THE CONSTITUTIVE HETEROCHROMATIN IN CHROMOSOMES OF *FRITILLARIA* SP., AS REVEALED BY GIEMSA BANDING

By L. F. LA COUR, F.R.S.

School of Biological Sciences, University of East Anglia, Norwich NR4 7TJ, U.K.

(*Received* 23 *May* 1978)

[Plates 1–6]

The incidence of C-bands (constitutive heterochromatin), as determined by differential Giemsa staining, was studied in the chromosomes of 56 species, varietal forms and subgenera of *Fritillaria* and 30 of them are illustrated. With the exception of the subgenera *Korolkowi*, a supposed link between lilies and fritillaries, the chromosome complements of all plants contained bands. There were wide differences in the size and number of these bands among species both within and between groups. In those with the largest and most abundant bands, there was a pronounced tendency for centromeric localization, both in Old and New World species. The Giemsa-positive centromeres were masked when this occurred. Heteromorphy in respect of banding occurred in most species. The relation of repetitive DNA sequences with heterochromatin is discussed, as is also the problem of evolution in *Fritillaria*.

Introduction

Fritillaria pudica, was one of the first plants in which heterochromatic regions (H-segments) were seen and mapped at metaphase in mitotic chromosomes (Darlington & La Cour 1941). In contrast to the euchromatic parts, they appeared as weakly stained regions in Feulgen squashes of pollen and roots taken from plants grown at 0–3 °C. They were not seen in such preparations of material taken from plants grown at more equable temperatures.

Later studies of the roots of *F. lanceolata* (La Cour & Wells 1974) showed that, in thin sections stained with toluidine blue, the H-segments alone were weakly stained at all stages of the mitotic cycle, irrespective of the temperature at which the plants were grown. Further to this, the staining behaviour was matched by the image seen with the electron microscope, in that the H-segments were at all times less opaque to electrons than euchromatin. This differential behaviour was shown to be due to less intense condensation (coiling) in the H-segments, a criterion not always applicable in other plants (e.g. in *Scilla sibirica*, as shown by the same study).

A new avenue of enquiry has now been opened up by the finding that after differential Giemsa staining the position of C-bands observed at metaphase in chromosomes of *F. lanceolata* (Schweizer 1973) correspond exactly with those of the weakly Feulgen stained H-segments seen in roots of this plant after cold treatment (La Cour 1951).

The sensitivity of the C-banding technique has led to a renewal of interest in the problem as to whether constitutive heterochromatin (H-segments) is absent from some fritillaries. An

earlier study using cold treatment suggested this possibility (La Cour 1951). It was also thought that the incidence of C-bands might throw some light on evolution and speciation in a genus in which polyploidy (other than triploidy) and interspecific hybridization is virtually non-existent.

The genus shows great diversity in form and can be usefully divided into subgenera (Rix 1975). It was therefore also of interest to see whether such a division was warranted by C-banding alone. A list of *Fritillaria* species and subgenera examined (classified according to Rix) is given in the body of the paper.

Materials and methods

The material used was from a collection *Fritillaria* species growing at the John Innes Institute, Norwich, amassed during the period when I was a member of staff.

Roots from potted plants were pretreated with colchicine (0.05 % aqueous solution) for 3–4 h at room temperature, fixed in absolute ethanol/glacial acetic acid (3 : 1) and stored overnight at 2–4 °C.

The Giemsa banding method described by Darlington & La Cour (1976) was employed.

Observations

1. *General morphology*

Table 1 lists the species of *Fritillaria* and subgenera examined for C-banding. Almost all of these (all except 3) have a basic haploid chromosome number of $n = 12$. The exceptions are *F. ruthenica* (probably identical to *F. tenella*) with a basic number of 9 and *F. pudica* and *F. glauca* with $n = 13$. These variations have most probably arisen by fusion and fragmentation of chromosomes, as indicated by their karyotypes (Darlington 1937). The complement of those with $2n = 24$ is composed of two pairs of M-chromosomes (metacentric) and 10 pairs of ST-chromosomes (subterminal); those with $2n = 18$ have five pairs of M's and 4 pairs of ST's and those with $2n = 26$, one M-pair and 12 pairs of ST's.

In conventional cytological preparations, the nucleolar chromosomes of some fritillaries are recognizable by the presence of secondary constrictions (Darlington 1937) which are situated in the euchromatin (La Cour 1951). These constrictions were not visible in the Giemsa preparations. The nucleolar chromosomes could therefore be recognized only in those species where the organizer region was indicated by a heterochromatic trabant.

2. *The C-bands*

(*a*) *General*

All of the species listed in table 1 were found to have these bands in their chromosome complements, some much more than others. It was not possible, however, to obtain from all of them complete metaphase plates that were free of cell wall and suitable for microphotography or complete analysis. In spite of this some indication of the amount and location of banding for most species will be given.

In order to avoid any ambiguity in respect of bands (heterochromatin) situated near the centromere, the common practice of describing such a location as 'proximal' has been avoided in the present paper. This is because in *Fritillaria*, as recently found (La Cour 1978*a*), heterochromatin is sometimes situated contiguous with the centric region (constriction), at one or

TABLE 1. LIST OF SPECIES AND SUBGENERA OF *FRITILLARIA* STUDIED FOR C-BANDING

Old World species	distribution
F. *caucasica* group	
alfredae Post	Lebanon
pinardi	NW Turkey
armena Boiss	NE Turkey
assyriaca Baker	Turkey, Iran
bithynica Baker	W Turkey
carduchorum Rix	SE Turkey
conica Boiss	SW Greece
forbesii Baker	SW Turkey
glaucoviridis Turrill	S Turkey
rhodia A. Hansen	Rhodes
stribrnyi	Bulgaria, Turkey in Europe
F. *graeca* group	
graeca purple netted form	Greece
graeca thessala	Balkans
graeca guicciardii	Attica
acmopetala Boiss	E Mediterranean
hermontis amana Rix	Mt Hermon
lusitanica	Spain, Portugal
tenella	SE France
crassifolia	NW and SW Turkey
messanensis (= *gracilis*)	Dalmatian coast, N Africa
olivieri Baker	W Iran
pontica	Greece, Bulgaria, Turkey
reuteri	W Iran
rhodocanakis	Hydra
pyrenaica	Pyrenees, NW Spain
straussii	Iran, Turkey
tuntasia	Kythnos
michailovskyi	NE Turkey
F. *meleagris* group	
aurea Schott	Turkey
delphinensis	SW Alps
latifolia	Caucasus, N Turkey
meleagris	Europe
pallidiflora Schrenk	Central Asia
F. *cirrhosa* group	
roylei	Himalaya
verticillata	China, Central Asia
subgenus *Theresia*	
persica	Iran
subgenus *Rhinopetalum*	
bucharica	Afghanistan–Turkestan
stenanthera Regel	Turkestan
subgenus *Petilium*	
imperialis	Turkey to Kashmir
raddeana	Turkestan
subgenus *Korolkowia*	
sewerzowii Regel	Central Asia
New World species	
subgenus *Liliarhiza*	
brandegii	California
lanceolata Pursh	California
falcata (Jepson) Beetle	California
recurva	California
biflora	California
glauca yellow form	California
liliacea	California
pudica	California
phaenanthera Purdey	California
purdyi Eastwood	California
roderickii W. Knight	California
viridia Kellog	California
pinetorum Davidson	California
camschatcensis	Alaska, Kamschatka, Japan

both sides, and occasionally within the constriction immediately adjacent to the centromere. In both situations the centromere itself, represented by a pair of Giemsa-positive dots (Eiberg 1974; Marks 1977), is then masked (La Cour 1978*b*).

(*b*) F. caucasica *group*

The bands of 8 members of the *F. caucasica* group are illustrated in figures 1–8. Of these, more bands were revealed in the chromosomes of *F. alfredae* (figure 1, plate 1) than of any other Old World species I have examined. At the other extreme, the complement of *F. conica* (figure 8, plate 2) had less bands than those of other members of the group. There was some variability among the 8 species of this group in respect of location of bands. They were predominantly intercalary in *alfredae* (figure 1) and *F. assyriaca* (figure 5, plate 2). In *F. bithynica* (figure 3) and *F. pinardii* (figure 4) the most prominent ones were terminal. In *F. glauca viridis* (figure 7) large bands are found in both intercalary and terminal positions.

Heteromorphy, in respect of the bands, was evident in two members of the group. In *F. carduchorum* (figure 2) heteromorphy is shown in one pair of M-chromosomes by the absence of a band in an arm of one of them, and was also seen in a pair of nucleolar chromosomes where a terminal band was present in only one of the chromosome long arms. In *F. forbesii* (figure 6), heteromorphy was apparent in one of the two pairs of M-chromosomes and was denoted by the presence of prominent distal bands in both arms of a single M-chromosome.

Because of close proximity of heterochromatin to the centric constriction, the pairs of centromeric dots were not always visible. Thus in *alfredae* (figure 1) only one chromosome showed them and in those of *carduchorum* (figure 2) only two. The centromeres can also be orientated so that only one of the two dots is in the plane of observation, as illustrated in figures 4 and 6. Relatively weaker Giemsa staining was most probably responsible for the centromeres not being visible in the chromosomes of *conica* (figure 8).

There were clearly differences within the group concerning the nucleolar chromosomes. In both *carduchorum* (figure 2) and *conica* (figure 8) pairs of such chromosomes were identified by the presence of thick heterochromatic trabants and in a pair of *pinardii* chromosomes (figure 4) by a tiny trabant emanating from a prominent band. They were not distinguishable in *alfredae*, *bithynica*, *assyriaca* and *forbesii*.

Triploid forms, usually of more robust stature, exist in a number of *Fritillaria* species. The bands in chromosomes of a triploid form of *assyriaca* ($2n = 36$) are shown in figure 5.

In addition to those members of the *F. caucasica* group in which bands are presently illustrated, bands were also seen in all the chromosomes of *F. rhodia*, some being terminal and some intercalary. There were relatively few bands in the chromosomes of *F. armena*, the most prominent ones being terminal. The nucleolar chromosomes were not recognized in either species.

(*c*) F. graeca *group*

The bands of 11 members of the *F. graeca* group are shown in figures 9–19. They were particularly numerous in *F. rhodocanakis* (figure 18), the chromosomes of which contained more than 100 bands. This species was further notable for a preponderance of larger bands situated next to and within the centric constriction, a feature displayed to less extent in some chromosomes of *F. hermontis amana* (figure 9), *F. lusitanica* (figure 11, plate 3), *F. involucrata* (figure 14) and *F. michailovskyi* (figure 15). The chromosomes of *F. straussii* (figure 16) contained

Figures 1–16 show metaphases from root tips of *Fritillaria*, differentially stained with Giemsa to show C-bands.

FIGURE 1. *F. alfredae* a fritillary with more bands than any other in the Old World; many of them are intercalary. (Magn. × 2500.)

FIGURE 2. *F. carductorum*, the nucleolar pair (N) show heteromorphy. (Magn. × 1650.)

FIGURE 3. *F. bithynica*. (Magn. × 1650.)

FIGURE 4. *F. pinardii*. (Magn. × 1650.)

(*Facing p.* 64)

Description of plate 2

Figure 5. A triploid form of *F. assyriaca*. (Magn. × 1650.)

