA contents established for 18 perennial and annual taxa of *Anthemideae* by NAGL & EHRENDORFER (1974) have been supplemented and corroborated by the present studies on *Anacyclus*. In regard to heterochromatin, data presented on interphase chromocentres (p. 126, Fig. 15) give additional proof for a correspondence with Giemsa C-bands already suggested for *Anemone* and *Hepatica* (MARKS & SCHWEIZER 1974) and *Nigella* (MARKS 1975). Furthermore, VERMA & REES (1974) have shown in *Secale cereale* that there is a 1 : 1 correlation between the area of metaphase chromosomes staining darkly with Giemsa (mainly terminal segments) and the area occupied by heterochromatin (chromocentres) in interphase nuclei as revealed by Feulgen staining. It appears that such a relationship also exists in *Anacyclus*—at least approximately—when our data on the percentage of Giemsa banded chromosome length are compared with the proportion of heterochromatin (vol. %) as measured on Feulgen stained G1 nuclei (NAGL & EHRENDORFER 1974: 43). An apparent gross deviation concerns the much too low value for *A. valentinus* (AC-130 B) obtained by NAGL & EHRENDORFER (1974: 43). Checking of the original sources suggests this to be due to a technical error rather than to a genuine difference.

Relationships between total karyotype length and Giemsa C-band material are rather obvious in *Anacyclus*. The situation illustrated in Fig. 16 is most simply explained by the assumption that in the evolution from the perennial *A. depressus* to the annuals *A. valentinus* and *A. clavatus*, and to *A.* "*coronatus*" mainly band material has been added on, while non-banding material has changed little. On the other hand, the line to the annuals *A. radiatus* and *A.* "*purpurascens*" appears to be characterized by the addition of much more non-banding than banding materials. However, these apparent trends need further confirmation by nuclear DNA content determinations of the same six provenances.

In many groups it is not yet clear whether C-bands are additional, substituted or modified chromosome segments. At least for some of the cold-sensitive heterochromatic segments (H-segments) in *Trillium grandiflorum* an accessory origin is likely (RUTISHAUSER 1956); these H-segments also stain darkly with Giemsa (SCHWEIZER 1973 b, TAKEHISA & UTSUMI 1973). Based on chromosome length measurements VOSA (1973) concluded that in *Scilla siberica* "heterochromatic segments may either replace . . . or be in addition to euchromatic regions". NAGL &

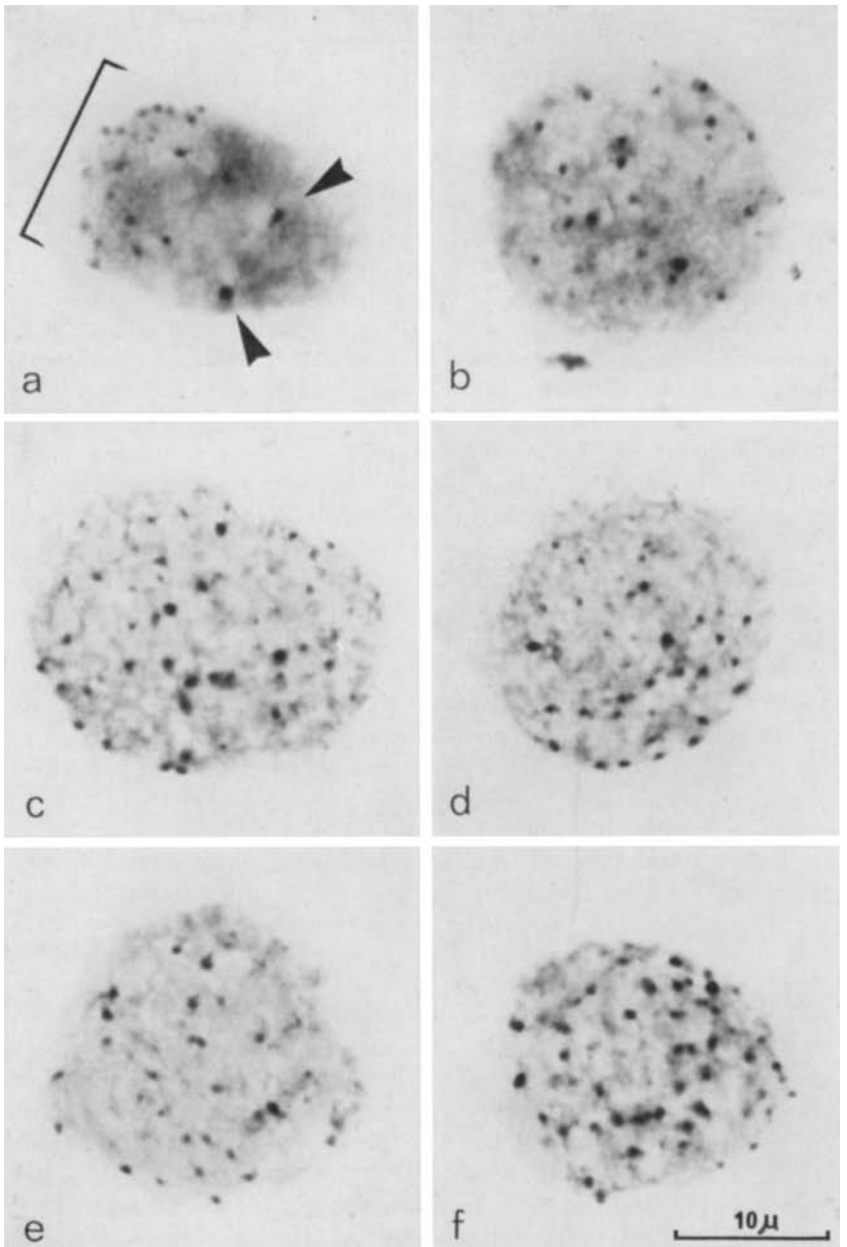

Fig. 14. *Anacyclus*, examples of Giemsa C-stained interphase nuclei showing chromocentres. *a* and *b* *A. depressus*, note in *a* the polar cluster of chromocentres which correspond to the centromeric bands (bracket). The two distantly located chromocentres marked by arrowheads most probably represent the terminal bands of nucleolar chromosomes 8 (Fig. 2; see also MARKS 1975). Nucleus *a* is from an intact cell, whilst the remaining nuclei *b–f* have been isolated through heavy squashing. *c* *A. clavatus*, *d* *A. valentinus*, *e* *A.* "*purpurascens*", *f* *A.* "*coronatus*" (compare also with Fig. 15)

