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# A survey of C-band patterns in chromosomes of *Lilium* (*Liliaceae*)*

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**Key words:** Angiosperms, *Liliaceae, Lilium.* – C-banding, heterochromatin, karyosystematics, karyotype.

**Abstract:** C-band patterns are described for 20 *Lilium* spp. distributed across six sections. All species have a similar basic karyotype (n = 12) but C-bands differ markedly between them. The patterns are characterized by a dispersed scattering of thin intercalary bands as well as centric and NOR bands. Only one species, *L. canadense,* shows a clear equilocal pattern with intercalary C-bands occurring proximally in all of the longer chromosome arms. Comparing species, similar patterns are revealed for *L. regale* and *L. sulphureum*, for *L. formosanum* and *L. longiflorum* (all in sect. *Leucolirion*) and to a lesser extent for *L. hansonii, L. martagon*, and *L. tsingtauense* (sect. *Martagon*). The pattern for *L. henryi* (previously classed in sect. *Sinomartagon*) matches those of *L. regale* and *L. sulphureum* quite well and its transfer to sect. *Leucolirion* is proposed. This is consistent with results from interspecies hybrids between *L. henryi* and *L. regale* (and related species) which are reportedly fertile. No other clear similarities in C-band patterns were seen across species. It seems that C-band patterns change rapidly in *Lilium* and hence their usefulness in classification will be restricted to identifying closely related species.

The genus *Lilium* L. (*Liliaceae*) includes up to 100 species widely distributed across Temperate Asia, Europe, and North America (WOODCOCK & STEARN 1950, SYNGE 1980). Early sub-divisions of the genus were clearly artificial as they were based solely on flower shape and aspect (see WOODCOCK & STEARN 1950). A more detailed classification was proposed by COMBER (1949) who defined seven sections based on a combination of 15 traits. Particular importance was placed on seed weight and germination patterns, leaf arrangement, and the habit of the bulb and bulb scales although floral characters were also considered.

COMBER's subdivision of the genus seems to have been generally accepted, although LIGHTY (1960) outlined some modifications based partly on the results of inter-species pollinations involving 44 species. He generated "crossing coefficients" by assigning various subjective weightings to the final stages of ovary development reached. However, the coefficients obtained were frequently inconsistent with COMBER's scheme and may have reflected the nature of the maternal parent more than relatedness of the two species involved. Much sounder guides to

* Dedicated to Prof. D. G. CATCHESIDE on the 80th anniversary of his birth.

interspecies relatedness are likely to be the production or otherwise of viable progeny and the fertility or sterility of any such hybrids produced.

Another property often used to deduce and clarify interspecies relationships is the karyotype (JACKSON 1971, GREILHUBER 1984). Unfortunately this has been of little help in *Lilium* because all species examined to date have $2n = 24$, with two large metacentric chromosomes and ten smaller acrocentrics (STEWART 1947, LIGHTY 1960). Even so the number and location of nucleolar organizing regions (NORs) within the karyotype has been of some use. Although quite diverse overall, closely related species often have NORs at the same locations (STEWART 1947).

More recently another aspect of the karyotype, the C-banding pattern, has been useful in classification of species. Within the *Liliaceae*, for example, comparative studies in *Allium* (VOSA 1976a, 1976b, BADR & ELKINGTON 1977, LOIDL 1983), *Fritillaria* (LA COUR 1978), *Scilla* (GREILHUBER & SPETA 1976, 1977, 1978), and *Tulipa* (BLAKELY & VOSA 1981, 1982) have shown that similar C-band patterns reflect relatively close relationships between species [although convergence cannot always be dismissed (GREILHUBER 1984)].

C-bands have been described for *Lilium longiflorum* (HOLM 1976, KONGSUWAN & SMYTH 1978) and *L. henryi* (WISUDHAROMN & SMYTH 1985). The patterns are quite dissimilar as expected for these two distantly related species (COMBER 1949). It seemed of interest to us to examine a wider range of species from all sections of *Lilium* to gauge the overall variation in C-banding patterns. Also several species apparently closely related on morphological grounds were banded to obtain an idea of the rate of change of patterns within the genus. At the same time it was hoped that C-bands might be useful in resolving some of the uncertainties which remain in COMBER's (1949) classification.

### Sources and methods

Twenty *Lilium* spp. were obtained from a range of sources (Table 1). The identification of each species was confirmed by reference to descriptions and illustrations in WOODCOCK & STEARN (1950) and SYNGE (1980). Voucher colour photographs have been deposited with the National Herbarium of Victoria, Melbourne (MEL), and with the Editorial Office of Plant Systematics and Evolution (Vienna).

Seeds were germinated and grown for several seasons until mature bulbs had formed. For C-banding, bulbs or bulbils were grown in vermiculite for several weeks at 20 °C and roots excised and treated in 0.02% (w/v) colchicine for 4 to 5 h. They were fixed in 45% (v/v) acetic acid at room temperature for 30 min, washed in distilled water and hydrolysed in 1 M HCl at room temperature for 7 – 8 min. After another rinse they were transferred to 45% acetic acid, squashed and coverslips removed after freezing. Slides were dunked in ethanol and air dried for between 3 and 18 h. They were then treated in freshly prepared 0.064 M $Ba(OH)_2$ for 3 to 5 min, washed with water and then immersed in 2 × SSC (0.3 M NaCl and 0.03 M trisodium citrate) at 60 °C for between 15 and 20 min. This time was important as the optimum varied between batches of slides. After a water rinse cells were stained in 4% Giemsa (buffered to pH 6.8) for 6 to 18 h, rinsed, dried, and mounted in GURR's DEPEX.

Silver banding of NORs followed the method of VON KALM & SMYTH (1984).

## Results

In this study karyotypes have been arranged following STEWART (1947) who labelled the 12 chromosomes A to L in order of the decreasing relative length of their short

Table 1. List of *Lilium* spp. studied, classified into sections following COMBER (1949), and accessions: *ALS* Australian Lilium Society, Vic.; *CHA* Mr G. CHANDLER, The Basin, Vic.; *DOY* G. DOYNE Pty. Ltd., Monbulk, Vic.; *GRG* Golden Ray Gardens (Mr B. HAYLER), Kallista, Vic.; *KEN* Mr. K. WATKINS, Hughesdale, Vic.; *KOO* Koonawarra Lilium Nursery (Mr D. MCPHERSON), Ferny Creek, Vic.; *LVN* Lilium Vale Nursery (Mr R. SINCLAIR), Kenthurst, N.S.W.; *ROB* Mr A. ROBINSON, Doncaster, Vic.; *VBB* VAN BERKEL BULBS, Monbulk, Vic.; *WIT* Dr R. WITHERS, Melbourne, Vic.