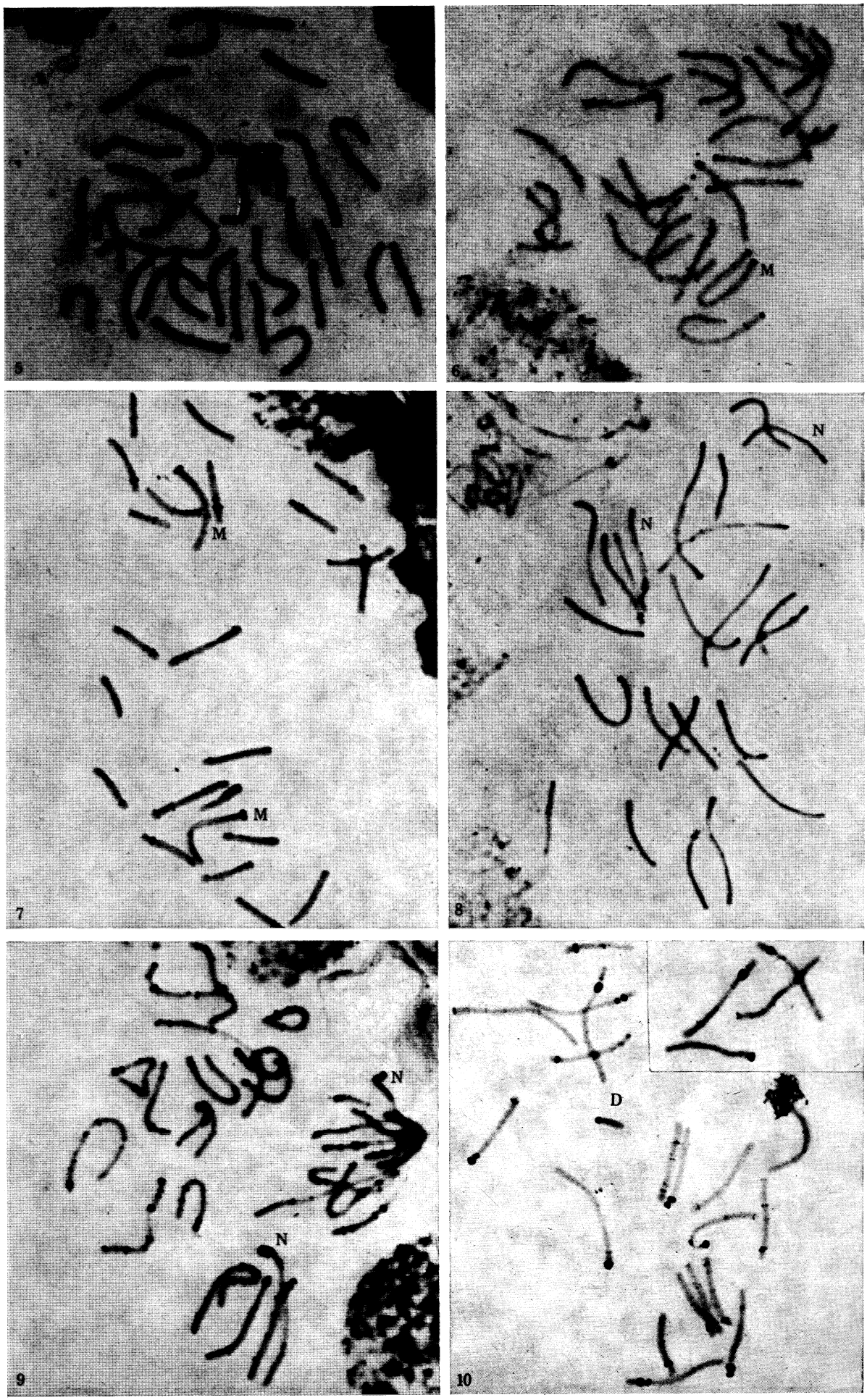
Figure 6. *F. forbesii*. One M-chromosome (M) has distal bands in both arms. (Magn. × 1650.)

Figure 7. *F. glauca viridis*. Two M-chromosomes (M) have prominent distal bands. (Magn. × 1650.)

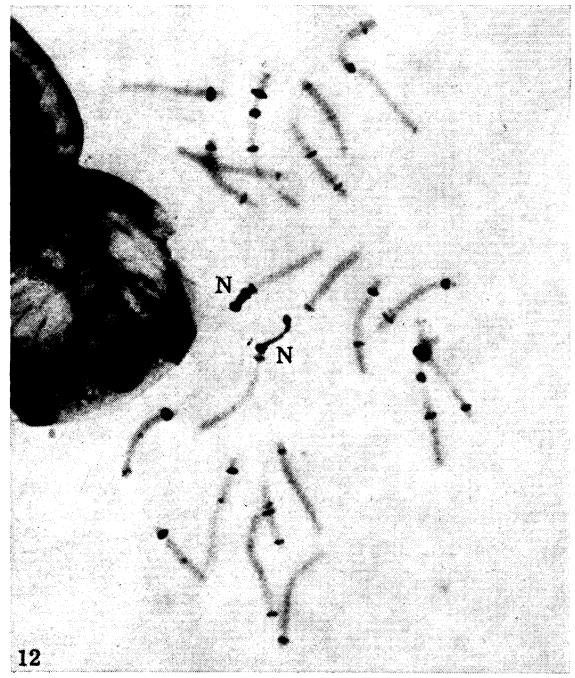
Figure 8. *F. conica*. The two nucleolar chromosomes (N) are recognizable. (Magn. × 1650.)

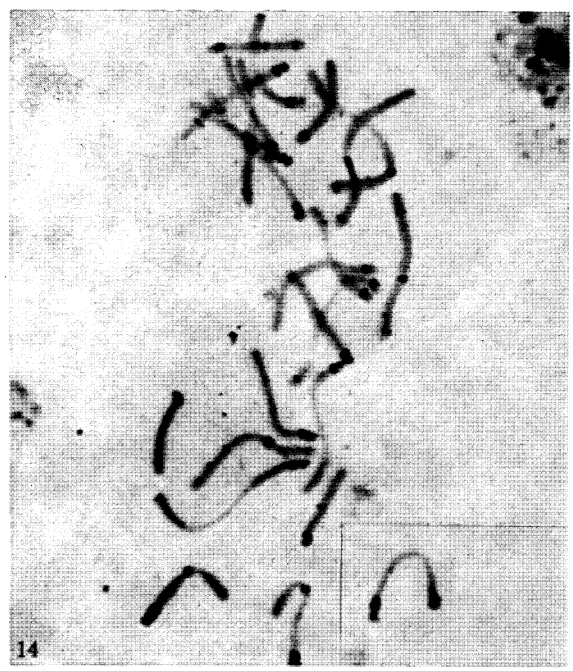
Figure 9. *F. hermonis amana*. The nucleolar chromosomes (N) are recognizable. (Magn. × 1650.)

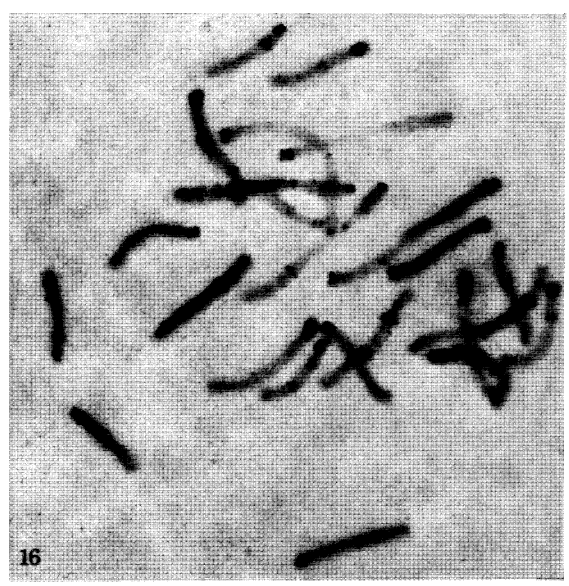
Figure 10. A purple netted form of *F. graeca*, with an ST-chromosome showing a deleted long arm (D). (Magn. × 1350.)



FIGURES 5–10. For description see opposite.







FIGURES 11–16. For description see opposite.

an assortment of bands, many being minute. The chromosomes of the purple netted form of *F. graeca* (figure 10), *F. messanensis* (figure 12) and *F. reuteri* (figure 17, plate 4) showed less bands than any others in the group. The short arms of some ST-chromosomes of the first species were entirely heterochromatic and one of this type was remarkable in that it had a deleted long arm.

Some ST-chromosomes with heterochromatic short arms were also observed in *F. gracilis* (figure 13) and *F. acmopetala* (figure 19). In the latter figure, which was partly included to show the centromeric dots at high magnification, a fine band can be seen intercalated between the centromere and a large distal band in the short arm of two other ST-chromosomes. Heteromorphy was also evident in this pair and was shown by the presence of a distal band in the long arm of only one of the pairs.

The nucleolar chromosomes could not be identified in all species of the group. They were identifiable, however, in *messanensis* (figure 12), *reuteri* (figure 17) and *rhodocanakis* (figure 18), and in all three the organizing region of these chromosomes was heterochromatic. Notably, heteromorphy was apparent at this region between pairs in all three species. In *reuteri* and *rhodocanakis* the heterozygosity was remarkable, in that in both it was of exactly similar form. Further heteromorphy was evident in the long arm of the nucleolar pair of *rhodocanakis*, as shown by the absence of a large band in one of them.

The bands in the chromosomes of *F. oliveri*, *F. pyrenaica*, *F. portica*, *F. tuntasia*, *F. graeca thessala* and *F. graeca guicciardii* were examined, but could not be photographed whole. Of these, *tuntasia* chromosomes contained the largest bands, some being situated next to the centric constriction and some distal. In the absence of masking, most chromosomes of both *oliveri* and *pyrenaica* showed the centromeres clearly. Their chromosomes also contained some distal bands as well as fine intercalary ones. Both pairs of M-chromosomes of *pontica* had a large distal band in one arm and in two pairs of ST-chromosomes the short arm was heterochromatic. The chromosomes of the *graeca gucciardii* form contained relatively less bands than those of the *graeca thessala* form. The short arms of some ST-chromosomes in both plants were heterochromatic. Pairs of nucleolar chromosomes with heterochromatic trabants of similar character were seen in both forms.

Further to these, the bands of a plant of *F. tenella* ($2n = 18$) with 10 accessory B-chromosomes of equal size and identical banding have already been illustrated (La Cour 1978*b*).

(*d*) F. meleagris *group*

The bands of *F. latifolia* and *F. delphinensis* belonging to the *F. meleagris* group are shown in figures 20 and 21, plate 5, respectively. In both plants the largest bands were mostly situated

---

Description of plate 3

Figure 11. *F. lusitanica*. Two M-chromosomes (M) have additional heterochromatin at the centromere, masking the centromeres. (Magn. × 1650.)

Figure 12. *F. messanensis*. The heterochromatic trabants of the nucleolar chromosomes (N) diverge in respect of condensation. (Magn. × 1650.)

Figure 13. *F. gracilis*. (Magn. × 1650.)

Figure 14. *F. involucrata*. The two chromosomes seen at the bottom left are possibly nucleolar chromosomes. (Magn. × 1650.)

Figure 15. *F. michailovskyi*. (Magn. × 1650.)

Figure 16. *F. straussii*. (Magn. × 1650.)

contiguous with the centric constriction or sometimes within it. In *latifolia* the two pairs of M-chromosomes each had a distal band in one arm. In three of them the centromere was orientated so that only one of the pairs of centromeric dots was in the plane of observation. The fourth, however, contained a large single body (band) within the centric constriction masking the centromere, and indicating heteromorphy in one M-pair.

The bands of three other species of this group were studied but could not be photographed whole. namely, *F. aurea*, *F. pallidiflora* and *F. meleagris*. The complement of the first contained numerous bands, many fine ones and some of larger size situated next to the centric constriction. In the second, one pair of M-chromosomes had prominent distal bands in one arm, while in two ST-pairs large bands were contiguous with the centric constriction. The centromeres were clearly defined in some chromosomes and fine intercalary bands occurred in most of them. Some of the complement of *meleagris* contained one or more fine intercalary bands and distal ones were seen in the short arms of 8 ST-chromosomes.

(e) Other Old World groups

Of two species belonging to the *F. cirrhosa* group only *F. roylei* had divisions suitable for study and these could not be photographed whole. The short arms on 3 pairs of ST-chromosomes were heterochromatic, including a nucleolar pair recognizable by a minute trabant on the short arm. Each of one pair of M-chromosomes had a prominent distal band on one arm. There were also a number of small intercalary bands and the centromeres were clearly apparent.

No divisions were available in the roots of *F. verticillata* from this group, but the chromosomes obviously contained heterochromatin, since about 12 deeply stained chromocentres were present in the interphase nuclei.