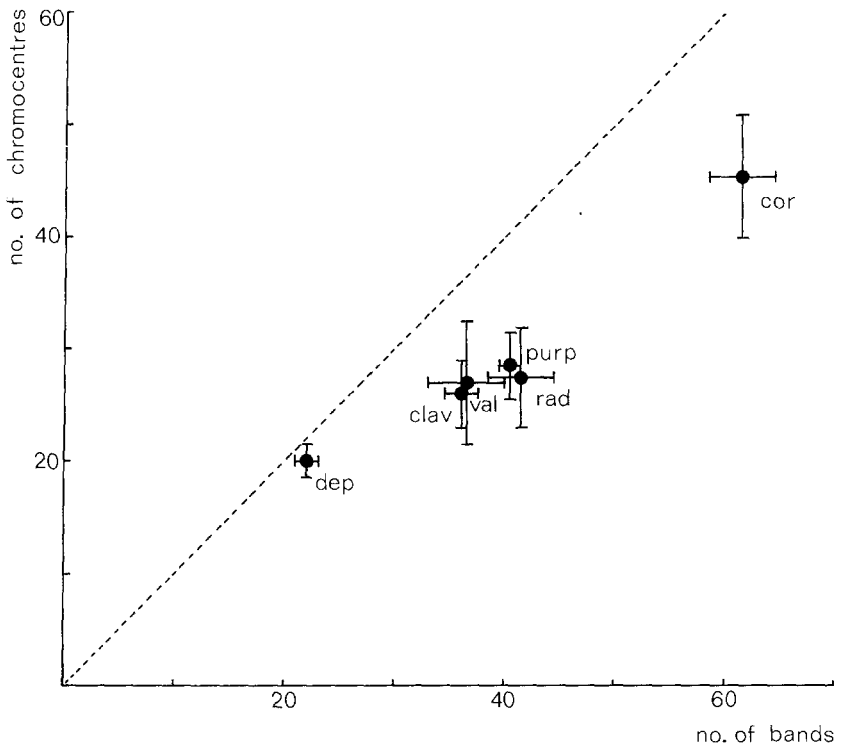

Fig. 15. Relation of the number of Giemsa C-bands per diploid complement of the *Anacyclus* species studied (mean derived from 5–8 karyotypes, and standard deviation) versus the number of chromocentres in Giemsa C-stained interphase nuclei (mean obtained from counts on 40 nuclei, or 60 nuclei for AC-147, and standard deviation)

EHRENDORFER (1974) were the first to point out that any discussion on changes in nuclear DNA content should take into consideration the relative changes of DNA in euchromatin and heterochromatin. Even if one assumes that C-bands do not have any proper functional cistrons and that C-band DNA is not transcribed in vivo, the mere addition of C-material may effect fitness and adaption by altering the "nucleotype" (see BENNETT 1972). GERAEDTS et al. (1975) demonstrated by means of Feulgen and UV-DNA measurements that the length polymorphisms for human chromosomes 1 and Y, which appear to be attributable to increased heterochromatin content, are paralleled by an increase in chromosomal DNA.

Nucleolus organizing chromosomes. It seems noteworthy that all annuals in *Anacyclus* have a higher number of main nucleolar chromosomes (six; pairs 7, 8, 9) than the perennials *A. pyrethrum* and *A. depressus* (four; pairs 8, 9). A detailed comparison suggests the homologies shown in Fig. 12. The submedian satellite pair no. 8 of the annuals therefore is a new acquisition. As it does not closely conform to a chromosome of the non-satellite A-group in the perennials, it could have originated from a translocation including a satellite and a non-satellite chromosome.

In other *Anthemideae* genera, available, but provisional data (UITZ 1970) do not clearly hint to a general trend for numerical increase of satellite, i.e. nucleolar, chromosomes from perennials to annuals. But other good examples for such a trend are available from *Dipsacaceae* (KACHIDZE 1929, EHRENDORFER 1962, 1965): In *Cephalaria* primitive perennials like *C. leucantha* or *C. graeca* have only one pair, more advanced species like the annual *C. transsylvanica* two, and the strongly derived therophyte *C. syriaca* with a reduced base number ($2n = 18 \rightarrow 10$) even has three satellite chromosome pairs. In *Scabiosa* again the semi-shrubby *S. cretica* has only one pair, the advanced annual *S. stellata* from the same section two (both with $2n = 18$), and the related but even more derived *Callistemma brachiatum* (with $2n = 14$) also two. In *Knautia* the same pattern is apparent: the perennials with $2n = 20$ have two pairs of nucleolar chromosomes, annuals like *K. integrifolia* ($2n = 20$) or *K. orientalis* ($2n = 16$) three.

It might be interesting to test the hypothesis that the transition from the perennial to the faster developing annual life habit is accompanied in certain groups by karyotypic changes leading to an increase in the number of nucleolar chromosomes, and thereby to an increase in rRNA gene content (see: FLAVELL & SMITH 1974, CULLIS 1975). Certainly, changing the number of major nucleolar chromosomes would be only one possible way of controlling rDNA content (MOHAN & FLAVELL 1974).