| Section and species | Accession |
|---|---|
| 1. Sect. *Martagon* REICHENBACH emend. COMBER | |
| *L. hansonii* LEICHTLIN ex. D. T. MOORE | KOO, 1 bulb |
| *L. hansonii* LEICHTLIN ex D. T. MOORE | KEN, 1 bulb |
| *L. martagon* L. | ALS, 1 seed |
| *L. tsingtauense* GILG | GRG, 3 bulbs |
| 2. Sect. *Pseudolirium* ENDLICHER emend. COMBER | |
| b. *L. pardalinum* KELLOGG | LVN, 3 bulbs |
| *L. pardalinum* KELLOGG "Santa Cruz" | ALS, sev. seeds |
| c. *L. canadense* L. | ROB, 2 bulbs |
| 3. Sect. *Lilium* (*Liriotypus* ASCH. & GRAEB. emend. COMBER) | |
| *L. candidum* L. | KOO, 2 bulbs |
| 4. Sect. *Archelirion* BAKER emend. COMBER | |
| *L. auratum* L. "Praecox" | CHA, 1 bulb |
| *L. speciosum* THUNB. | CHA, 1 bulb |
| *L. speciosum* THUNB. "Album Novum" | KEN, 1 bulb |
| 5. Sect. *Sinomartagon* COMBER | |
| a. *L. henryi* BAKER | VBB, many bulbs |
| *L. lankongense* FRANCHET | GRG, 2 bulbs |
| *L. tigrinum* var. *flaviflorum* (MAKINO) STEARN | KEN, 2 bulbils |
| b. *L. amabile* PALIBIN | ALS, sev. seed |
| *L. concolor* SALISBURY | GRG, 2 bulbs |
| c. *L. nepalense* D. DON "Robusta" | ALS, 1 bulb |
| *L. wardii* (STAPF ex) STERN | ALS, sev. seed |
| 6. Sect. *Leucolirion* WILSON emend. COMBER | |
| a. *L. regale* WILSON | ALS, 2 seed |
| *L. regale* WILSON | VBB, many bulbs |
| *L. sulphureum* BAKER apud HOOKER | WIT, 1 bulbil |
| b. *L. formosanum* WALLACE | KEN, sev. bulbs |
| *L. longiflorum* THUNB. | DOY, many bulbs |
| 7. Unassigned | |
| *L. mackliniae* SEALY | ALS, 1 seed |

arm. He reported carmine-stained karyotypes of 35 species, with NORs identified by relict nucleolus attachment at prophase. Of the 20 species studied here, STEWART (1947) recorded all except *L. hansonii* (see NODA 1973), *L. lankongense, L. mackliniae, L. nepalense* (all karyotyped by LIGHTY 1960) and *L. tigrinum* var. *flaviflorum* (see NODA 1978).

C-banded karyotypes are shown either as drawings or using photographs of

representative chromosomes. Individual bands sometimes varied in size between homologoues, in which case the larger size is usually illustrated. Also, a few bands were detected in only one homologue of an individual, or in only one accession of a species. Such heteromorphic bands (or band locations) are indicated with dots on the karyotypes.

The order of presentation of species follows COMBER's (1949) subdivision of the genus.

**Sect. 1: *Martagon.*** COMBER's first section includes five species with characters considered to be present in early ancestors of the genus. We C-banded three species.

*L. hansonii*, a Korean species, has a scattering of thin intercalary bands on most chromosomes, with one large band distal on the long arm of chromosome C (Fig. 1). Two different J chromosomes were identified in accession KOO (Table 1). NOR locations have not been reported previously for *L. hansonii*, but silver banding of another accession (KEN) showed bands on chromosomes B, C, D, E, G, J, and K (Fig. 2). The sites in chromosomes E and J were silver banded on only one homologue, making a total of 12 NORs per plant. C-bands occur at all these locations (Fig. 1). NODA (1973) reported secondary constrictions at all NOR sites in two collections of *L. hansonii* (as well as one apparently corresponding to the large intercalary C-band on chromosome C). As in our plants, his individuals were heteromorphic for constrictions on chromosomes E and J. This is consistent with the proposal that the cultivated strain of *L. hansonii*, which is self-incompatible, is a single clone (NODA 1973).

*L. martagon* has a quite different distribution of thin, intercalary bands (Fig. 1). For this species, STEWART (1947) reported NORs on chromosomes A, B, C, F, and K. Although his figure shows no secondary constriction on K, we could clearly

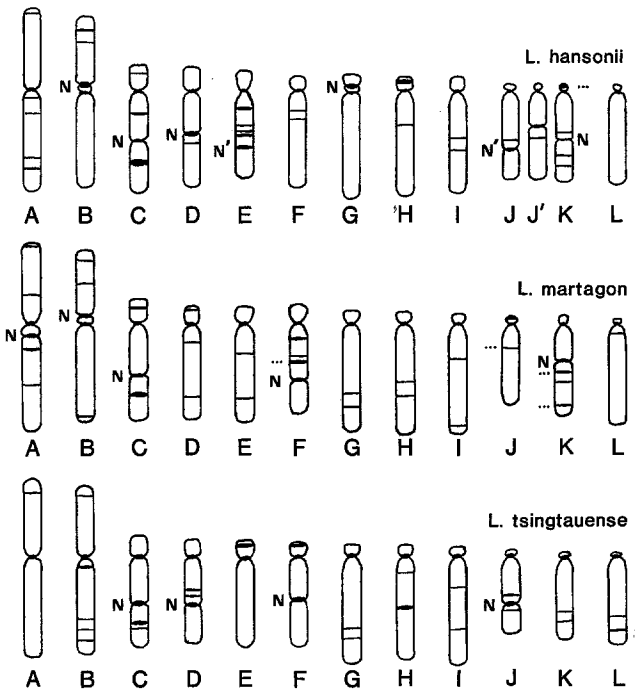


Fig. 1. C-banded idiograms of three species from sect. *Martagon*. Heteromorphic bands (i.e., bands absent in one or more homologues) are indicated with dots. *N* Confirmed nucleolus organizing regions in all figures. *N'* Heteromorphic NORs

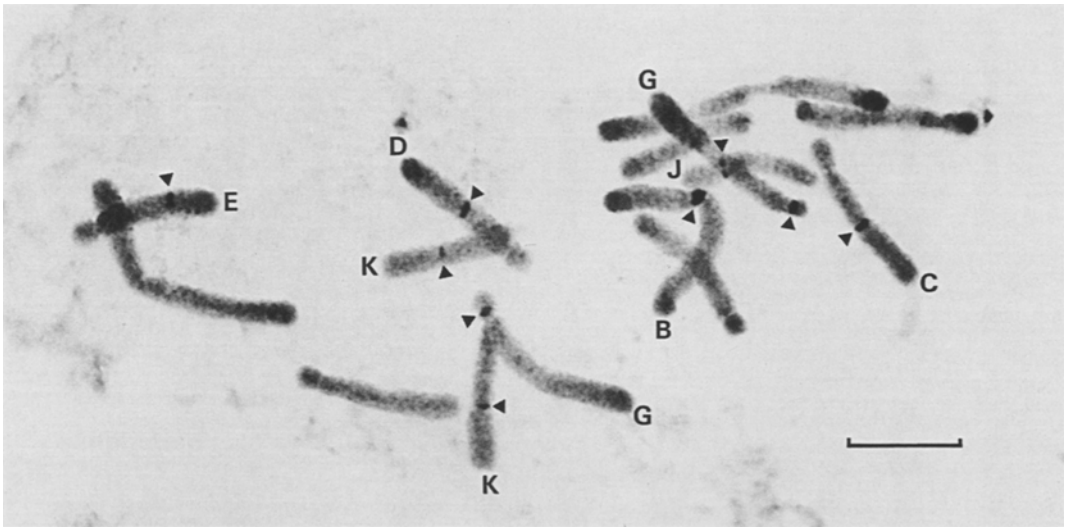


Fig. 2. Silver banded chromosomes of *L. hansonii* (prepared by Dr L. VON KALM). Bands are indicated with arrowheads. Bar: 10 µm

see one medial in the long arm in our accession (Fig. 1). All five NOR sites carried C-bands.