The bands in the chromosomes of *F. persica*, a solitary member of the subgenus *Theresia*, are shown in figure 22. Some of the largest bands were distal, with others next to the centric constriction. The large size of some single dots (bands) at the latter site suggests that heterochromatin additional to that of the centromeres was also sometimes contained within the constriction. The nucleolar chromosomes were recognizable as a pair with minute trabants, the short arm carrying them being heterochromatic.

The bands on the chromosomes of *F. bucharica*, belonging to the subgenus *Rhinopetalum* are shown in figure 23. The most prominent bands in this plant were mostly sited at an intercalary position in the two pairs of M-chromosomes. The centromeres were not visible because of poor differential staining. In *F. stenanthera*, another member of the same subgenus, the bands and centromeres were more clearly differentiated but could not be photographed. Each of one pair of M-chromosomes of this plant had a prominent distal band in one arm, while in one arm of each of the other pair of M-chromosomes there was an intercalary band. There were also distinctive bands next to the centric constriction in the short arms of 3 pairs of ST-chromosomes.

The nucleolar chromosomes could not be identified in either of the two species described above.

The chromosomes of *F. imperialis*, a member of the subgenus *Petilium*, and together with *meleagris*, the two most common garden fritillaries are shown in figure 25. The plant examined contained two accessory B-chromosomes in addition to the normal complement. These were

Figures 17–30 show metaphases from root tips of *Fritillaria*, differentially stained with Giemsa to show C-bands.

FIGURE 17. *F. reuteri*. Heteromorphy is evident in the nucleolar pair (N). (Magn. × 1650.)

FIGURE 18. *F. rhodocanakis*. The centromeres are masked in many chromosomes by heterochromatin additional to that of the centromeres, as indicated in some ST-chromosomes by arrows. Heteromorphy is evident in the nucleolar pair (N). (Magn. × 1650.)

FIGURE 19. Some chromosomes of *F. acmopetala*, taken at high magnification to show the centromeric dots, representing the centromeres. (Magn. × 3000.)

FIGURE 20. From *F. latifolia*. The M-chromosomes (M) show differences at the centromere; in 3 of them only one of the two centromeric dots is in view and in the fourth the centromere is masked by a contiguous band. (Magn. × 1650.)

(*Facing p.* 66)

Description of plate 5

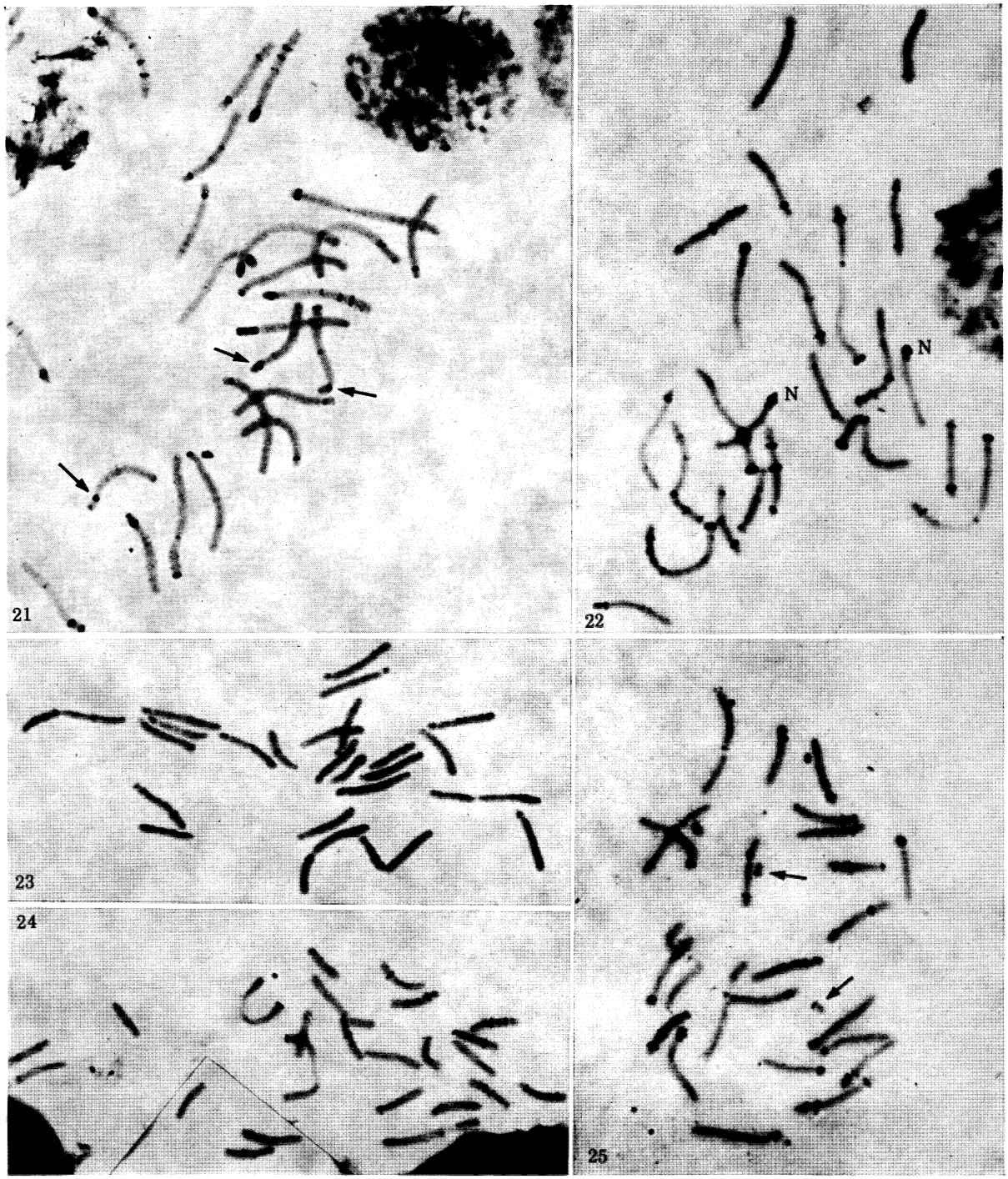
Figure 21. *F. delphinensis*. The arrows indicate chromosomes with additional heterochromatin at the centromere. (Magn. × 1650.)

Figure 22. *F. persica*. The nucleolar chromosomes (N) are recognizable. (Magn. × 1650.)

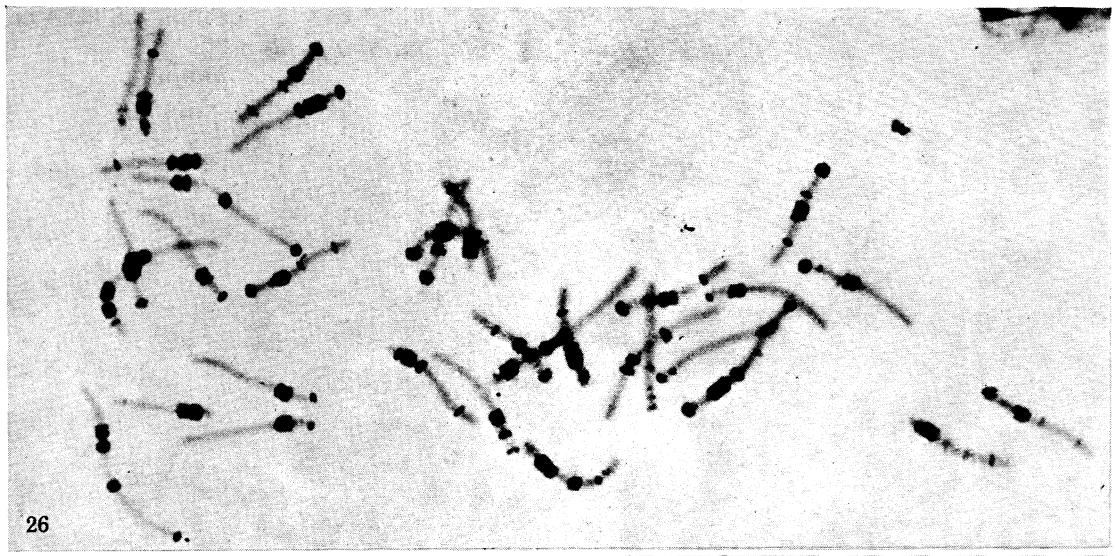
Figure 23. *F. bucharica*. (Magn. × 1650.)

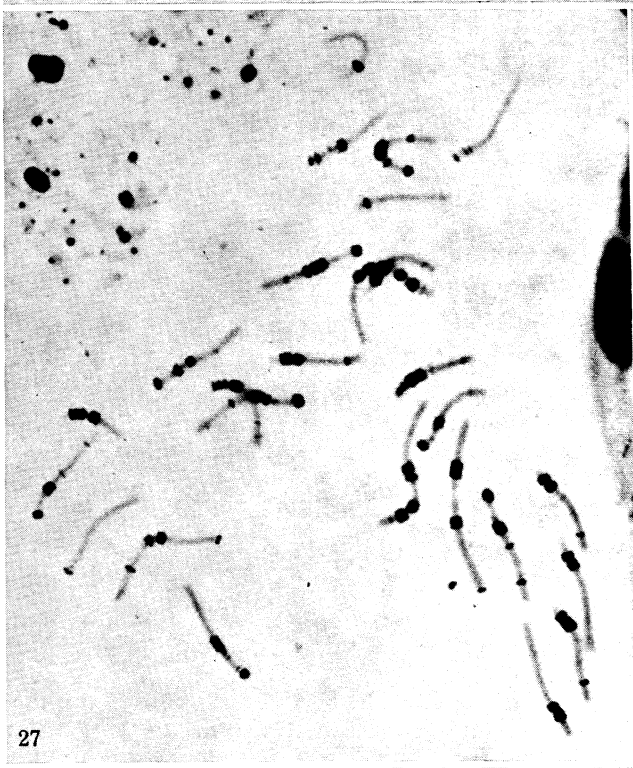
Figure 24. *F. purdyi*, an American species with few C-bands. (Magn. × 1650.)

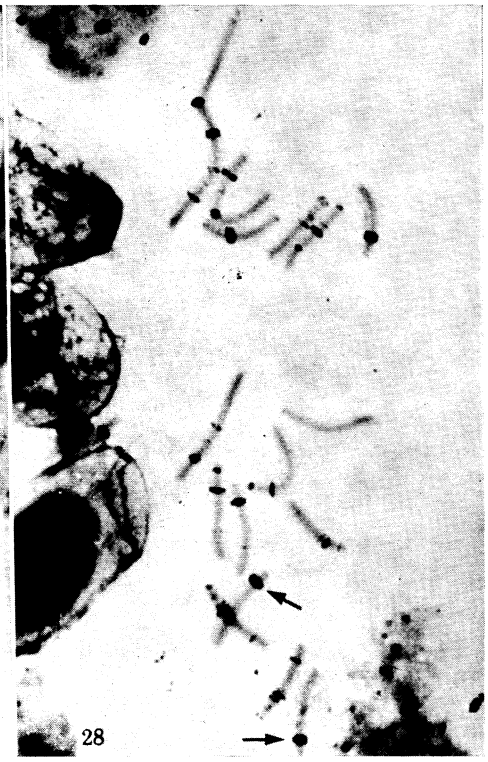
Figure 25. *F. imperialis*. The complement contains two (arrowed) telocentric B-chromosomes. (Magn. × 1650.)

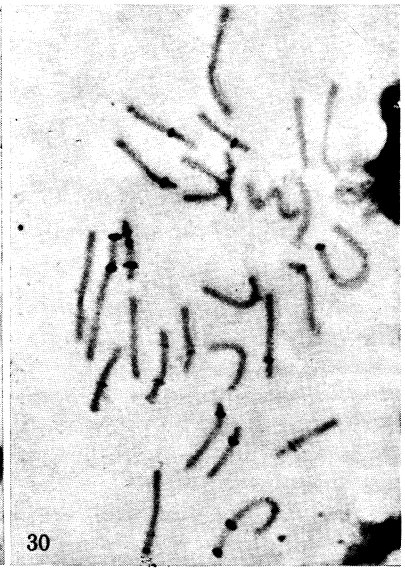


FIGURES 21–25. For description see opposite.