C-Banding and karyotype evolution. C-band material and heterochromatin in general appear to be relatively plastic nuclear components which may be subjected to considerable quantitative and qualitative changes during karyotype evolution, whilst C-band negative (and generally euchromatic) chromosome segments may exhibit higher degrees of conservatism. This phenomenon is already well known for plants (cf. "knobs" in *Zea*, references in HADLACZKY & KÁLMÁN 1975, or cold-sensitive segments in *Trillium*, e.g. RUTISHAUSER 1956) and recently has become particularly evident in certain mammalian systems where specific staining reactions such as Q-, G- or R-banding also allow

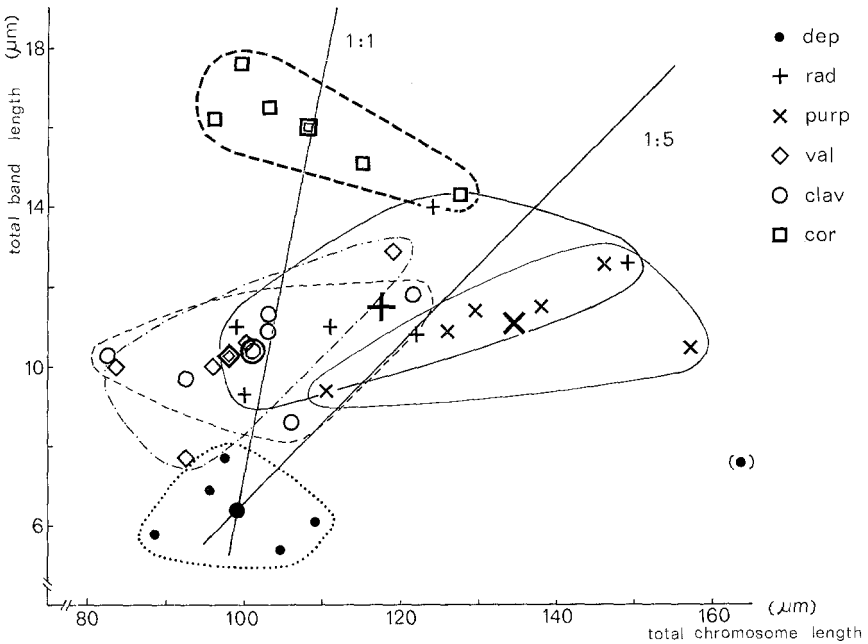

Fig. 16. Relationships between total chromosome (2 n) length and total length of Giemsa bands (absolute values in µm) in *Anacyclus*. For each sample 5–6 individual karyotypes and the mean (large symbols) have been entered (one value of *A. depressus* derived from an early pro-metaphase has been excluded from this calculation). The two lines start from *A. depressus* and indicate increase of total chromosome length due to addition of only banded (heterochromatic) material (1 : 1), or banded and non-banded (euchromatic) material (ratio 1 : 4) giving rise to a slope of 1 : 5 of the plot

to discriminate between other chromosome segments than C-bands (see Hsu 1973). A recent study on species of the American rodent genus *Peromyscus* showed that karyotype differences between the two species *P. crinitus* (2 n = 48, 56 chromosome arms per diploid complement) and *P. boylei* (2 n = 48, 96 chromosome arms) are solely due to differences in C-band content: the second arms, which differ in number, are totally heterochromatic in both species whereas the G-banding patterns of the C-negative arms were identical (Arrighi et al. 1976). Similarly, evolution of the phenotypically almost indistinguishable and still ± cross-compatible members of the *Drosophila virilis* group has mainly proceeded through appearance, shifting or disappearance of heterochromatic blocks consisting of strongly repetitive DNA sequences (Holmquist 1975).

Only the intercalary (distal half to subterminal) and to a lesser degree the terminal non-satellite Giemsa C-bands are truly variable in their presence and position in *Anacyclus* (p. 124–125). In contrast proximal and nucleolar (satellite) bands appear stable and omnipresent (only the size of the latter changes). Similar phenomena seem to be widespread in plants and animals. Obviously, it is the special function and structure of Giemsa C-stained heterochromatin in the centromere (YUNIS & YASMINEH 1971) and the nucleolus organizing zone which stabilize these regions. The more variable Giemsa C-regions may be under less rigorous selection. That they are located in the distal half of non-satellite chromosome arms could very well be linked to the localization of PMC chiasmata to this region in *Anacyclus* and in other *Anthemideae* (UITZ 1970, DELAY & PETIT 1971, HUMPHRIES 1975).

A remarkable facet in our *Anacyclus* studies is that banding in the two arms of a single chromosome or among the various chromosomes of a set clearly is not independent but follows certain superimposed and correlating influences. Already by 1933 HEITZ had observed similar patterns of heterochromatin distribution in *Vicia* and *Pellia*, and called the phenomenon "äquilokale Heterochromatie". Also, double bands in *Anacyclus* appear only in positions where there are already single bands.

Explanations for lability and polymorphism, localization and "equilocality" of banding and heterochromatin are necessarily still speculative. The well known tendency of heterochromatin for non-specific interphase (ectopic) and meiotic fusion, the higher frequency of chromosome breaks and reunions in heterochromatin rich regions (see RIEGER & MICHAELIS 1967), the proximity of ± isobrachial chromosome arms in mitotic telophase and interphase, the localization of chiasma to certain chromosome regions, the susceptibility of non-vital chromosome segments to unequal crossover and further repetition (SMITH 1976), and observed cases of band proliferation (see e.g. HENNIG 1973: 31, WHITE et al. 1975) could be listed as possible components of relevant but still hypothetical explanations.