The final species examined, *L. tsingtauense*, has yet another pattern of scattered thin bands (Fig. 1). The sites of four NORs reported by STEWART (1947), on C, D, F, and J, are relatively heavily C-banded. In the three bulbs examined one of each of chromosomes A, C, and D were absent and three grossly different chromosomes were observed. These apparently have resulted from a complex three way rearrangement. Certainly, a sample of pollen from another bulb of this accession showed 55% (275 out of 500) of grains to be shrivelled and empty as is expected from unbalanced haploid complements.

**Sect. 2: *Pseudolirium.*** All North American species fall into this section.

Two accessions of *L. pardalinum,* a Western species from COMBER's (1949) group b, gave similar patterns (Fig. 3). Three bulbs of the LVN accession (Table 1) were heteromorphic for the proximal C-band on chromosome L. The form "Santa Cruz" (ALS accession) lacked a medial band and had a more distal C-band on the B short arm, and revealed a further band distal on the K long arm (Fig. 3). In each accession NORs on chromosomes H, I, and K (STEWART 1947) were heavily banded.

The Eastern species *L. canadense* (in COMBER's group c) has a markedly different pattern (Fig. 3). One to three very prominent intercalary bands are close to and roughly equidistant from centromeres on nearly all the longer chromosome arms. The only exceptions involved heteromorphisms for the absence of such bands on the long arms of A and B chromosomes in the two bulbs studied. The two NORs, close to the telomeres on short arms of E and G (STEWART 1947), are also heavily banded.

**Sect. 3: *Lilium.*** Because this section includes *L. candidum,* the type species of the genus, its name should be that of the genus rather than *Liriotypus* ASCH. & GRAEB. emend. COMBER which COMBER (1949) used. This follows Article 22 of

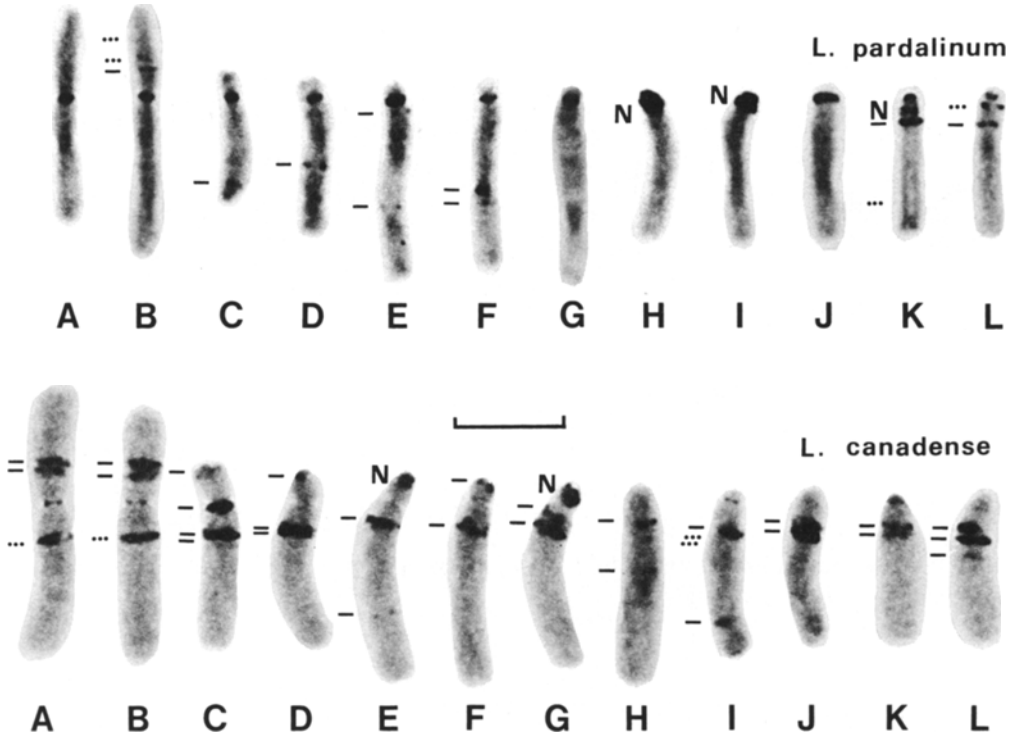


Fig. 3. C-banded karyotypes of two species from sect. *Pseudomartagon*. Bands always present (−), locations of heteromorphic bands (···). Bar: 10 μm

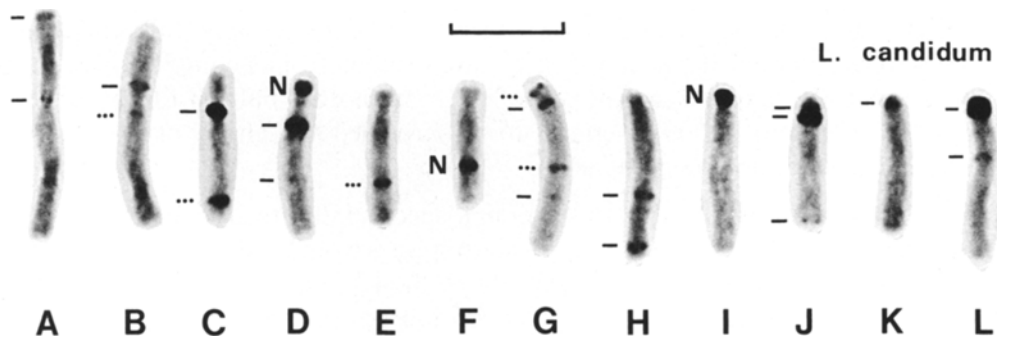


Fig. 4. C-banded karyotype of *L. candidum* from sect. *Lilium*. Bar: 10 μm

the International Code of Botanical Nomenclature and was originally pointed out by LIGHTY (1960). The section includes most W. Asian and European species but we examined only the type.

*L. candidum* has relatively thick intercalary C-bands close to centromeres of chromosomes C, D, J, and L (Fig. 4). The NORs on chromosomes D, F, and I (STEWART 1947) are also heavily C-banded. The two bulbs examined were polymorphic for the presence of five intercalary bands on chromosomes B, C, E, and G (Fig. 4).