FIGURES 26–30. For description see opposite.

of equal size and each had a terminal band, which suggests that they were probably telocentric and identical.

In the A-chromosomes most bands were closely associated with the centric constrictions. However, two prominent bands were most probably contained in a pair of nucleolar chromosomes, next to a secondary constriction. The other pair of such chromosomes known to be present in this species could not be identified.

A specimen of the plant sometimes called *F. sewerzowi*, but more correctly known as *Korolkowia* and said to form a link between *Fritillaria* and *Lilium*, was used for banding but unblemished metaphases suitable for photography could not be obtained. This plant was of particular interest because it provided the only example of one without any apparent bands, though all the Giemsa positive centromeres were clearly defined.

(*f*) *New World species*

The bands observed in the chromosomes of 6 American fritillaries, belonging to the subgenus *Liliarhiza*, are illustrated in figure 24 and figures 26–30. It is immediately apparent that, from these illustrations, great differences exist among the New World fritillaries in the amount of heterochromatin their chromosomes contain. They also diverged somewhat in the reaction of their chromosomes to the Giemsa stain. Under exactly similar conditions of treatment the species with most bands were inclined to overstain.

Figure 26, plate 6 shows the bands of a triploid form of *F. phaenanthera* ($2n = 36$), the diploid form of which is sometimes thought to be of hybrid origin. Meiosis has never been examined to determine whether this is so or not. Most bands, in all but 3 ST-chromosomes, were large and situated either next to the centric constriction or distally. The former location was responsible for masking of the centromeres, which were visible only in the 3 ST-chromosomes. Heteromorphy was evident among these 3 in respect of the number of fine bands. There was also evidence of similar heterozygosity in another ST-type.

The bands of a yellow form of *F. glauca* are shown in figure 27. This plant, like *F. pudica* (Darlington 1937), is notable in having a basic chromosome number of 13. It is clear from figures 26 and 27 that most of its bands have a somewhat similar location to those of *phaenanthera*. An interesting feature was that the complement of *glauca* contained a pair of ST-chromosomes with a pattern of banding virtually indistinguishable from that of the *phaenanthera* chromosomes of this type mentioned above.

Of the American species with relatively fewer bands, the complement of *F. purdyi* (figure 24) contained the least. Apart from a distal band in one arm of a pair of M-chromosomes, the remainder were intercalary. The centromeres, most probably because of weak staining, were not clearly defined in all chromosomes.

Description of plate 6

Figure 26. A triploid form of *F. phaenanthera* ($2n = 36$). (Magn. × 1650.)

Figure 27. *F. glauca* yellow form ($2n = 26$). (Magn. × 1650.)

Figure 28. *F. liliacea*. The 17 chromosomes show a gradation in the size of bands, some being tiny and others extremely large, as indicated by arrows in some ST-chromosomes adjacent to the centric constriction. (Magn. × 1650.)

Figure 29. *F. pinetorum*. (Magn. × 1650.)

Figure 30. *F. roderickii*. (Magn. × 1350.)

Only 17 chromosomes of the *F. liliacea* complement are shown in figure 28. This plant was notable for some extremely fine bands in one pair of ST-chromosomes, and large ones in both long and short arms of some others next to the centric constriction. The short arms of another pair were heterochromatic.

The majority of bands in the *F. pinetorum* complement (figure 29) were intercalary. Both pairs of M-chromosomes contained large bands and, in the plate illustrated, the centromeres in 3 of them were orientated so that only a single dot is in view. In the fourth the size of the centric inclusion suggests that additional heterochromatin was contiguous with the centromere. Further heteromorphy was indicated by the presence of a large band in a single ST-chromosome.

The complement of *F. roderickii* (figure 30) contained only slightly more bands than that of *purdyi*. One of the M-pairs had 2 prominent bands in their longer arm, of distal and intercalary locations. Heteromorphy was evident as shown by the presence of 2 prominent bands in a single ST-chromosome.

Among other American fritillaries, the bands in *F. brandegii*, *F. viridia* and *F. camschatcensis* were studied, but could not be photographed entire. All of the chromosomes in the first of these contained from 1–3 bands and a total comparable to that of *liliacea*. A pair of M-chromosomes each contained a prominent distal band in one arm. Similarly distinctive distal ones were also observed in one arm of a pair of M-chromosomes in *viridia*, the complement of which contained relatively fewer bands.

Of the remaining species mentioned, *camschatcensis* has a wider distribution and is found in both the Old and New Worlds – in Asia and Alaska. All of its chromosomes contained bands, with as many as 4 small ones in most chromosomes. Except for larger distal ones on one arm of each of an M-pair, they were all intercalary. The centromeres were well defined. Thus there was no localization of bands next to the centric constriction.

The nucleolar chromosomes could not be identified in any of the American species.

Discussion

The present observations have shown that out of 56 species and subgenera of *Fritillaria* examined for C-bands, the subgenera *Korolkowi* was the only one without them. All of its chromosomes, however, showed Giemsa positive centromeres. The absence of C-bands in this plant supports the widely held view that it is not a true *Fritillaria*. An examination of the frequency of C-bands in the chromosomes of *Lilium* may perhaps prove of interest in respect of the supposition that *Korolkowi* is a link between *Lilium* and *Fritillaria*.

The observations also provide convincing proof of the efficiency of the Giemsa banding technique in the detection of constitutive heterochromatin, as shown by its now obvious presence in some fritillaries where hitherto it was unsuspected (La Cour 1951). One particular aspect of the technique is that in many plants to which it has been applied, the dots representing the centromeres have not always been seen. This is most probably due to the fact that, in some species, even closely related ones as presently studied, the C-bands take up the Giemsa stain before the centromeres. However, as we have seen from the present observations, the centromeres are masked when other heterochromatin is in close proximity, as is also clear in other plants, e.g. *Anacyclus depressus* and *A. valentinus* (Schweizer & Ehrendorfer 1976).

A recent Giemsa study (La Cour 1978*a*) has revealed in the chromosomes of *F. crassifolia* and a form of *graeca* from Attica, a new class of heterochromatin in addition to that commonly

found in fritillaries. Because of its intermediate staining behaviour, the new type is consistently thought to be less condensed than the other. It can now be said that this new definitive type has not been seen in other fritillaries presently studied, including other forms of *graeca*, nor has it apparently been reported to occur in other organisms elsewhere. It can be assumed that this mutant class has arisen more recently in evolution.

The fact that some heteromorphy in respect of banding was found in some of the species is scarcely surprising. Heteromorphy was first reported in early studies of H-segments in *Trillium* (Darlington & La Cour 1940). The Giemsa technique shows how widespread the phenomenon is and provides a very efficient means of detecting it (e.g. Vosa 1973; Marks & Schweizer 1974; Schweizer & Ehrendorfer 1976).

It is apparent from this study that wide differences exist between fritillaries in the distribution and amount of detectable heterochromatin their chromosomes contain. This applies not only between groups but also within them, e.g. the *graeca* group (figures 9–18) and the *Liliarhiza* (American) group (figure 24 and figures 26–30). The divergence is extreme in the latter group, as is also that between *alfredae* (figure 1) and other Old World species studied. What this means in terms of evolution is at the moment obscure.

The answer may possibly lie in the evolution of DNA with highly repetitive sequences which the constitutive heterochromatin seems most likely to contain. The evidence for localization of such sequences to heterochromatin is becoming increasingly strong, though at the moment more particularly in respect of heterochromatin associated with the centromere. This was established by *in situ* cytological hybridization of satellite DNA, e.g. in the mouse (Pardue & Gall 1970; Jones 1970) in the salamander (Macgregor *et al.* 1973) and in *Drosophila melanogaster* where four distinct and homogeneous satellites were found to localize with the chromocentre (Peacock *et al.* 1973). In *Drosophila virilis* three satellites found in heterochromatin may correspond with three differently staining blocks of heterochromatin, as shown by fluorochrome and Giemsa staining (Holmquist 1975).

Both short and long repeated sequences have been found in chromosomal DNA of plants (Flavell & Smith 1976; Narayan & Rees 1976; Smith & Flavell 1977). Unfortunately at present there is no record of cytological hybridization of DNA in plants. In 7 species of *Anemone* no correlation was evident between the percentage of repeated DNA sequences and the percentage of heterochromatin, as estimated from C-banding (Cullis & Schweizer 1974). This is possibly a reflection of differences between them in the length, organization and dispersal of such sequences within the genome.

The present observations clearly show that, in those fritillaries with an abundance of heterochromatin, there was a marked tendency for it to accumulate next to the centric constriction or sometimes contiguously with the centromere and also distally at the telomere, though to a lesser extent. In respect of the centromere, it may be significant that the less condensed type of heterochromatin recently found in *F. crassifolia* and a form of *F. graeca*, as mentioned above, was likewise centromerically localized.

A possible explanation for the accumulation of repetitive DNA near the centromere in the evolution of the eukaryotes, is that the centromeres were themselves composed of DNA with repeated sequences. It is a reasonable assumption that such DNA is non-transcriptional, as evidence suggests (Flamm, Walker & McCullam 1969; Walker 1971). A certain amount of constitutive heterochromatin at this vital site, and indeed at the telomere, may be necessary for their protection. It is not clear, however, from the C-banding technique whether

heterochromatin is always present at the telomere or not always detectable because it is sometimes too small to detect.

A similar difficulty arises in nucleolar chromosomes in respect of the nucleolar organizing region, at which the presence of constitutive heterochromatin is thought to have a protective rôle (see Yunis & Yasmineh 1971). As was clear in many fritillaries, the organizing regions (secondary constrictions) were not visible in Giemsa preparations because in some fritillaries they occur in euchromatin with no apparent near-by heterochromatin (La Cour 1951). Constrictions, primary or secondary are often difficult to visualize after Giemsa staining when situated in the weakly stained euchromatin.

If indeed all constitutive heterochromatin contains DNA with repeated sequences, the mechanism by which it tends to accumulate at such sites as the centromere and telomere is not clear. Clearly at these regions it could not have been increased by unequal crossover at meiosis, as might occur at intercalary positions (Smith 1976). We have no knowledge as to whether there was a rapid duplication of repeated sequences early in evolution or whether it was a gradual process. Loss of repeated DNA sequences could also occur. The loss, unlike that involving unique sequences within structural genes, might not be detrimental to the organism.

One further point perhaps worthy of consideration, particularly since there is little interspecific hybridization in fritillaries, is that the American fritillaries, with an abundance of heterochromatin, may possibly be relicts. The forest or forest border habitats in northwest California where most of the American fritillaries occur have many tertiary relicts (Axelrod, personal communication). The climate of the area has been favourable for survival and the rocky serpentine type of terrain may have enabled some to survive free of competition.

I am grateful to the Royal Society for a grant from the Parliamentary Grant-in-aid of Scientific Investigations.

References

Cullis, C. A. & Schweizer, D. 1974 Repetitious DNA in some *Anemone* species. *Chromosoma* **44**, 417–421.

Darlington, C. D. 1937 Recent advances in cytology. London: Churchill.

Darlington, C. D. & La Cour, L. F. 1940 Nucleic acid starvation of chromosomes in *Trillium*. *J. Genet.* **40**, 185–213.

Darlington, C. D. & La Cour, L. F. 1941 The detection of inert genes. *J. Hered.* **32**, 115–121.

Darlington, C. D. & La Cour, L. F. 1976 The handling of chromosomes (6th Edition). London: Allen & Unwin.

Eiberg, H. 1974 New selective techniques for human chromosomes, Cd staining. *Nature, Lond.* **248**, 55.

Flamm, W. G., Walker, P. M. B. & McCallum, M. 1969 Some properties of the single strands isolated from the DNA of the nuclear satellite of the mouse (*Mus musculus*). *J. molec. Biol.* **40**, 423–443.