So far little is known about the origin and genetical significance, the functions—if any—, and the biochemistry of Giemsa bands in plants. It has been suggested that Giemsa C-bands contain highly repeated DNA sequences but not all repeated sequence DNA is localized in C-bands (for discussion see: CULLIS & SCHWEIZER 1974, SCHWEIZER & NAGL 1976). Recently a correlation between a highly repeated satellite DNA and C-banding positive heterochromatin was demonstrated in *Scilla siberica* (TIMMIS et al. 1975). For highly reiterated simple sequence DNA some models of evolution have been proposed and it was suggested that highly repeated DNA, such as satellite DNA

is evolving more rapidly than unique sequence DNA (BRITTEN & KOHNE 1968, FLAMM 1972, HOLMQUIST 1975; see also LEWIN 1974, NAGL 1975). If the above correlation between highly repetitive DNA and C-banding is generally true for plant chromosomes, it may be possible to draw analogous lines for the evolutionary trends of C-band material in plants.

FLAVELL et al. (1974) considered whether highly repetitive sequence DNA may be responsible for the considerable variation in the nuclear DNA content of higher plants (C-value paradox). From DNA reassociation experiments on 23 species with DNA contents ranging from 1.7–98 pg they obtained evidence that most of the variation in nuclear DNA mass in higher plant chromosomes can be accounted for by variation in repetitious DNA. For annual *Lathyrus* species NARAYAN & REES (1976) have demonstrated positive relationships between heterochromatin, repetitive DNA, and total nuclear DNA.

The evolutionary direction of increase or decrease in overall nuclear DNA, heterochromatin and banding within certain groups often remains uncertain, e.g. for the *Drosophila virilis* group (HOLMQUIST 1975). For *Cichoriaceae*, i.e. *Microseridinae* (PRICE & BACHMANN 1975) and *Crepis* (JONES & BROWN 1976), or *Ranunculus* (SMITH & BENNETT 1975) the suggested DNA-reduction from perennials to annuals appears well founded. For *Lathyrus* REES & HAZARIKA (1969) have advocated a similar decrease, but now NARAYAN & REES (1976) are pleading rather for an evolutionary increase of DNA (predominantly repetitive) and heterochromatin. In *Lolium* nuclear DNA increase from perennials to annuals is likely (REES & JONES 1972). For the Angiosperms as a whole BENNETT (1972) has advanced the generalization that annuals on the average have less nuclear DNA because this corresponds to shorter cell cycles and thereby contributes to faster development.

The evolutionary trend from perennials to annuals in *Anthemideae* is generally accompanied by a shift to shorter cell cycles and faster development, but karyotype length, nuclear DNA and heterochromatin either increase or (more rarely) decrease (NAGL & EHRENDORFER 1974). In *Anacyclus* there is some increase (or very slight decrease) of karyotype lengths and nuclear DNA from perennials to annuals, and a clear increase of banding and heterochromatin. This corroborates the suggestions by NAGL & EHRENDORFER (1974) and NAGL (1974a, b) that an increase of nuclear DNA is normally linked to an increase in heterochromatin in the group, and that in annuals such an increase in heterochromatin may not slow down but rather accelerate cell cycles and development.

"Banding style" as a systematic character. From its stable chromosome number, relative uniformity of the medium sized chromosomes,

and the very slight structural differences discernible with classical staining methods between its taxa, *Anacyclus* certainly has not the appearance of a suitable and attractive object for karyosystematic studies. Nevertheless, Giemsa C-banding made possible an easy and clear separation of all the 6 samples studied in detail, and their hierarchical grouping on the basis of such karyological criteria alone (Fig. 17). Indeed, the remarkably aberrant banding of AC-150 (Morocco), initially classified as *A.* "*clavatus*", has subsequently induced a more detailed morphological analysis, the finding of well marked differences in fruits, involucral bracts etc., and the provisional recognition as a separate species *A.* "*coronatus*" (cf. p. 110).

All this confirms what a powerful new tool for karyosystematic research in plants Giemsa C-banding is. Its broader application should open up considerable new interest in this well established but recently somewhat stagnant field of biosystematics.

What makes chromosome banding so discriminative? First, the greatly enlarged possibilities to recognize homologous chromosomes and to compare them between related species, as we have done for the satellite pairs no. 7, 8 and 9 in *Anacyclus* (Fig. 12). These chromosomes may turn out to be particularly useful markers for karyosystematic studies in *Anthemideae*. Second, the fact that intensity and distribution of bands over the chromosome set constitute a differential syndrome. This syndrome, which we would like to call "banding style", can be broken down into its components and is open to quantitative analysis and graphic illustration as we have shown in Figs. 11 and 13. Relevant aspects have been mentioned on p. 124–131, and 136–139 and comprise relationships of proximal, intercalary and terminal banding, distribution of bands over chromosome arms, certain chromosome types, and the whole chromosome set.

For systematic purposes it is essential to gain an understanding of the stability or variability of a character relative to others in a given group. In regard to Giemsa C-banding in *Anacyclus* it is relevant that certain bands are remarkably stable, while others appear quite variable (p. 124 f., 128 ff). Particularly stable "generic" features are the omnipresence of proximal bands, the lack of intercalary bands in the proximal half of all chromosomes, and a certain balance between subterminal-intercalary and terminal banding (apart from satellite-carrying arms). From own unpublished banding studies it appears that in *Anthemis* subg. *Cota* proximal bands often are much weaker and that there is a clear preponderance of terminal over subterminal-intercalary banding. On the other hand, there is also a lack of intercalary banding in the proximal half on longer chromosome arms, while this is very typical for the "banding style" e.g. in *Tulipa* (FILION 1974). Such stable features

obviously are "ancient" components of the banding syndrome and may be useful for the karyosystematic differentiation of higher taxonomic categories. In contrast, the frequency of terminal, and particularly of intercalary or the amount of satellite banding appears increasingly labile within *Anacyclus*. The structural changes responsible for these differences are evidently of more recent origin and relate to the taxonomic separation of species groups (e.g. perennials and annuals, and subgroups within annuals), species (e.g. *A. valentinus* and *A. clavatus*) or even infraspecific taxa (possibly *A. radiatus* and *A. "purpurascens"*; cf. p. 130).