**Sect. 4: *Archelirion.*** Of the six Oriental species in this section, we have examined two. One bulb of the form "Praecox" of *L. auratum* was relatively homomorphic,

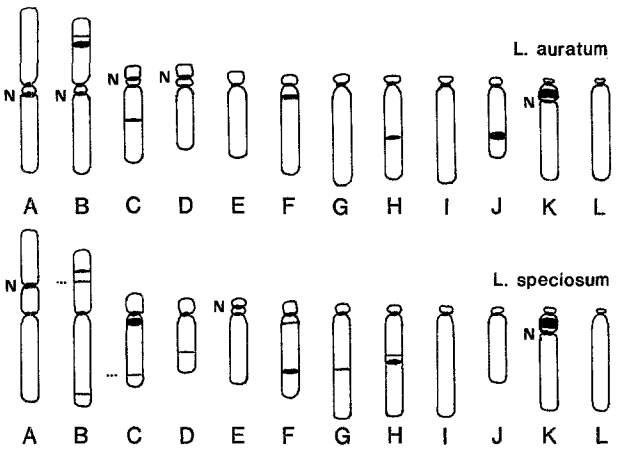


Fig. 5. C-banded idiograms of two species from sect. *Archelirion*

with prominent intercalary bands on chromosomes B, J, and K (Fig. 5, where the chromosomes within each of STEWART's (1947) pairs C and D, E and F, and K and L have been interchanged). Five NORs were all C-banded, but that on chromosome C (STEWART's D) is apparently in the short rather than the long arm (VON KALM & SMYTH 1984).

Two accessions of *L. speciosum* were banded (Fig. 5). They showed closely similar patterns, although the cultivar "Album Novum" (KEN accession) had an additional band proximal in the short arm of chromosome B and lacked the distal, intercalary band of C present in the CHA accession. STEWART (1947) located four NORs in *L. speciosum,* but we could not see a constriction or C-band at one of these reported sites (in the short arm of chromosome C). Further, no rRNA genes could be detected here by in situ hybridization using *L. speciosum* "Gilrey" (VON KALM & SMYTH 1984).

**Sect. 5: *Sinomartagon.*** This is the largest section and includes a heterogeneous collection of Asiatic species. COMBER (1949) subdivided it into three groups.

Within group a we examined *L. henryi, L. lankongense*, and *L. tigrinum* var. *flaviflorum* (Fig. 6). The pattern in *L. henryi* is relatively simple, with thick intercalary bands on chromosomes C and L and a few other thinner bands including NOR bands on A and F (WISUDHAROMN & SMYTH 1985). C-bands in *L. lankongense* are very different, and also rather sparse and thin except for the large intercalary band on chromosome E (Fig. 6). The two bulbs examined had similar homomorphic patterns. NOR locations have not been established in *L. lankongense*. Finally the pattern in *L. tigrinum* var. *flaviflorum* is again very different (Fig. 6). Many of the very thin bands were visible in only a proportion of spreads. However, the large intercalary band medial on chromosome H was always seen as were bands at NORs on chromosomes A, B, and G. [NORs were identified by silver staining (C. M. CORRICK, pers. comm.) and did not include one on chromosome K reported by NODA (1978).] The larger C-bands correspond well with those previously described by SON (1977) in the diploid set of chromosomes of the allo-triploid *L. tigrinum* KER-GAWLER (*L. lancifolium* THUNB.).

In COMBER's (1949) grouping b of small-flowered species, we investigated *L. amabile* and *L. concolor* (Fig. 7). Both species have relatively thin intercalary bands, although *L. amabile* has thicker bands distal on chromosomes A and D. Of the

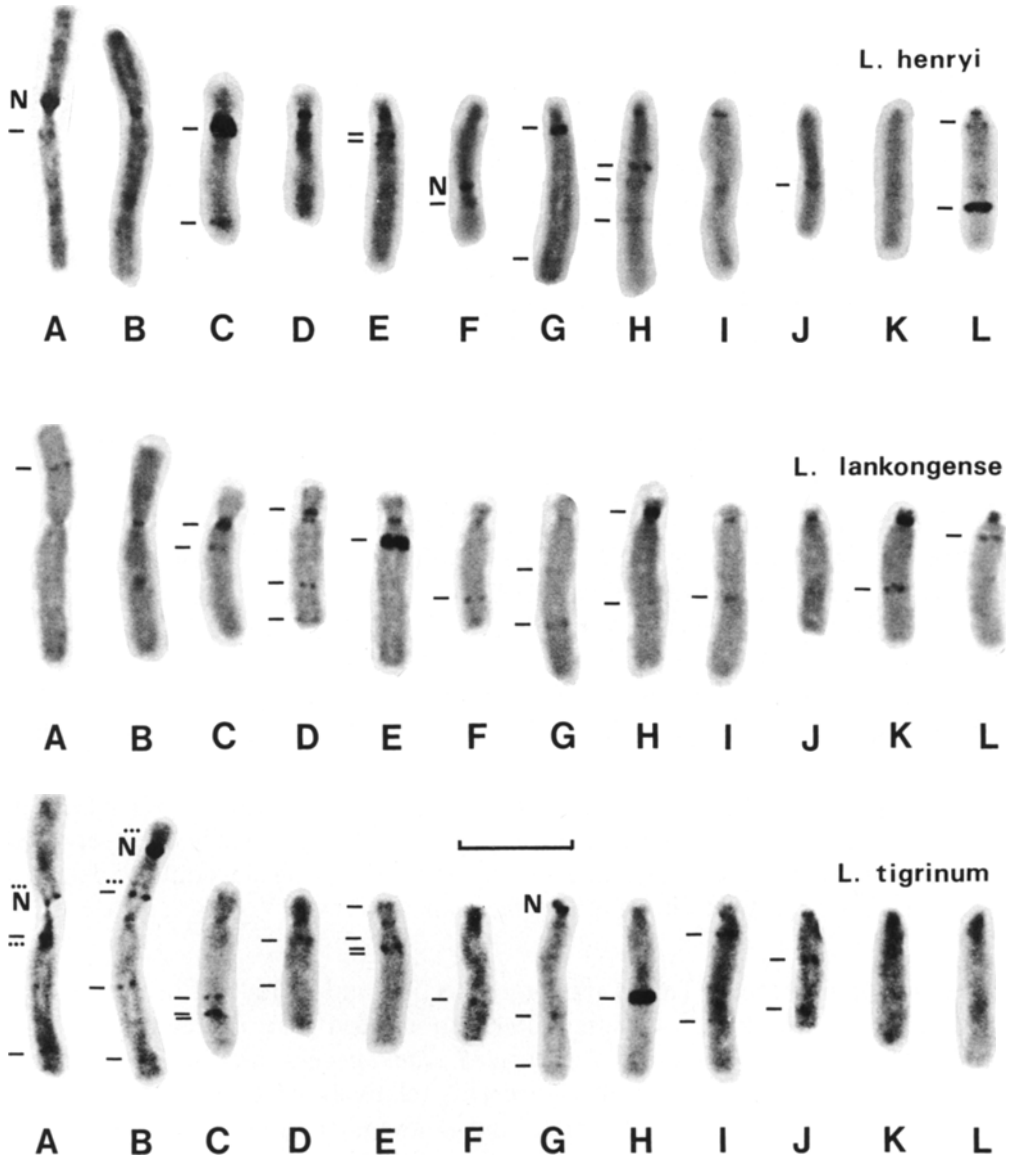


Fig. 6. C-banded karyotype of three species from group a of sect. *Sinomartagon* (*L. tigrinum* prepared by Dr C. CORRICK). Bar: 10 μm

NORs (STEWART 1947), the three in *L. amabile* are relatively heavily C-banded, while of the five in *L. concolor,* those on chromosomes A, B, and G (the latter recorded as chromosome I by STEWART), were thicker than those on F and K.