Flavell, R. B. & Smith, D. B. 1976 Nucleotide sequence organisation in the wheat genome. *Heredity* **37**, 231–252.

Holmquist, G. 1975 Organisation and evolution of *Drosophila virilis* heterochromatin. *Nature, Lond.* **257**, 503–506.

Jones, K. W. 1970 Chromosomal and nuclear location of mouse satellite DNA in individual cells. *Nature, Lond.* **225**, 912–915.

La Cour, L. F. 1951 Heterochromatin and the organisation of nucleoli in plants. *Heredity* **5**, 37–50.

La Cour, L. F. 1978*a* Two types of constitutive heterochromatin in the chromosomes of some *Fritillaria* species. *Chromosoma*. **67**, 67–75.

La Cour, L. F. 1978*b* Differential Giemsa staining of B-chromosomes of *Fritillaria tenella*. *Heredity*. **41** (1), 101–103.

La Cour, L. F. & Wells, B. 1974 Fine structure and staining behaviour of heterochromatic segments in two plants. *J. Cell Sci.* **14**, 1–17.

Macgregor, H. C., Horner, H., Owen, C. A. & Parker, I. 1973 Observations on centromeric heterochromatin and satellite DNA in salamanders of the genus *Plethodon*. *Chromosoma* **43**, 329–348.

Marks, G. E. 1977 The nature of centromeric dots in *Nigella* chromosomes. *Chromosoma* **62**, 369–373.

Marks, G. E. & Schweizer, D. 1974 Giemsa banding: karyotype differences in some species of *Anemone* and in *Hepatica nobilis*. *Chromosoma* **44**, 405–416.

Narayan, R. K. J. & Rees, H. 1976 Nuclear DNA variation in *Lathyrus*. *Chromosoma* **54**, 141–154.

Pardue, M. L. & Gall, J. G. 1970 Chromosomal localization of mouse satellite DNA. *Science* **168**, 1356–1358.

Peacock, W. J., Brutlag, D., Goldring, E., Appels, R. A., Hinton, C. W. & Lindsley, D. L. 1973 The organization of highly repeated DNA sequences in *Drosophila melanogaster* chromosomes. *Cold. Spr. Harb. Symp. Quart. Biol.* **38**, 405–416.

Rix, M. 1975 Fritillary cooperative exhibit. In *Lilies and other Liliaceae* (Ed. F. Napier & J. Platt) pp. 45–53. London: Royal Horticultural Society.

Schweizer, D. 1973 Differential staining of plant chromosomes with Giemsa. *Chromosoma* **40**, 307–320.

Schweizer, D. & Ehrendorfer, F. 1976 Giemsa banded karyotypes, systematics, and evolution in *Anacyclus* (Asteraceal-Anthemideae) *Plant Syst. Evol.* **126**, 107–148.

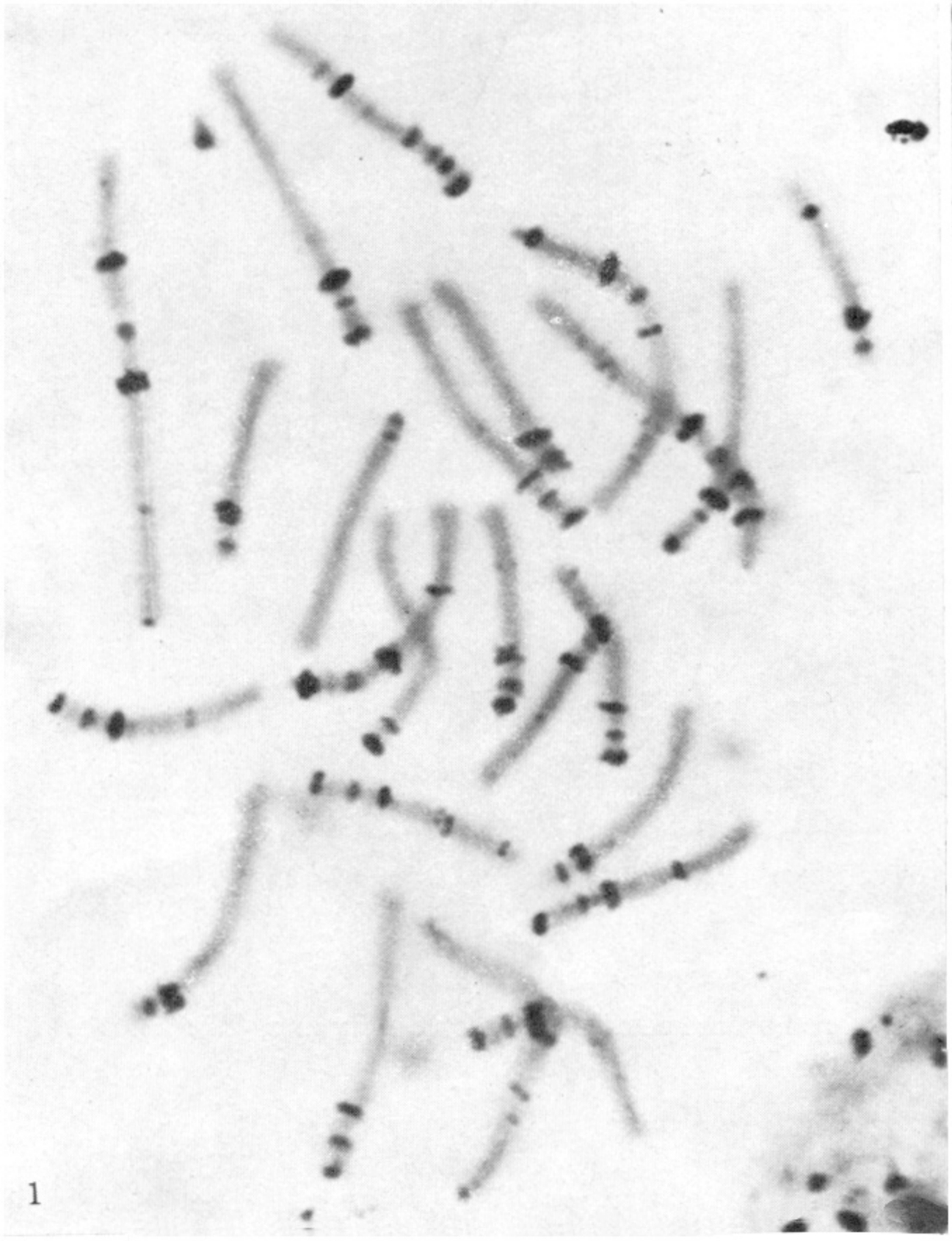
Smith, G. P. 1976 Evolution of repeated DNA sequences by unequal crossover. *Science* **191**, 528–535.

Smith, D. B. & Flavell, R. B. 1977 Nucleotide sequence organisation in the *Rys* genome. *Biochem. biophys. Acta* **474**, 82–97.

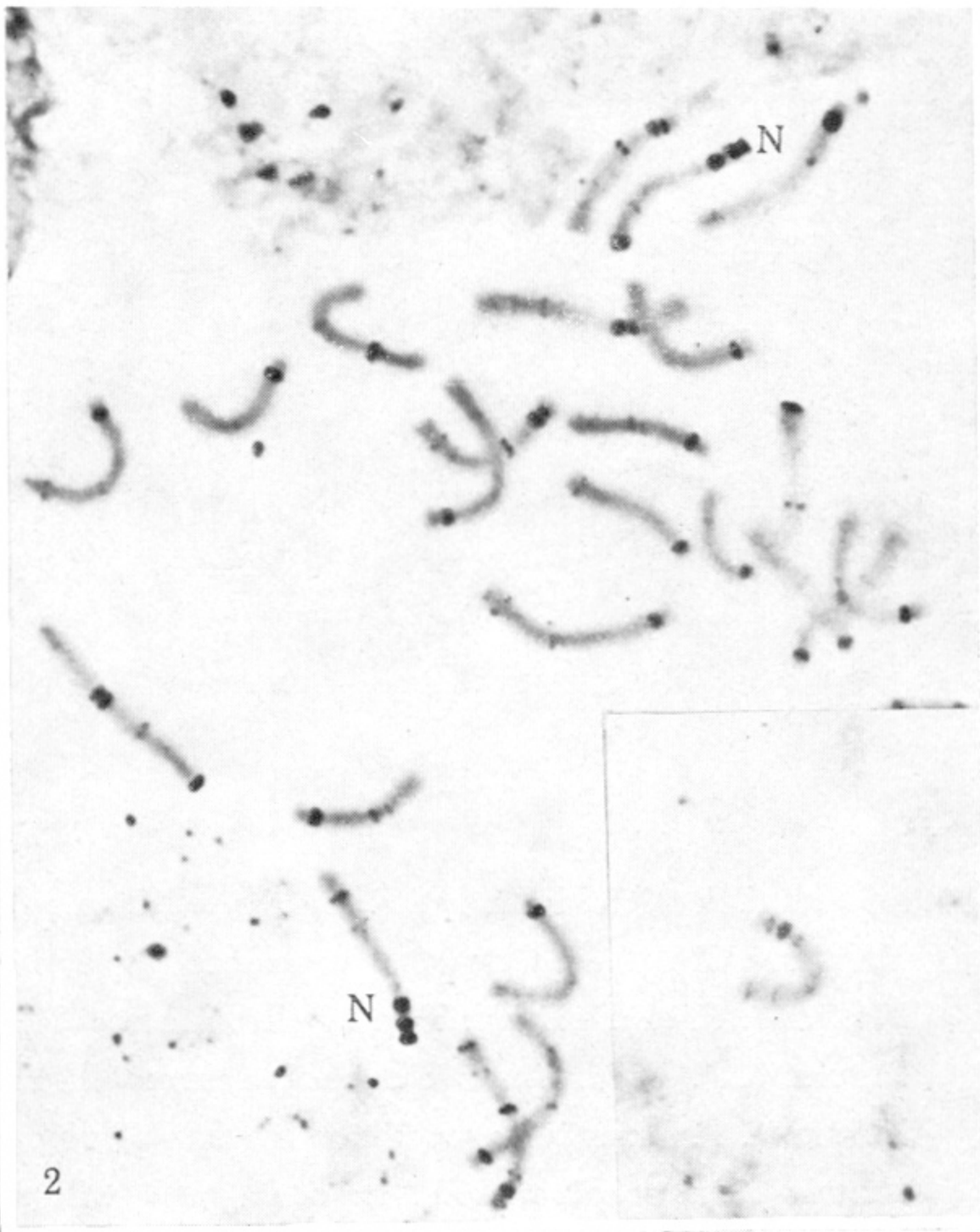
Vosa, C. G. 1973 Heterochromatin recognition and analysis of chromosome variation in *Scilla sibrica*. *Chromosoma* **43**, 269–278.

Walker, P. M. B. 1971 Origin of satellite DNA. *Nature, Lond.* **229**, 306–308.