The amplitudes of banding variation as compared with the taxonomically decisive amplitudes of morphological variation differ between families and genera. In *Scilla* (Vosa 1973, Greilhuber & Speta 1976), *Allium* (Fiskesjö 1975) or *Triticum* (Gill & Kimber 1974, Hadlaczky & Belea 1975) banding variation may be comparable to *Anacyclus* and has backed suggestions about the evolutionary divergence or the hybrid resp. allopolyploid origin of taxa. In other groups, e.g. *Trillium* (Schweizer 1973 b, Takehisa & Utsumi 1973), *Anemone blanda* (Marks & Schweizer 1974, Marks 1974, 1976), or *Leopoldia comosa* (Bentzer & Landström 1975) excessive banding polymorphism may be a handicap for taxonomic conclusions. In such groups only careful studies will allow to separate the infraspecific from the interspecific variation amplitudes of the "banding-style".

Karyosystematics and evolutionary strategies in *Anacyclus*. To what an extent does our present (and still quite limited) knowledge of karyotypes and Giemsa C-banding patterns contribute to the interpretation of relationships and possible evolutionary differentiation of *Anacyclus*? As a point of reference the purely karyologically based grouping of our samples proposed on p. 128–131 is shown as an abstract 2-dimensional graph in Fig. 17. The most important conclusions from this are: a) very close affinities between the perennials (sect. *Pyrethraria* DC.), b) another natural group consists of all the annuals studied (Sect. *Anacyclus* = sect. *Diorthodon* DC.), and c) two subgroups within the annuals, i.e. one with the very closely related *A. radiatus* and *A. "purpurascens"* (possibly conspecific), the other with the more distantly related *A. valentinus*, *A. clavatus* and *A. "coronatus"*. While this represents the major groups of the small genus, one nevertheless has to keep in mind that there are altogether about 13 species (Humphries pers. comm.) of which only 5–6 have been included in our karyological study.

A comparison of this hierarchy of karyological affinities with the morphological characters discussed on p. 109 f. and with an independently and morphologically derived scheme of systematic affinities between

Anacyclus (HUMPHRIES unpubl.) reveals a remarkable and near-too-perfect correspondence. Furthermore, comparative studies on the flavonoid glycoside distribution within *Anacyclus* (GREGER unpubl.) convincingly and again independently show the same hierarchy of relationships. Can there be better proof for the systematic relevance of the karyotype and banding characters and vice versa of the morphological and phytochemical criteria?

Concerning the evolutionary relationships of the *Anacyclus* taxa discussed one might suggest that—parallel to many other genera of *Anthemideae* (and Angiosperms in general)—the perennials of sect. *Pyrethraria* are more primitive and have given rise to the annuals of sect. *Anacyclus*. The latter evidently can not be brought into a linear sequence, but rather have evolved in a parallel and diverging fashion corresponding to the subgroups and their elements.

Comparable to other *Anthemideae* genera (see e.g. EHRENDORFER 1970, UITZ 1970, NAGL & EHRENDORFER 1974) perennials and annuals in *Anacyclus* have developed different "evolutionary strategies". Annuals develop faster from seed than perennials. This is due to shorter mitotic cell cycles (NAGL 1974) and more cell elongation in annuals, and must be regarded as an adaptation to their labile pioneer habitats. Increasing specialization in fruit structure and dispersal in annuals is another related and wide-spread phenomenon, also observed in *Anacyclus*. As regards flower biology, perennial *A. pyrethrum* has been found to be self-sterile and therefore allogamous, while the annuals *A. radiatus* and *A. clavatus* are self-fertile and $\pm$ autogamous (UITZ 1970). This switch in reproductive biology from perennials to annuals is typical for many other *Anthemideae* and Angiosperms, and evidently adaptive too (lower recombination rate and better fertility in annuals). The increased rate of chiasmata in PMC's of annuals (UITZ 1970: *A. pyrethrum* = 1.58; *A. clavatus* = 1.75; *A. radiatus* = 1.86) may be a compensation for autogamy. In any case the relatively high frequency of banding polymorphism and heterozygosity clearly demonstrates that the annual *Anacyclus* populations are far from genetic uniformity. Another wide-spread tendency in annual Angiosperms and in *Anacyclus* is decreasing karyotype homogeneity and karyotype symmetry (cf. STEBBINS 1958, GRANT 1958). The increase in number of nucleolar chromosomes from perennials to annuals may be related to their faster development (cf. p. 136). Total karyotype length and DNA mass per nucleus has increased (or slightly decreased) from perennials to annuals in *Anacyclus* (cf. p. 133) (as in several other annual *Anthemideae*: NAGL & EHRENDORFER 1974), and this has been accompanied by an increase in banding, interphase chromocentres and heterochromatin. As the frequency of chromosome breakage is usually higher in hetero-