COMBER (1949) conceded that his final group c contains a wide range of species which may require further subdivision. The two, very different species we surveyed, *L. nepalense* and *L. wardii*, have markedly different patterns (Fig. 8). *L. nepalense* is relatively heavily banded, especially on the short arms of A and B and the long arms of F and G. (NORs have not been identified in this species although LIGHTY 1960 reported two secondary constrictions, on the short arms of B and C.) By contrast, *L. wardii* has many fewer intercalary bands although three (on C, F, and

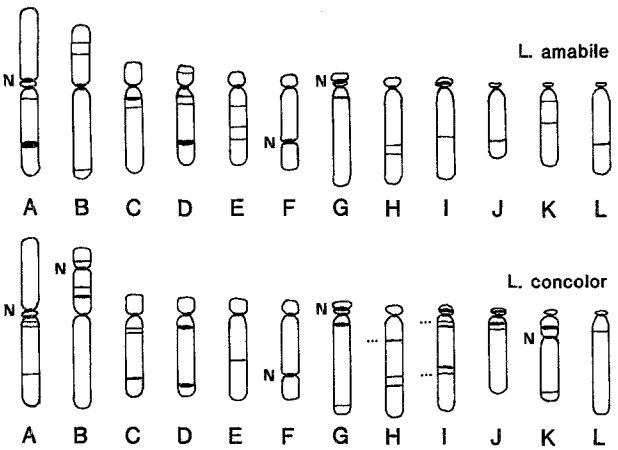


Fig. 7. C-banded idiograms of two species from group b of sect. *Sinomartagon* (*L. concolor* prepared by Dr J. JOSEPH)

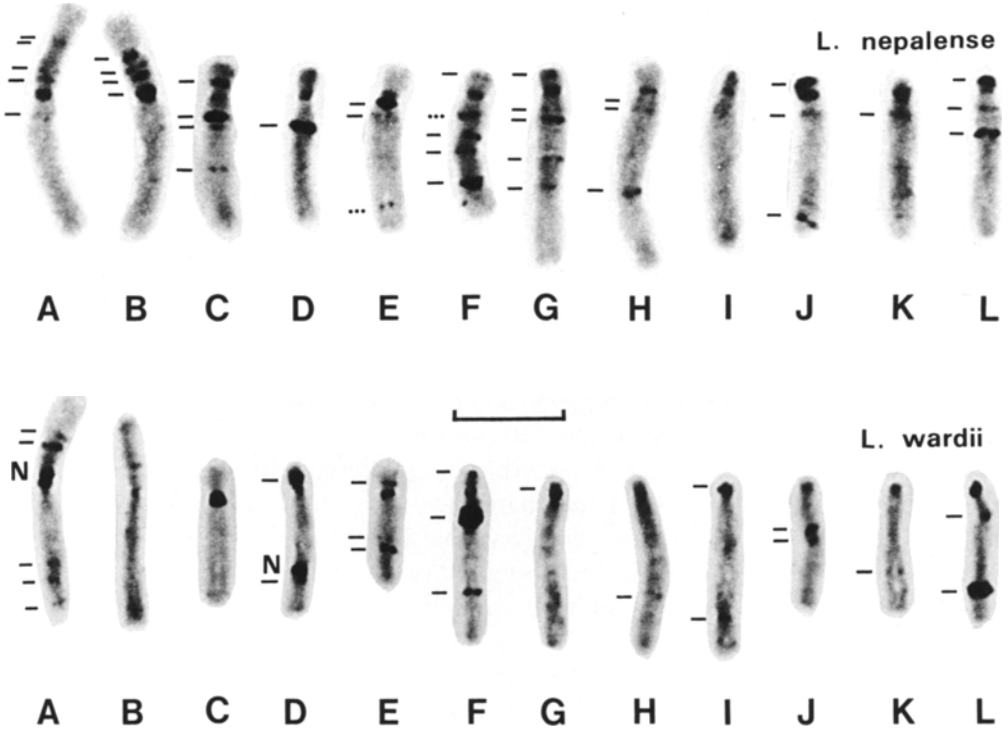


Fig. 8. C-banded karyotypes of two species from group c of sect. *Sinomartagon*. Bar: 10 µm

L) are very large (Fig. 8). The NORs on A and D (STEWART 1947) are associated with medium-sized C-bands in this species.

**Sect. 6: *Leucolirion.*** This section contains the trumpet lilies which fall into two, well-circumscribed groups. In COMBER's (1949) group a, we examined two species, *L. regale* and *L. sulphureum* (*L. myriophyllum* FRANCHET). Banding patterns of the two species are very similar (Fig. 9). In *L. regale* there is a prominent intercalary band on chromosome L and other scattered, less intense bands. One medium intercalary band, the more proximal of the two located distally on chromosome

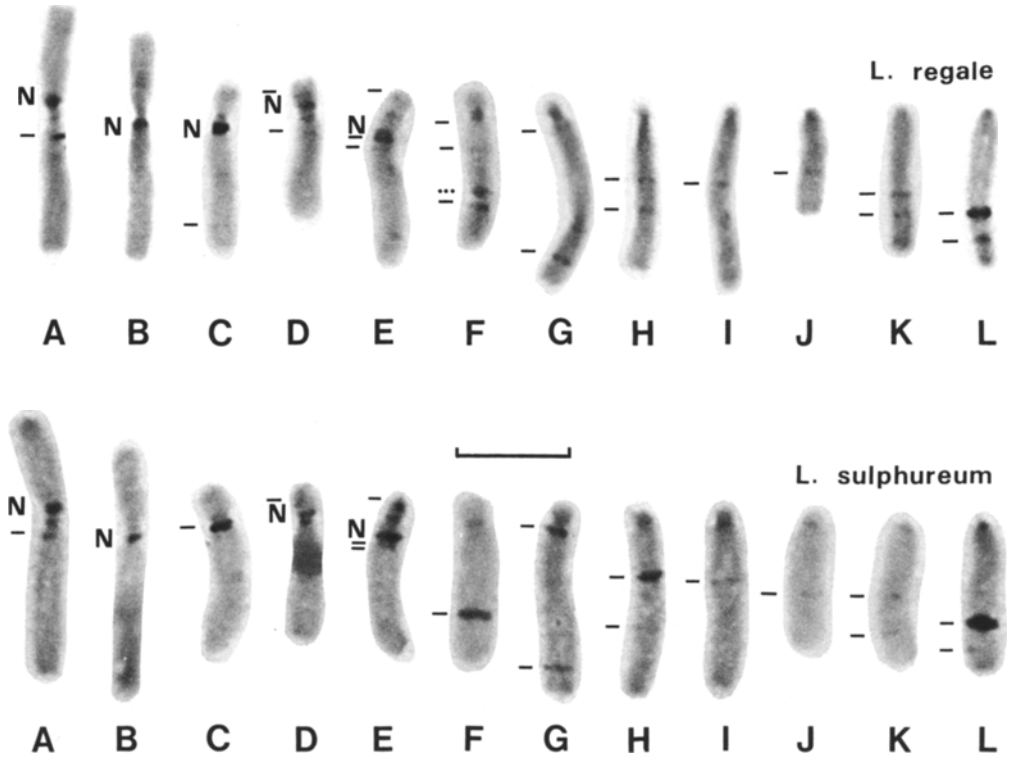


Fig. 9. C-banded karyotypes of two species from group a of sect. *Leucolirion*. Bar: 10 μm

F, was seen on only one homologue of one bulb of the two in the ALS accession (Table 1). It was not detected in the VBB strain.