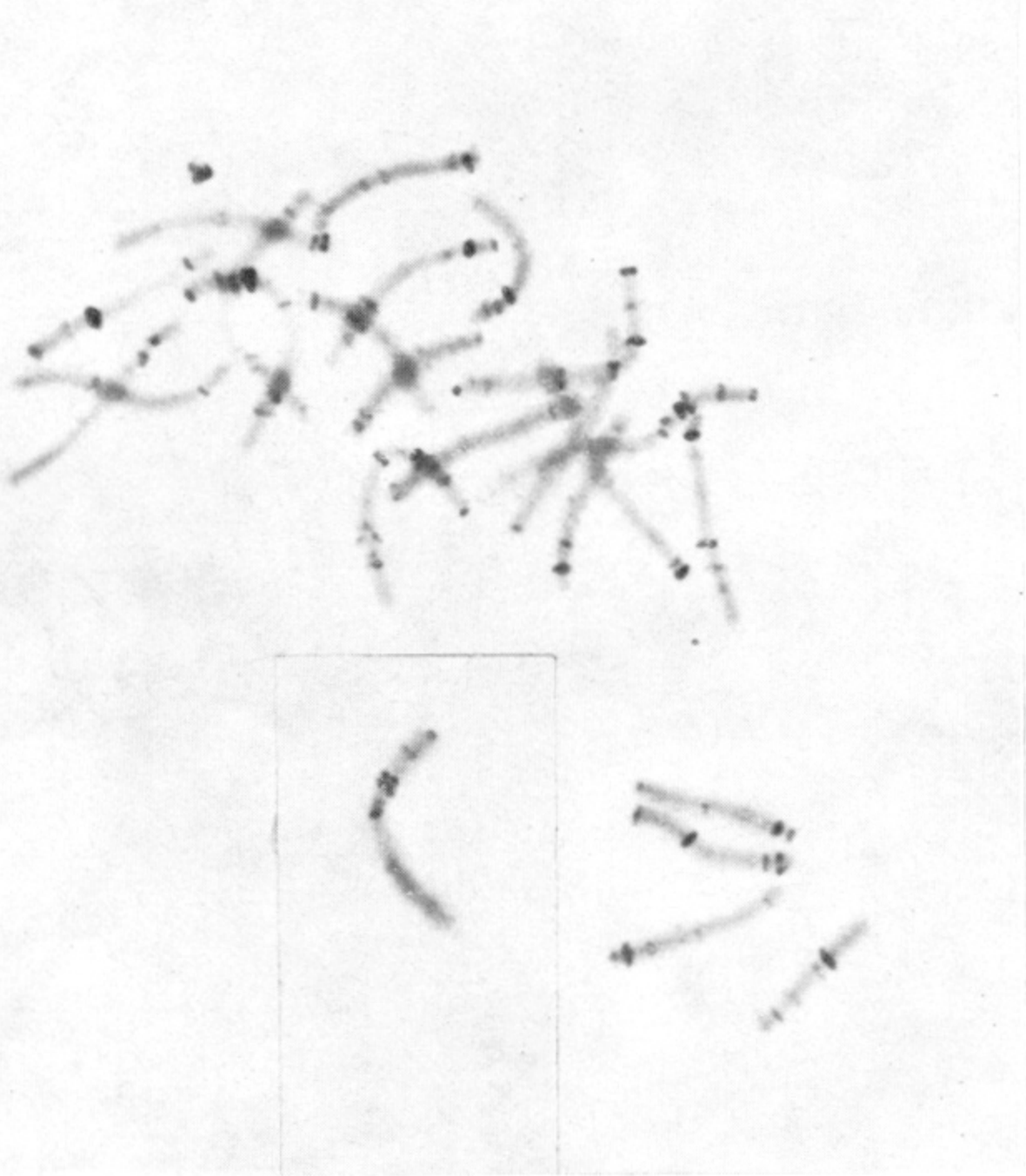
Yunis, J. J. & Yasmineh, W. G. 1971 Heterochromatin, satellite DNA and cell function. *Science* **174**, 1200–1209.



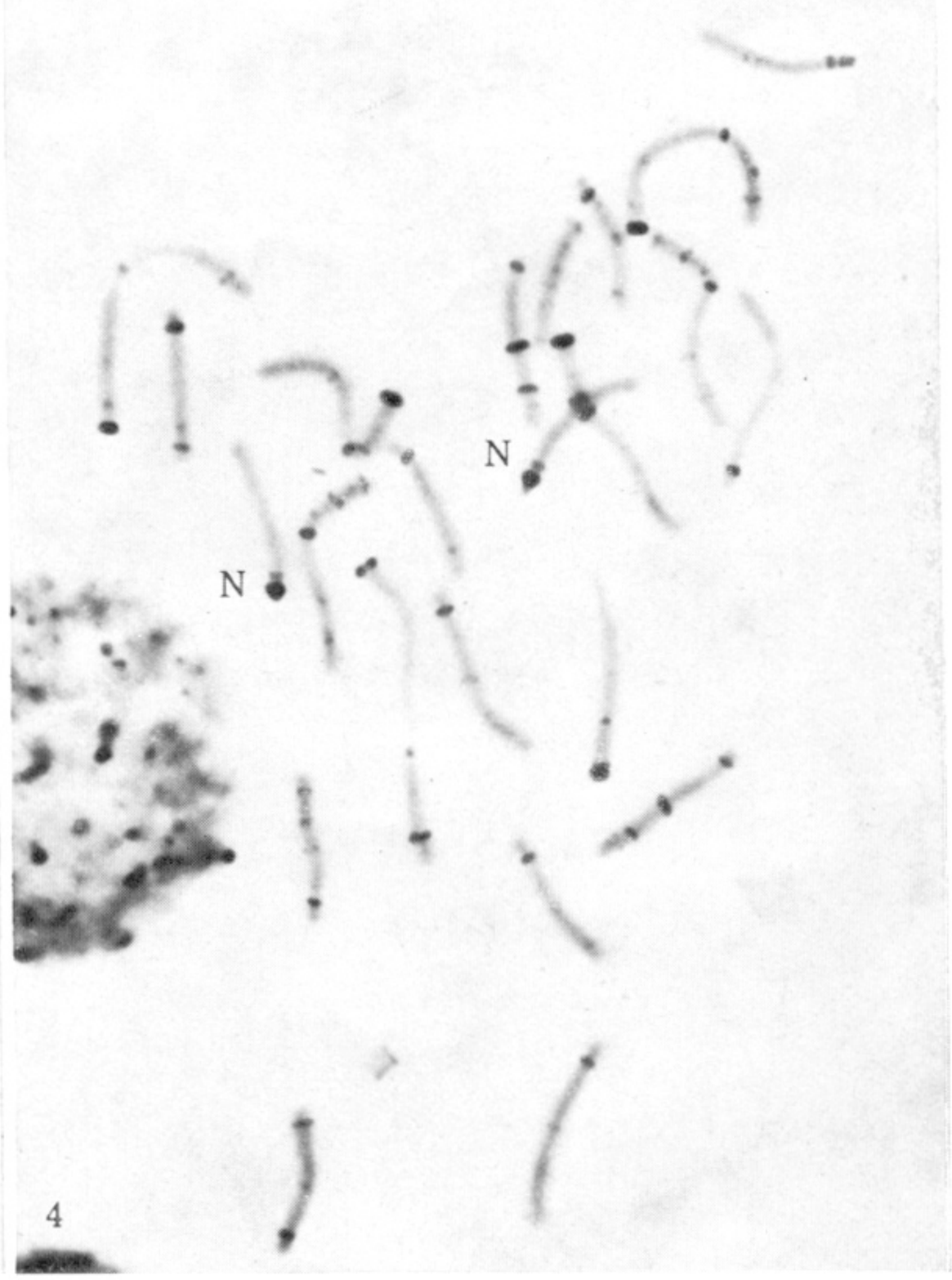
1



2



3



4

Figures 1–16 show metaphases from root tips of *Fritillaria*, differentially stained with Giemsa to show C-bands.

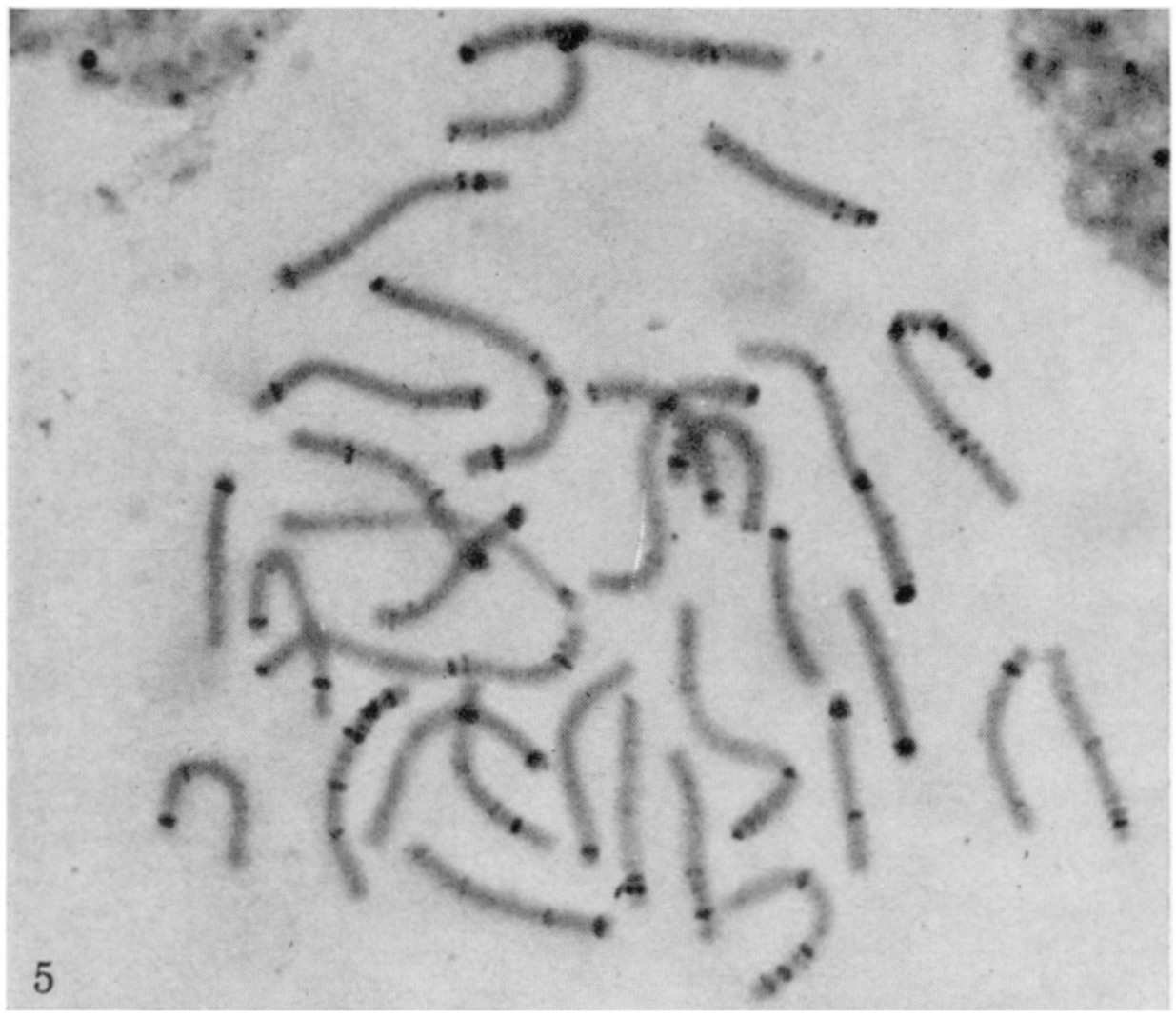
FIGURE 1. *F. alfredae* a fritillary with more bands than any other in the Old World; many of them are intercalary. (Magn. × 2500.)

FIGURE 2. *F. carductorum*, the nucleolar pair (N) show heteromorphy. (Magn. × 1650.)