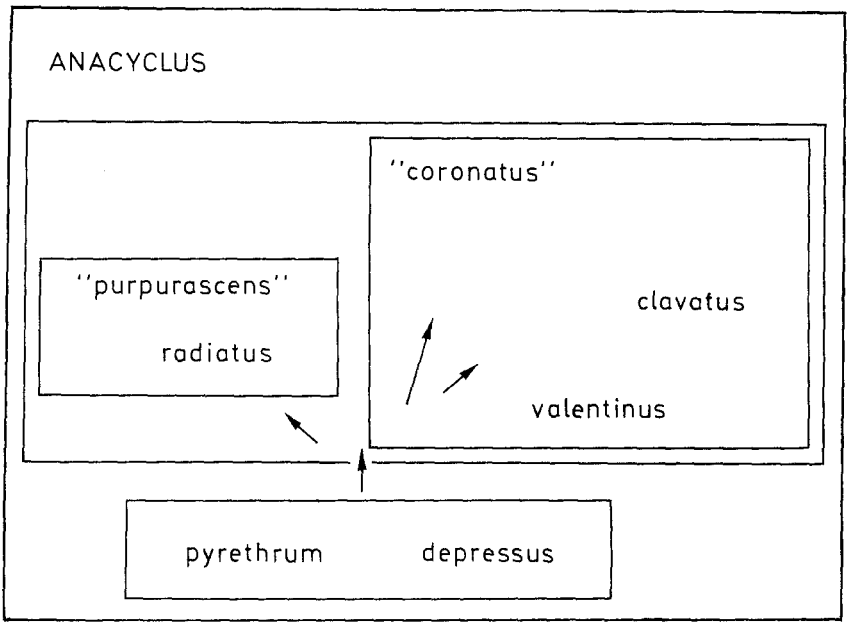

Fig. 17. Hierarchical grouping of *Anacyclus* taxa according to graded similarities of their Giemsa C-banded karyotypes (cf. p. 128–131). Arrows indicate probable lines of evolutionary differentiation

chromatin rich segments as compared with entirely euchromatic regions (p. 138) certain relationships may exist between the heterochromatin accumulation and the greater structural karyotype variation in annuals, and their more progressive and divergent evolutionary strategy as compared to the rather conservative and more intensively hybridizing perennials.

Summary

1. Six samples representing the small W. Mediterranean *Asteraceae-Anthemideae* genus *Anacyclus* (Tab. 1) have very similar, uniform, and medium sized mitotic karyotypes (2 n = 18).

2. Giemsa C-staining reveals the omnipresence of bands at centromere and nucleolar regions in *Anacyclus*, and a variety of specific banding patterns in the distal half of chromosome arms (Figs. 1–10).

3. "Banding style" allows to recognize a number of homologue chromosome pairs (cf. Fig. 12), to separate easily all Giemsa karyotypes studied (Figs. 11, 13, Tables. 2, 3), and to group them according to their graded similarities (Fig. 17). The result corresponds remarkably with

morphological and biochemical similarities in *Anacyclus*, and underlines the general importance of banding data for future karyosystematic work.

4. There is some banding polymorphism within samples and banding heterozygosity, even in self-fertile annuals (e.g. Figs. 4, 5, 8, 10).

5. Giemsa C-banding corresponds to heterochromatic interphase chromocentres (Fig. 14, 15).

6. From perennials to annuals there is an increase in the number of nucleolar chromosomes (2 → 3) and in the amount of banding (6.2% → 8.3–15%) (Figs. 11, 13, Tables 2, 3). The addition of band material in some annuals corresponds strongly, in others partly to changes of total chromosome (2 n) length (Fig. 16).

7. Aspects and relationships of banding, "equilocal" band distribution over chromosomes, heterochromatin, variation in nuclear DNA content, karyotype changes, and different evolutionary strategies in perennials and annuals are discussed.

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References

ARRIGHI, F. E., STOCK, A. D., and PATHAK, S., 1976: Chromosomes of *Peromyscus* (*Rodentia, Cricetidae*). V. Evidence of pericentric inversions. Chromosomes Today **5**, 323—329.

BAUMBERGER, H., 1970: Chromosomenzahlbestimmungen und Karyotypanalysen bei den Gattungen *Anemone*, *Hepatica* und *Pulsatilla*. Ber. Schweiz. Bot. Ges. **80**, 17—95.

BENNETT, M. D., 1972: Nuclear DNA content and minimum generation time in herbaceous plants. Proc. Roy. Soc. Lond. **B 181**, 109—135.

BENTZER, B., and LANDSTRÖM, T., 1975: Polymorphism in chromosomes of *Leopoldia comosa* (*Liliaceae*) revealed by Giemsa staining. Hereditas **80**, 219—232.

BOBROW, M., and CROSS, J., 1974: Differential staining of human and mouse chromosomes in interspecific cell hybrids. Nature **251**, 77—79.

BRITTEN, R. J., and KOHNE, D. E., 1968: Repeated sequences in DNA. Science **161**, 529—540.

BURKHOLDER, G. D., and COMINGS, D. E., 1972: Do the Giemsa banding patterns of chromosomes change during embryonic development? Exp. Cell Res. **75**, 268—271.

CASPERSSON, T., DE LA CHAPELLE, A., SCHRÖDER, J., and ZECH, L., 1972: Quinacrine fluorescence of metaphase chromosomes. Identical patterns in different tissues. Exp. Cell Res. **72**, 56—59.