In *L. sulphureum* most of the *L. regale* bands are present although not necessarily of the same thickness (Fig. 9). A new band was seen on each of chromosomes H and K more proximal than thin bands visible in the same vicinity in *L. regale* chromosomes. Only one of the four intercalary bands on chromosome F of *L. regale* was seen in its homologue in *L. sulphureum,* the large, most distal of the bands.

STEWART (1947) reported five NORs in both these species, close to centromeres in the short arms of chromosomes A and D and in the long arms of B, C, and E (using our numbering system in which we have interchanged STEWART's C and D chromosomes). However, the site near the centromere in chromosome C, although C-banded like that of the other NORs, was not nucleolar organizing in our accession of *L. sulphureum* (Fig. 9). In situ hybridization with $^{3}$H-rRNA showed no detectable genes in this region although the other four NORs were heavily labelled (results not shown). The *L. regale* plants were not tested.

We also examined two species from COMBER's (1949) group b of this section – *L. formosanum* and *L. longiflorum* (Fig. 10). C-bands in the latter species have already been described (HOLM 1976, KONGSUWAN & SMYTH 1978) and the pattern on *L. formosanum* is very similar (Fig. 10). The only differences involved three intercalary C-bands on chromosomes G, I, and L of *L. longiflorum* which were not seen in our plants of *L. formosanum*.

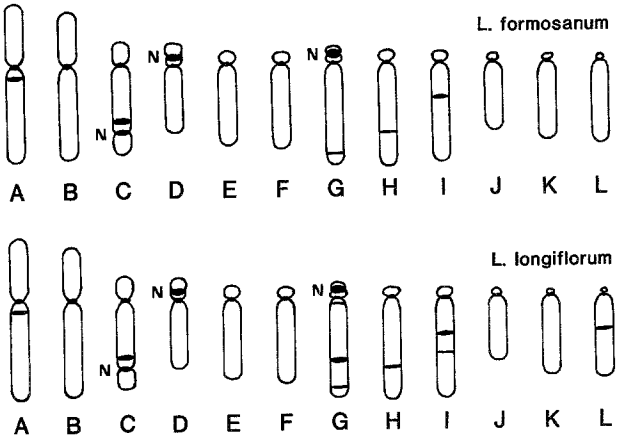


Fig. 10. C-banded idiograms of two species from group b of sect. *Leucolirion*

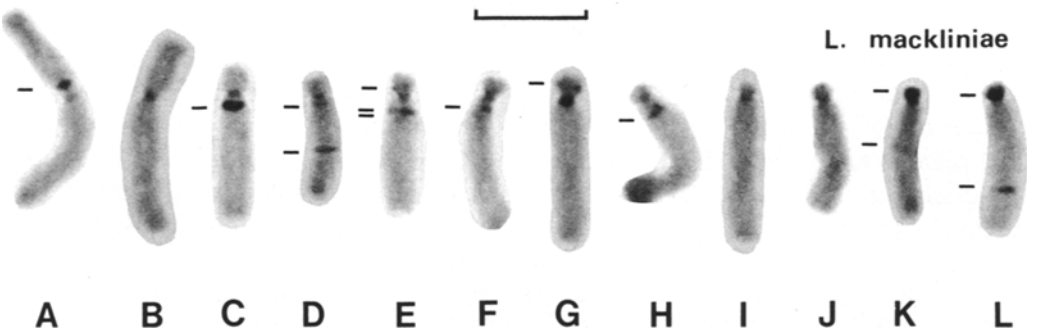


Fig. 11. C-banded karyotype of *L. mackliniae*, a species not yet assigned to a section. Bar: 10 μm

**Unclassified.** *L. mackliniae* was first described about the same time as COMBER's (1949) classification appeared and was not formally placed in a section. Its C-banded karyotype, which reveals relatively few bands, is shown in Fig. 11. Nine of the 11 intercalary bands are located close to the centromeres on one or both sides. NOR numbers and locations have not previously been reported for *L. mackliniae*. We observed a maximum of four nucleoli per diploid interphase nucleus and, like LIGHTY (1960), saw two secondary constrictions, one proximal in the short arm of chromosome A and the other medial in the long arm of D. These are also C-banded (Fig. 11) and are likely to be the NORs of *L. mackliniae*. All C-bands of the one plant examined were homomorphic.

## Discussion

**C-bands and the classification of *Lilium* species.** The most striking generalization which can be made about *Lilium* C-band patterns is their great inter-species diversity. Clearly C-bands have evolved relatively rapidly in size, number and location. That being so, the closely similar pattern obtained for *L. regale* and *L. sulphureum* on the one hand (Fig. 9) and for *L. formosanum* and *L. longiflorum* on the other (Fig. 10) implies close relationships within these pairs. All four fall into COMBER's (1949) sect. *Leucolirion*. The first two are in COMBER's group a, and all four species

in this group [which includes *L. leucanthum* (BAKER) BAKER and *L. sargentiae* WILSON] are all closely similar in appearance (WOODCOCK & STEARN 1950, SYNGE 1980). All four have indistinguishable unbanded karyotypes (STEWART 1947), and all can be intercrossed readily to produce fertile hybrids (WOODCOCK & STEARN 1950, LIGHTY 1960). *L. formosanum* and *L. longiflorum* (in COMBER's group b) are also morphologically similar and produce fertile hybrids (WOODCOCK & STEARN 1950, SYNGE 1980). However, the two groups are not closely related, at least as judged by the lack of success with intercrosses, even using cut style and embryo culture techniques (MYODO & ASANO 1977). Their C-banding patterns are also very dissimilar (Figs. 9, 10).

Within COMBER's other sections, some C-banding similarities are probable between *L. hansonii* and *L. martagon* within sect. *Martagon* (Fig. 1). The C chromosomes have similar patterns which include NORs. Also E and I chromosomes of *L. hansonii* resemble F and H, respectively, of *L. martagon*. The homoeologous A and B chromosomes also share some C-band locations between species. These two species have been intercrossed on many occasions (WOODCOCK & STEARN 1950) and the resulting hybrids are fertile, with almost complete synapsis of homoeologues at meiosis (BROCK 1954). Even so, chiasma frequencies are reduced, and bridges and fragments, resulting from inversion differences between the genomes, are seen at anaphase I (RICHARDSON 1936). There appear to be at least six small inverted regions, present in each of the metacentric homoeologues A and B as well as in the smaller acrocentric chromosomes. C-bands have not provided sufficiently stable or detailed landmarks to recognize these in somatic chromosomes.