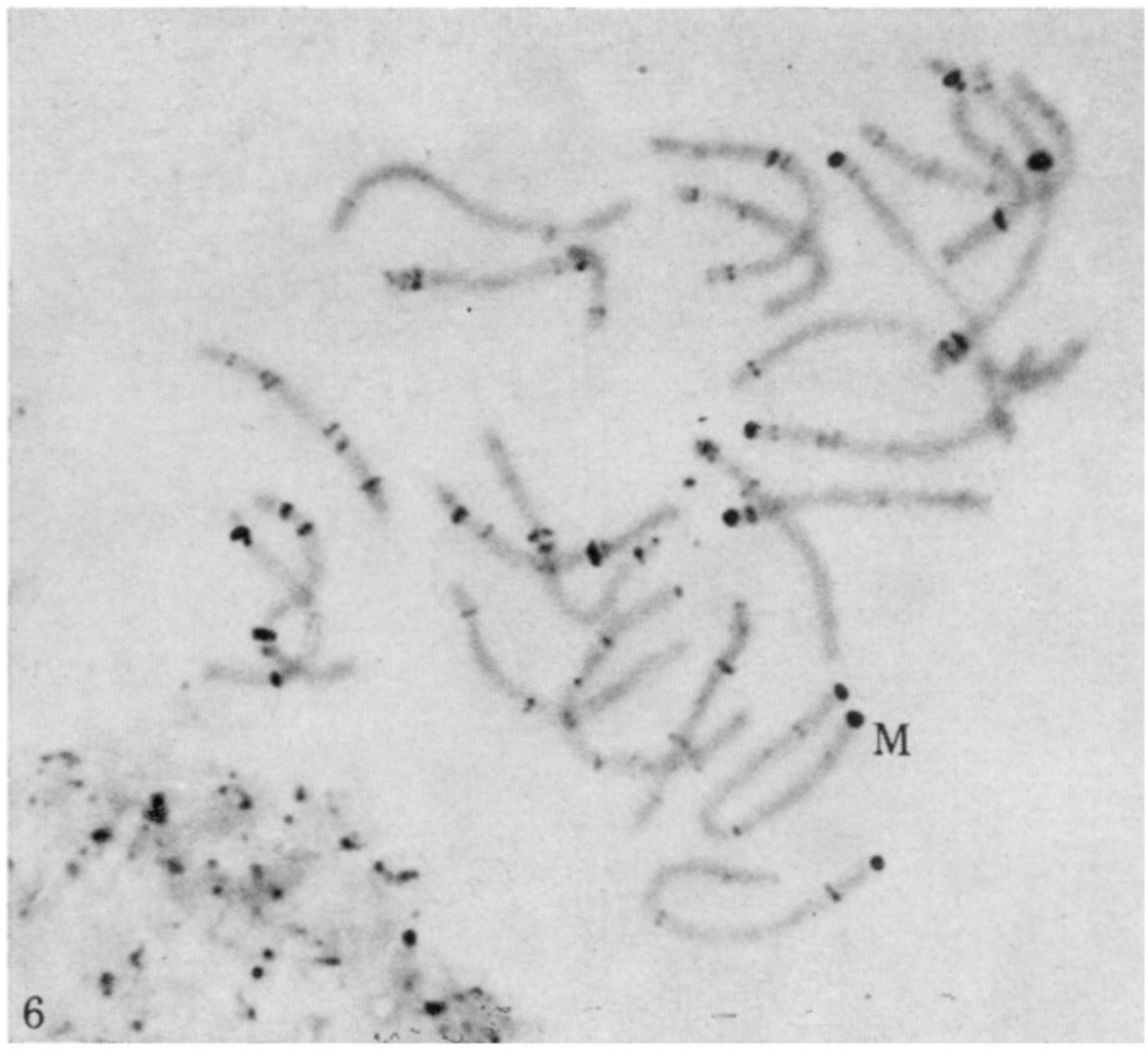
FIGURE 3. *F. bithynica*. (Magn. × 1650.)

FIGURE 4. *F. pinardii*. (Magn. × 1650.)

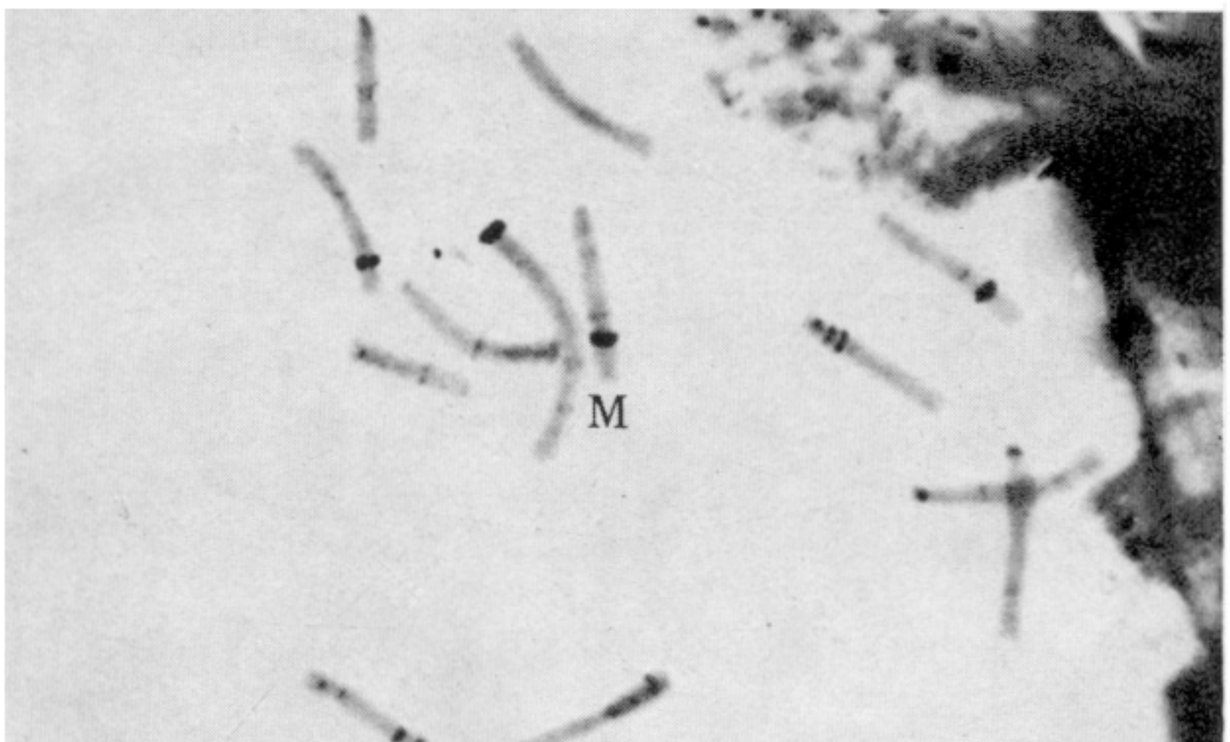
(*Facing p.* 64)