CULLIS, C. A., 1975: Ribosomal RNA cistron number in *Nicotiana* species and derived haploids. Chromosoma **50**, 435—441.
— and SCHWEIZER, D., 1974: Repetitious DNA in some *Anemone* species. Chromosoma **44**, 417—421.
DELAY, J., et PETIT, D., 1971: Littoral atlantique du Maroc. Inf. Ann. Caryosyst. Cytogénét. **5**, 1—16.
DEV, V. G., MILLER, D. A., and MILLER, O. J., 1973: Chromosome markers in *Mus musculus*: strain differences in C-banding. Genetics **75**, 663—670.
DÖBEL, P., RIEGER, R., and MICHAELIS, A., 1973: The Giemsa banding patterns of the standard and four reconstructed karyotypes of *Vicia faba*. Chromosoma **43**, 409—422.
EHRENDORFER, F., 1959: Differentiation-hybridization cycles and polyploidy in *Achillea*. Cold Spring Harb. Symp. Quant. Biol. **24**, 141—152.
— 1962: Beiträge zur Phylogenie der Gattung *Knautia* (*Dipsacaceae*), I. Cytologische Grundlagen und allgemeine Hinweise. Österr. Bot. Z. **109**, 276—343.
— 1965: Evolution and karyotype differentiation in a family of flowering plants: *Dipsacaceae*. In: Genetics Today (Proc. XI. Intern. Congr. Genetics, The Hague 1963) **2**, 399—407.
— 1970: Evolutionary patterns and strategies in seed plants. Taxon **19**, 185—195.
FERNANDES, A., and QUEIRÓS, M., 1971: Contribution à la connaissance cytotaxinomique des Spermatophyta du Portugal. II. *Compositae*. Bol. Soc. Brot. **45**, 5—121.
FILION, W. G., 1974: Differential Giemsa staining in plants. I. Banding patterns in three cultivars of *Tulipa*. Chromosoma **49**, 51—60.
FISKESJÖ, G., 1975: Chromosomal relationships between three species of *Allium* as revealed by C-banding. Hereditas **81**, 23—32.
FLAMM, W. G., 1972: Highly repetitive sequences of DNA in chromosomes. Internat. Rev. Cytol. **32**, 1—51.
FLAVELL, R. B., and SMITH, D. B., 1974: Variation in nucleolar organiser rRNA gene multiplicity in wheat and rye. Chromosoma **47**, 327—334.
— and BENNETT, M. D., SMITH, J. B., and SMITH, D. B., 1974: Genome size and the proportion of repeated nucleotide sequence DNA in plants. Biochem. Gen. **12**, 257—269.
GERAEDTS, J. P. M., PEARSON, P. L., VAN DER PLOEG, M., and VOSSEPOEL, A. M., 1975: Polymorphisms for human chromosomes 1 and Y. Feulgen and UV DNA measurements. Exp. Cell Res. **95**, 9—14.
GILL, B. S., and KIMBER, G., 1974: Giemsa C-banding and the evolution of wheat. Proc. Nat. Acad. Sci. U.S.A. **71**, 4086—4090.
GRANT, V., 1958: The regulation of recombination in plants. Cold Spring Harb. Symp. Quant. Biol. **23**, 337—363.
GREILHUBER, J., 1974: Hy-banding: a new quick technique for heterochromatin staining in plant chromosomes. Naturwiss. **61**, 170—171.
— 1975: Heterogeneity of heterochromatin in plants: comparison of Hy- and C-bands in *Vicia faba*. Plant Syst. Evol. **124**, 139—156.
— and SPETA, F., 1976: C-banded karyotypes in the *Scilla hohenackeri* group, *S. persica*, and *Puschkinia*. Plant Syst. Evol. **126**, 149—188.
HADLACZKY, GY., and BELEA, A., 1975: C-banding in wheat evolutionary cytogenetics. Plant Science Letters **4**, 85—88.
— and KÁLMÁN, L., 1975: Discrimination of homologues chromosomes of maize with Giemsa staining. Heredity **35**, 371—374.

HEITZ, E., 1933: Die Herkunft der Chromocentren. Dritter Beitrag zur Kenntnis der Beziehung zwischen Kernstruktur und qualitativer Verschiedenheit der Chromosomen in ihrer Längsrichtung. Planta **18**, 571—636.

HENNIG, W., 1973: Molecular hybridization of DNA and RNA *in situ*. Intern. Rev. Cytol. **36**, 1—44.

HOLMQUIST, G., 1975: Organisation and evolution of *Drosophila virilis* heterochromatin. Nature **257**, 503—506.

HSU, T. C., 1973: Longitudinal differentiation of chromosomes. Ann. Rev. Genet. **7**, 153—176.

HUMPHRIES, C. J., 1975: Cytological studies in the macaronesian genus *Argyranthemum* (*Compositae*: *Anthemideae*). Bot. Notiser **128**, 239—255.

— 1976: A revision of the macaronesian genus *Argyranthemum* WEBB ex SCHULTZ BIP. (*Compositae–Anthemideae*). Bull. Brit. Mus. (Nat. Hist.) Bot. **5**, 145—240.

JALAL, S. M., CLARK, R. W., HSU, T. C., and PATHAK, S., 1974: Cytological differentiation of constitutive heterochromatin. Chromosoma **48**, 391—403.

JONES, R. N., and BROWN, L. M., 1976: Chromosome evolution and DNA variation in *Crepis*. Heredity **36**, 91—104.

KACHIDZE, N., 1929: Karyologische Studien über die Familie der *Dipsacaceae*. Planta **7**, 482—502.

LEWIN, B., 1974: Gene Expression. Vol. 2, Eucaryotic Chromosomes. London: John Wiley & Sons.

LOON, J. C. VAN, GADELLA, T. W. J., and KLIPHUIS, E., 1971: Cytological studies in some flowering plants from southern France. Acta Bot. Neerl. **20**, 157—166.

MARKS, G. E., 1974: Giemsa banding of meiotic chromosomes in *Anemone blanda* L. Chromosoma **49**, 113—119.

— 1975: The Giemsa-staining centromeres of *Nigella damascena*. J. Cell Sci. **18**, 19—25.

— 1976: Variation of Giemsa banding patterns in the chromosomes of *Anemone blanda* L. Chromosomes Today **5**, 179—184.

— and SCHWEIZER, D., 1974: Giemsa banding: karyotype differences in some species of *Anemone* and in *Hepatica nobilis*. Chromosoma **44**, 405—416.

MARTÍNEZ VÁZQUEZ, M., 1960: Estudio cariologico de especies del genero "*Anacyclus*". Genét. Ibér. **12**, 187—204.

— 1962: Estudios cariologicos en especies del genero *Anacyclus*. Genét. Ibér. **14**, 19—37.

MITSUOKA, S., and EHRENDORFER, F., 1972: Cytogenetics and evolution of *Matricaria* and related genera. Österr. Bot. Z. **120**, 155—200.

MOHAN, J., and FLAVELL, R. B., 1974: Ribosomal RNA cistron multiplicity and nucleolar organizers in hexaploid wheat. Genetics **76**, 33—44.

NAGL, W., 1974a: Role of heterochromatin in the control of cell cycle duration. Nature **249**, 53—54.

— 1974b: Mitotic cycle time in perennial and annual plants with various amounts of DNA and heterochromatin. Developm. Biol. **39**, 342—346.