The other species examined from sect. *Martagon, L. tsingtauense,* may also show a few possible C-band similarities (Fig. 1). Its chromosome C has a large intercalary C-band and a NOR equivalent to those of *L. hansonii* and *L. martagon* C chromosomes, its G has a similar pattern to that of *L. martagon* and the J resembles one of the Js in *L. hansonii*. It is interesting that *L. tsingtauense* has been reportedly crossed with the *L. martagon* × *L. hansonii* hybrid, *L.* × *dalhansonii* POWELL, to produce fertile offspring (ROBINSON 1975).

Within sect. *Pseudomartagon,* the patterns obtained for the two N. American species are markedly different (Fig. 3). *L. pardalinum* is a Western species (which all fall into COMBER's groups a and b) while *L. canadense* is Eastern (groups c and d). It is clear that these two assemblages within sect. *Pseudomartagon* are only distantly related. Many interspecies hybrids have been raised between Western species and between Eastern species but none reported between them (WOODCOCK & STEARN 1950, LIGHTY 1960, SYNGE 1980).

*L. candidum*, the sole member of sect. *Lilium* banded, has a unique pattern (Fig. 4). It is clearly different from all other species examined, including those from sect. *Martagon* (Fig. 1) which COMBER (1949) suggests is the most closely related section.

Fertile hybrids are readily obtained between the two Oriental species examined from sect. *Archelirion, L. auratum* and *L. speciosum* (WOODCOCK & STEARN 1950, LIGHTY 1960, SYNGE 1980). C-band patterns here are not close (Fig. 5). The homoeologous K chromosomes are indistinguishable, but prominent intercalary bands on the other chromosomes are species specific.

Within COMBER's sect. *Sinomartagon*, C-band patterns are extremely diverse. COMBER (1949) placed *L. henryi* in this section, although he realized it was "a

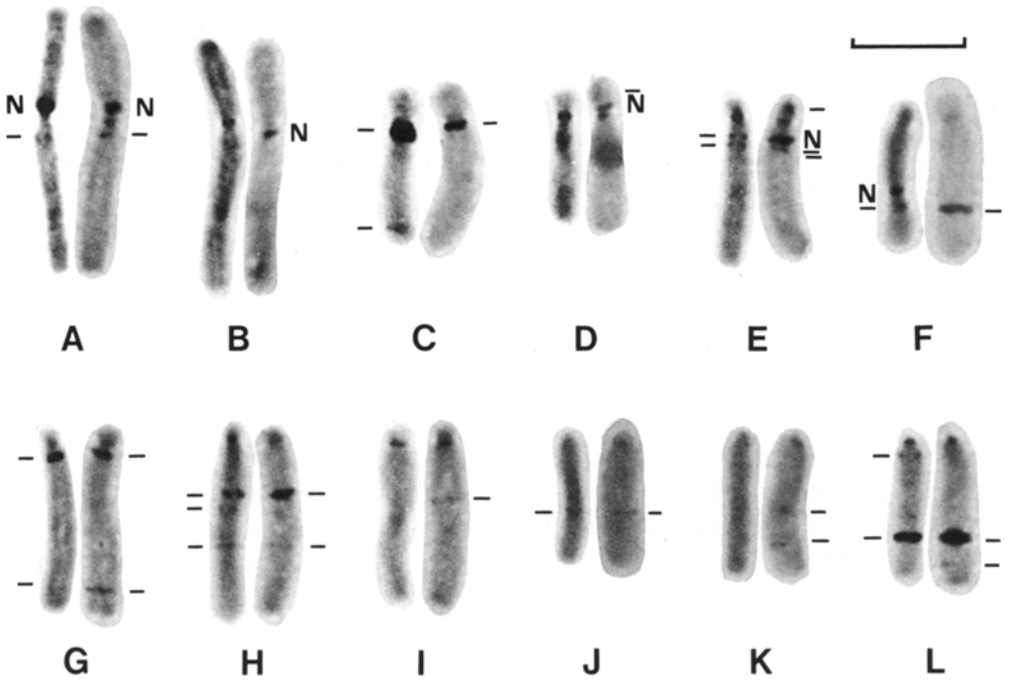


Fig. 12. Individual C-banded chromosomes of *L. henryi* (left) and *L. sulphureum* (right) showing close equivalence of C-band locations in the two species. Bar: 10 μm

somewhat anomalous species". Upon careful comparison of its C-bands (Fig. 6) with other species we could find no similar pattern in this section (Figs. 6–8). However, we did discover striking similarities with patterns in *L. regale* and *L. sulphureum* (e.g., Fig. 12) of sect. *Leucolirion*. This is surprising as at first sight there is little morphological resemblance between *L. henryi* and these species. *L. henryi* has orange, reflexed flowers with prominent papillae and pubescent nectaries whereas *L. regale* and *L. sulphureum* (and their close relatives *L. leucanthum* and *L. sargentiae*) have predominantly white, trumpet-shaped flowers, few obtuse papillae and smooth nectaries (SYNGE 1980). However, all species share epigeal germination, scattered leaves, non-jointed bulb-scales, heavy seeds (in *L. henryi* and *L. regale*) and coloured bulbs. [*L. henryi* is alone in having heavy seeds and deeply coloured bulbs if placed in sect. *Sinomartagon* (COMBER 1949).] Even so, the marked morphological differences make it surprising that C-band patterns are so close, given that banding patterns can differ considerably between species known to be closely related (e.g., *L. auratum* and *L. speciosum*) (Fig. 5).

It is possible that similar C-band patterns have arisen fortuitously through convergent evolution (GREILHUBER 1984). However, results from interspecific hybridizations also strongly support a close relationship between *L. henryi* and these four trumpet species. One of the first successful crosses made was with *L. sargentiae* to produce *L.* × *aurelianense* DEBRAS (WOODCOCK & STEARN 1950). This, and other crosses of *L. henryi* with *L. leucanthum, L. regale,* and *L. sulphureum,* have yielded vigourous, horticulturally important hybrids whose fertility has allowed much further intercrossing and back crossing (LIGHTY 1960, SYNGE 1980). By contrast, no verified crosses between *L. henryi* and species in sect. *Sinomartagon* (where COMBER

placed it) seem to have been recorded (LIGHTY 1960). Also those few crosses between *L. henryi* and either *L. auratum* or *L. speciosum* (in sect. *Archelirion*) which have been made have only succeeded with great difficulty and have produced sterile progeny. Chromosome paring at meiosis is much reduced, with no more than four bivalents occurring per meiocyte (ASANO 1983). LIGHTY (1960) proposed moving *L. henryi* to sect. *Archelirion* on unspecified morphological grounds. However, based mainly on hybridization results and C-band evidence we consider it fits better into sect. *Leucolirion* (with *L. regale* and its relatives in group a) and formally propose its transfer. The morphological differences, while considerable (see above), are not so great as to preclude such a transfer.