— 1975: I. Replication: Organization and replication of the eukaryotic chromosome. In: ELLENBERG, H., ESSER, K., MERXMÜLLER, H., SCHNEPF, E., and ZIEGLER, H., (Eds.): Progr. Bot./Fortschr. Bot. **37**, 186—210. Berlin: Springer.

Nagl, W., and Ehrendorfer, F., 1974: DNA content, heterochromatin, mitotic index, and growth in perennial and annual *Anthemideae* (*Asteraceae*). Plant Syst. Evol. **123**, 35—54.

Narayan, R. K. J., and Rees, H., 1976: Nuclear DNA variation in *Lathyrus*. Chromosoma **54**, 141—154.

Ockey, C. H., and Bateman, A. J., 1974: The linear differentiation of chromosomes. Nature **252**, 95—97.

Papeš, D., 1975: Cytotaxonomy of the genus *Leucanthemum* in Yugoslavia. In: Walters, S. M., and King, C. J., (Eds.): European Floristic and Taxonomic Studies, 90—100 (B.S.B.I. Conference report **15**).

Price, M. J., and Bachmann, K., 1975: DNA content and evolution in the *Microseridinae*. Amer. J. Bot. **62**, 262—267.

Przywara, L., 1974: Biosystematical studies on the collective species *Leucanthemum vulgare* from Poland (Pol., with Engl. summary). Fragm. Florist. Geobot. **20**, 413—470.

Rees, H., and Hazarika, M. H., 1969: Chromosome evolution in *Lathyrus*. Chromosomes Today **2**, 158—165.

— and Jones, R. N., 1972: The origin of the wide species variation in nuclear DNA content. Intern. Rev. Cytol. **32**, 53—92.

Rieger, R., and Michaelis, A., 1967: Die Chromosomenmutationen. In Stubbe, H., (Ed.): Genetik: Grundlagen, Ergebnisse und Probleme in Einzeldarstellungen, Beitrag 6. Jena: Fischer.

Rodrigues, J. E. de Mesquita, 1953: Contribução para o conhecimento cariológico das halófitas e psamófitas litorais. Diss. Univ. Coimbra 1—210.

Rutishauser, A., 1956: Chromosome distribution and spontaneous chromosome breakage in *Trillium grandiflorum*. Heredity **10**, 367—407.

Schweizer, D., 1973a: Vergleichende Untersuchungen zur Längsdifferenzierung der Chromosomen von *Vicia faba* L. Verhandl. Naturf. Ges. Basel **83**, 1—75.

— 1973b: Differential staining of plant chromosomes with Giemsa. Chromosoma **40**, 307—320.

— 1974: An improved Giemsa C-banding procedure for plant chromosomes. Experientia **30**, 570—571.

— 1976: Giemsa and fluorochrome banding of polytene chromosomes in *Phaseolus vulgaris* and *P. coccineus*. In: Jones, K., and Brandham, P. E., (Eds.): Current Chromosome Research, 51—56. Amsterdam: Elsevier/North-Holland.

— and Nagl, W., 1976: Heterochromatin diversity in *Cymbidium* and its relationship to differential DNA replication. Exp. Cell Res. **98**, 411—423.

Shchapova, A. I., and Bautina, T. A., 1974: Differential staining of the chromosomes of rye and wheat-rye amphidiploids. Proc. Siber. Sect. Acad. Sci. USSR **18**, 134—136.

Smith, G. P., 1976: Evolution of repeated DNA sequences by unequal crossover. Science **191**, 528—535.

Smith, J. B., and Bennett, M. D., 1975: DNA variation in the genus *Ranunculus*. Heredity **35**, 231—239.

Stebbins, G. L., 1958: Longevity, habitat, and release of genetic variability in the higher plants. Cold Spring Harb. Symp. Quant. Biol. **23**, 365—378.

TAKEHISA, S., and UTSUMI, S., 1973: Heterochromatin and Giemsa banding of metaphase chromosomes in *Trillium kamtschaticum* PALLAS. Nature New Biol. **243**, 286—287.

TANAKA, R., and SHIMOTOMAI, N., 1968: A cytogenetic study on the F_1-hybrid of *Chrysanthemum makinoi* × *Ch. vulgare*. Cytologia **33**, 241—245.

TIMMIS, J. N., DEUMLING, B., and INGLE, J., 1975: Localisation of satellite DNA sequences in nuclei and chromosomes of two plants. Nature **257**, 152—155.

UITZ, H., 1970: Cytologische und bestäubungsexperimentelle Beiträge zur Verwandtschaft und Evolution der *Anthemideae* (*Asteraceae*). Diss. Univ. Graz.

UTSUMI, S., and TAKEHISA, S., 1974: Heterochromatin differentiation in *Trillium kamtschaticum* by ammoniacal silver reaction. Exp. Cell Res. **86**, 398—401.

VERMA, S. C., and REES, H., 1974: Giemsa staining and the distribution of heterochromatin in rye chromosomes. Heredity **32**, 118—122.

VILLARD, M., 1971: Contribution à l'étude cytotoxinomique et cytogénétique du genre *Leucanthemum* ADAMS. em BRIQ. et CAV. Bull. Soc. Bot. Suisse **80**, 96—188.

VOSA, C. G., 1973: Heterochromatin recognition and analysis of chromosome variation in *Scilla sibirica*. Chromosoma **43**, 269—278.

WEIMARCK, A., 1975: Heterochromatin polymorphism in the rye karyotype as detected by the Giemsa C-banding technique. Hereditas **79**, 293—300.

WHITE, R., PASZTOR, L. M., and HU, F., 1975: Mouse satellite DNA in noncentromeric heterochromatin of cultured cells. Chromosoma **50**, 275—282.

YUNIS, J. J., and YASMINEH, W. G., 1971: Heterochromatin, satellite DNA and cell function. Science **174**, 1200—1209.

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