Turning to the remaining species in sect. *Sinomartagon,* C-banding does not throw much light on their subdivision by COMBER (1949) into group a (papilliferous perianth, pubescent nectary, white bulb, winged seed), group b (small bulb, wingless seed) and group c (smooth perianth, glabrous nectary, more or less coloured bulb, winged seed). The other two banded species remaining in group a, *L. lankongense* and *L. tigrinum* (Fig. 6), have very little C-band similarity. Rather, *L. tigrinum* has some possible matches with *L. concolor* in group b (Fig. 7), especially the homoeologues A, B, G, and I. *L. concolor* is a somewhat specialized species which does not readily cross with other group a species (MARSHALL 1983 a). By contrast the other group b species banded, *L. amabile* (Fig. 7), intercrosses readily (MARSHALL 1981) with *L. tigrinum* even though its C-banding pattern predicts a more distant relationship.

It would be of interest to C-band the chromosomes of *L. bulbiferum* L. and *L. dauricum* KER-GAWLER (*L. pensylvanicum* KER-GAWLER) which also intercross with many of COMBER's (1949) group a species of sect. *Sinomartagon* (SYNGE 1980, MARSHALL 1981). COMBER (1949) placed the former species in sect. *Lilium* and created a new section, *Daurolirion,* for the latter. However, both fit readily in group a of his sect. *Sinomartagon* on morphological grounds if less importance is attached to their hypogeal germination (epigeal in *Sinomartagon*) and the jointed bulb scales of *L. dauricum* (entire in *Sinomartagon*). Following LIGHTY (1960), we support their transfer to sect. *Sionomartagon.*

The C-band patterns of *L. nepalense* and *L. wardii* (Fig. 8) in COMBER's (1949) group c of *Sinomartagon* are very dissimilar. *L. wardii* has some morphological similarities with *L. lankongense* but C-band patterns do not support a close relationship (Figs. 6 and 8). Neither species intercrosses with any other readily (MARSHALL 1983 b) although *L. lankongense* has been hybridized with *L. davidii* DUCHARTRE (also in group a of sect. *Sinomartagon*) using embryo culture (NORTH & WILLS 1969). *L. nepalense* has a C-band pattern (Fig. 8) so unlike any other reported so far that its situation must await banding of additional possible relatives such as *L. primulinum* BAKER and *L. bakerianum* COLLET & HEMSLEY.

Finally, the position of *L. mackliniae* can be examined. The discovery of this species in 1946 forced a reconsideration of the generic limits of *Lilium.* While it is similar in appearance to species in the related genus *Nomocharis* FRANCHET, *L. mackliniae* has as its closest relatives *L. amoenum* SEALY, *L. henrici* FRANCHET and perhaps *L. sempervivoideum* LÉVEILLÉ (SEALY 1950). COMBER (1949) placed this last species in group c of sect. *Sinomartagon,* but he conceded that it, along with its relatives, "may well constitute a separate sub-section". The C-band pattern of *L. mackliniae* (Fig. 11) is not helpful in this regard in that it does not seem to match

any species in sect. *Sinomartagon* (Figs. 6 – 8) or, indeed, any other species banded. *L. mackliniae* and its relatives may well require the establishment of a new section. Alternatively, further study may suggest that they, along with *L. georgei* (W. E. EVANS) SEALY and *L. souliei* (FRANCHET) SEALY, be added to the four other species already in sect. *Oxypetala* (BALFOUR f.) SEALY, transferred from *Nomocharis* to *Lilium* by SEALY (1950).

## Significance of C-bands

The C-banding "style" (GREILHUBER & SPETA 1976, SCHWEIZER & EHRENDORFER 1976) of *Lilium* spp. across all sections is exemplified by a scattering of relatively thin intercalary bands. C-bands also occur at centromeric regions, but except for a few cases where large blocks adjoin the constriction on one side (e.g., in *L. candidum* and *L. wardii)* (Figs. 4 and 8), they are relatively thin. Perhaps the thickest centric C-bands were seen in *L. pardalinum* (Fig. 3) (KONGSUWAN & SMYTH 1977).

NORs were also universally C-banded in *Lilium,* and the possibility that NOR heterochromatin includes a reservoir of ribosomal RNA genes has been examined elsewhere (VON KALM & SMYTH 1984). It is likely that centric and nucleolar C-bands have a different structural basis from other C-bands in that these classes respond differentially to C-banding procedures (KONGSUWAN & SMYTH 1977).

It is interesting that intercalary C-bands of most species do not seem to show an equilocal distribution (i.e., occurring at similar locations on most non-homologous chromosomes) (GREILHUBER 1984). Such a pattern has been reported, for example, in groups of species within the *Tulipa* subg. *Leiostemones* (BLAKEY & VOSA 1982), from sect. *Codonoprasum* of *Allium* (VOSA 1976 b, LOIDL 1983), from the *Scilla hohenackeri* group (GREILHUBER & SPETA 1976) and the *Scilla siberica* group (GREILHUBER & SPETA 1978) and probably in some American species at least of *Fritillaria* (LA COUR 1978). The only clear case in *Lilium* is in *L. canadense* (Fig. 3), where thick intercalary bands lie close to the centromeres on nearly all the longer arms. (*L. mackliniae*, Fig. 11, may show a similar pattern although bands are fewer and thinner.) LOIDL (1983) has suggested that equilocal heterochromatin of this type could arise by transfer between chromosome arms held in a relict anaphase pattern during interphase (i.e., in a RABL orientation). The regularly distributed C-bands of *L. canadense*, as in the other cases cited, tend to be relatively large. LOIDL's (1983) proposal therefore may well be valid but relevant only to the sporadic additions of such large blocks on a background of smaller, more dispersed intercalary bands.

Very few *Lilium* chromosomes have telomeric C-bands. Some are present on the short arms of acrocentrics of *L. canadense* (Fig. 3), *L. candidum* (Fig. 4), and *L. nepalense* (Fig. 8), all in different sections. Even so this paucity is somewhat surprising given that telomeres are often C-band "initiation sites" (SCHWEIZER & EHRENDORFER 1983). Also they do seem to have arisen more frequently in the closely related genus *Fritillaria* (LA COUR 1978).

The significance of C-bands remains obscure. In *Lilium,* as in other cases, their variable size, number and location across species make it unlikely that they have a role in determining overall phenotype. Rather they are likely to be involved in genomic architecture and to influence nuclear and cellular properties of the plant. *Lilium* chromosomes are relatively large (STEWART 1947) but C-band DNA can

contribute only minor amounts to this bulk, at least in the 20 species surveyed here. It has been suggested that one specific function of C-band DNA is to regulate recombination (JOHN & MIKLOS 1979). To test this it may be of interest to undertake a comparative study of chiasma distributions in the large bivalents of *Lilium* spp.

In conclusion, this survey has shown that C-band patterns can be useful in deducing relationships between *Lilium* spp. although their rapid rate of change limits this to closely related species. This great variation, however, does mean that C-bands will be useful markers in tracing the ancestry of interspecies hybrids of horticultural importance (SON 1977, SMYTH & KONGSUWAN 1980).

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