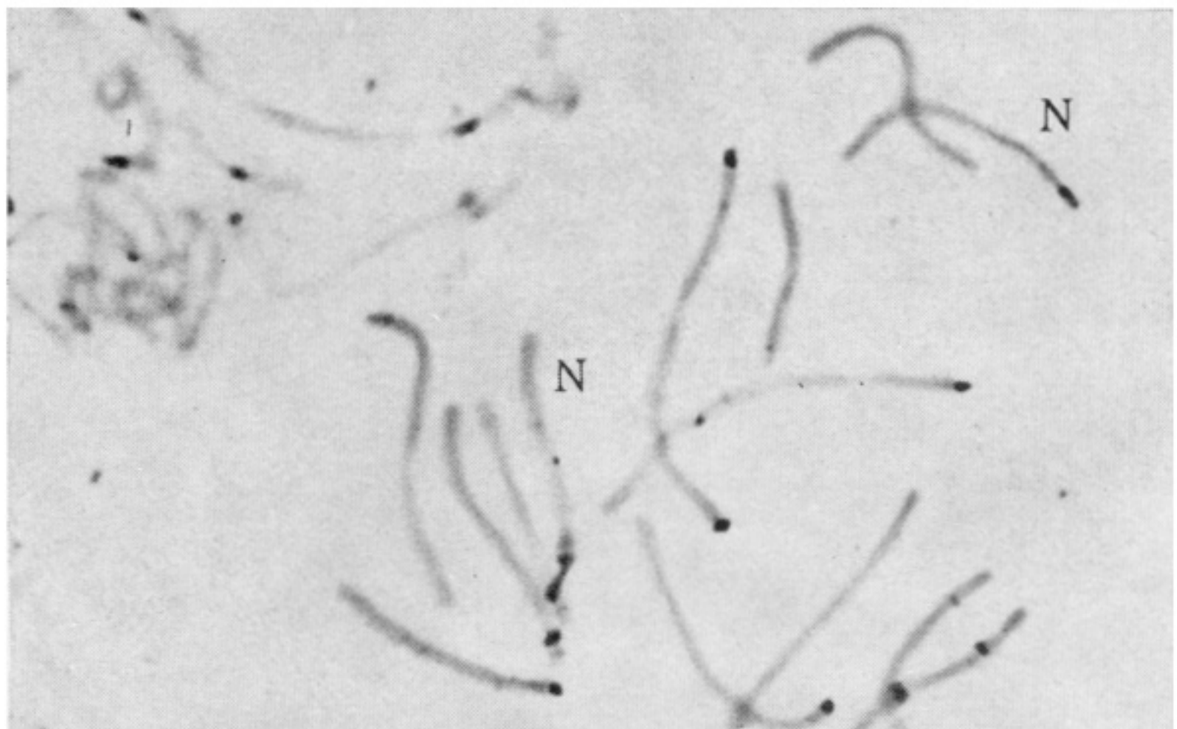
5



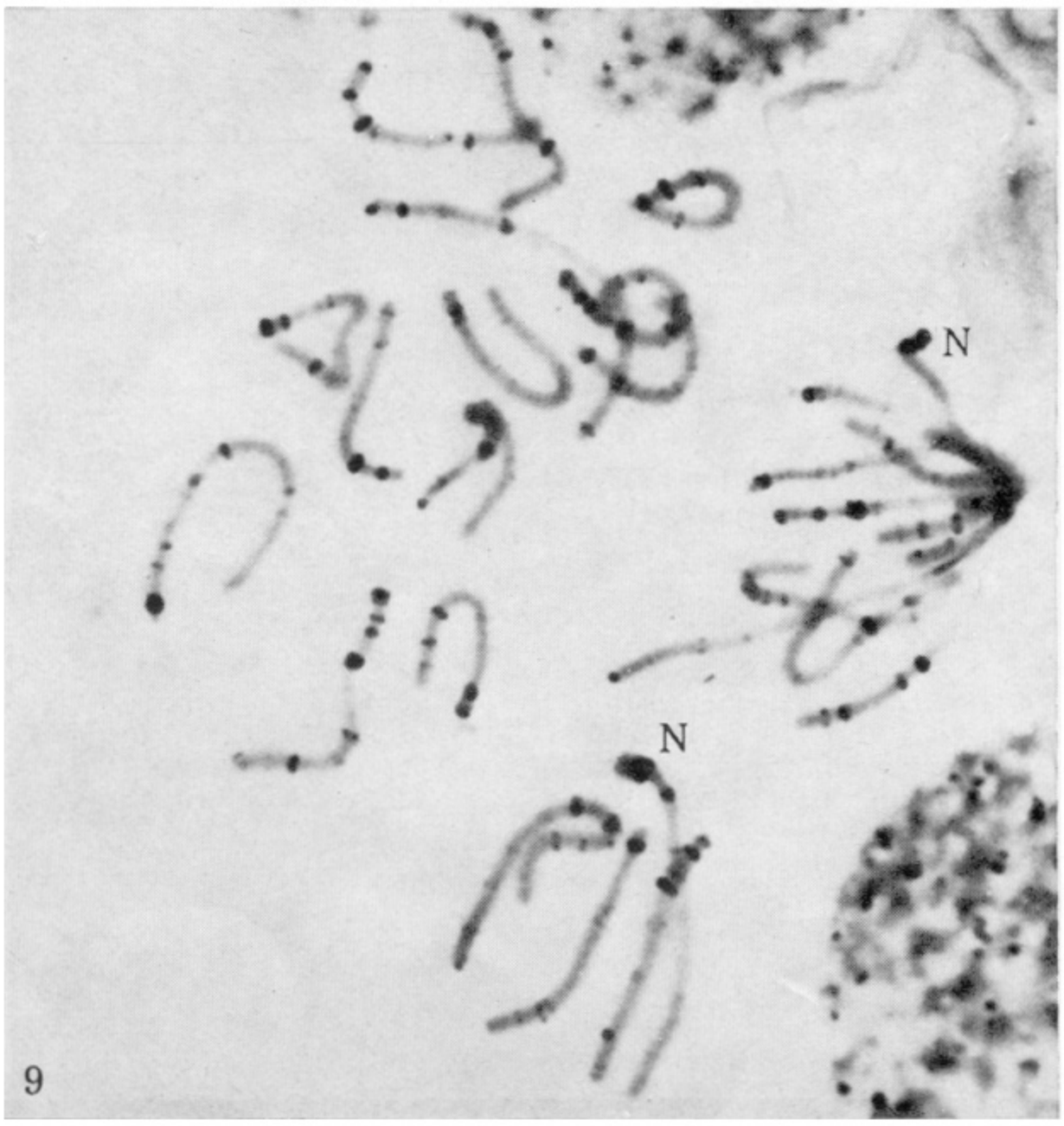
6



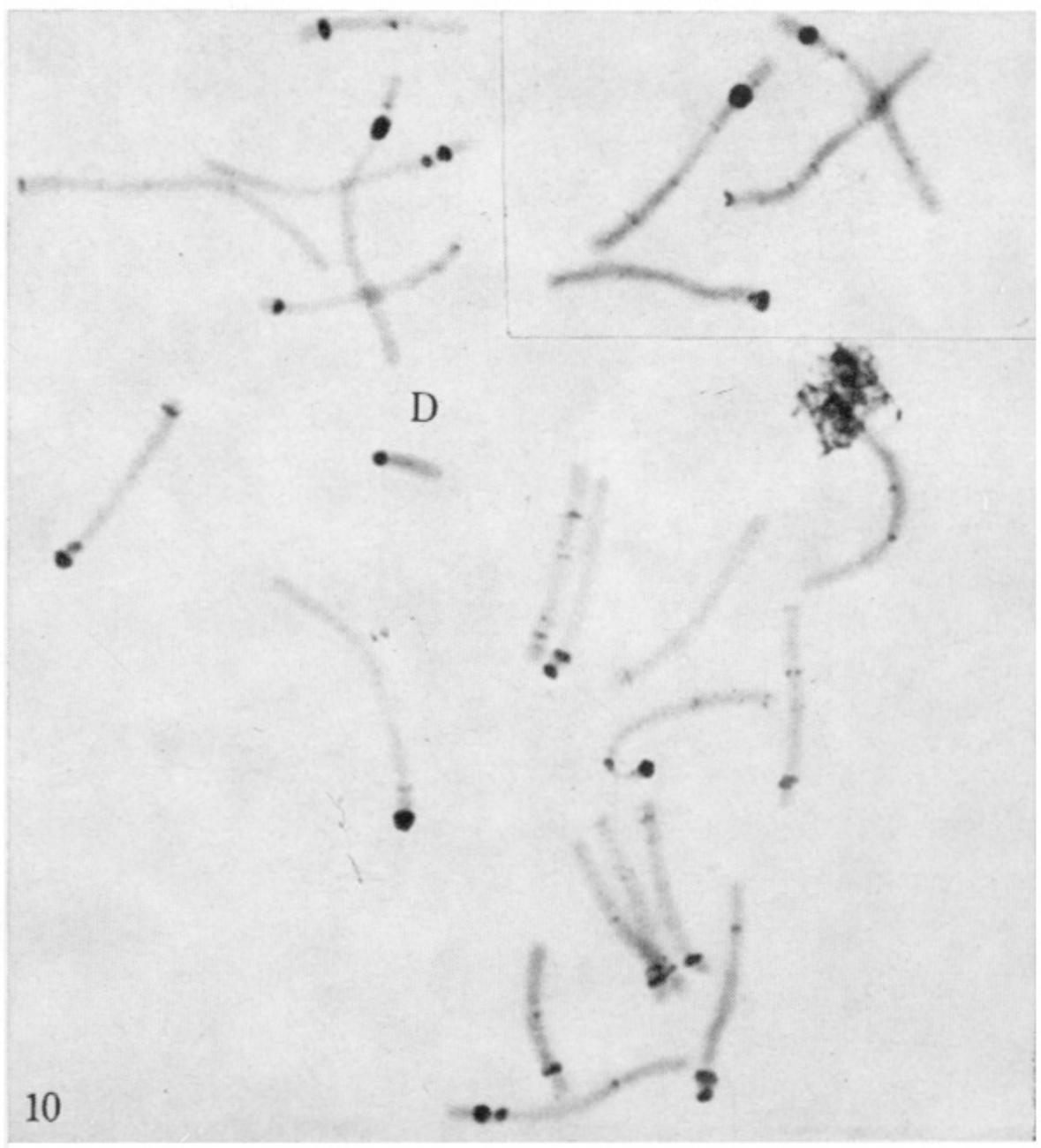
7



8

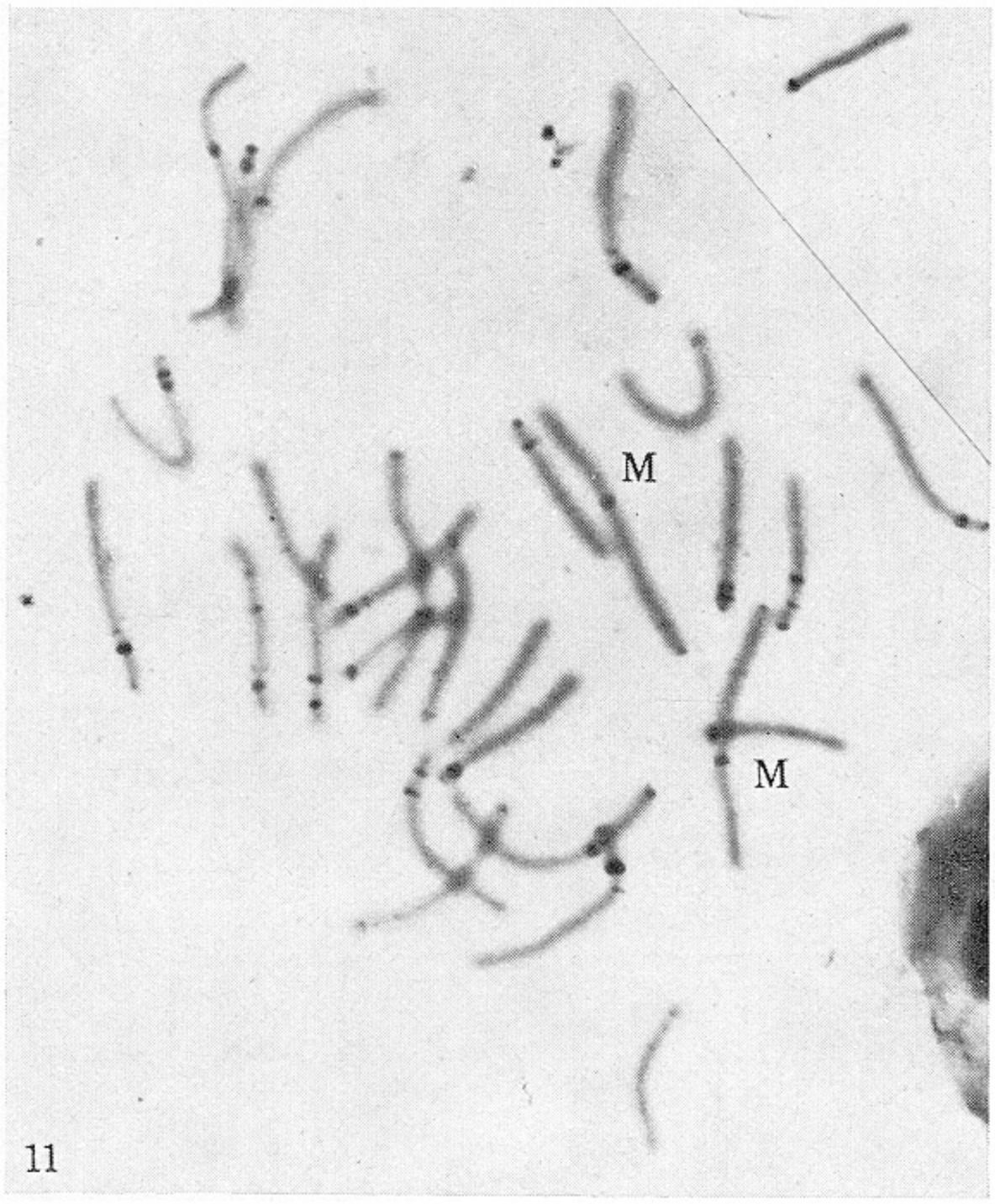


9

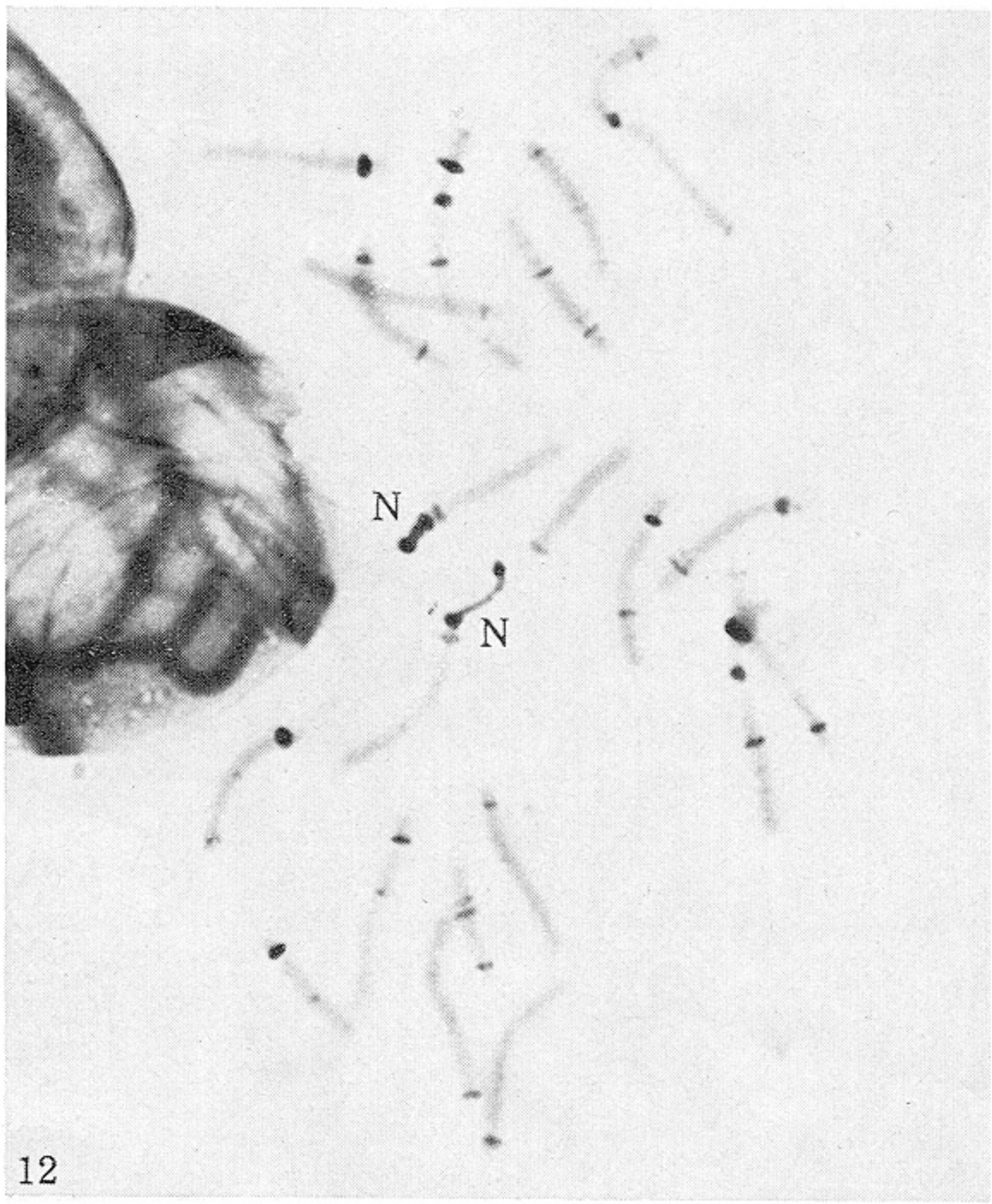


10

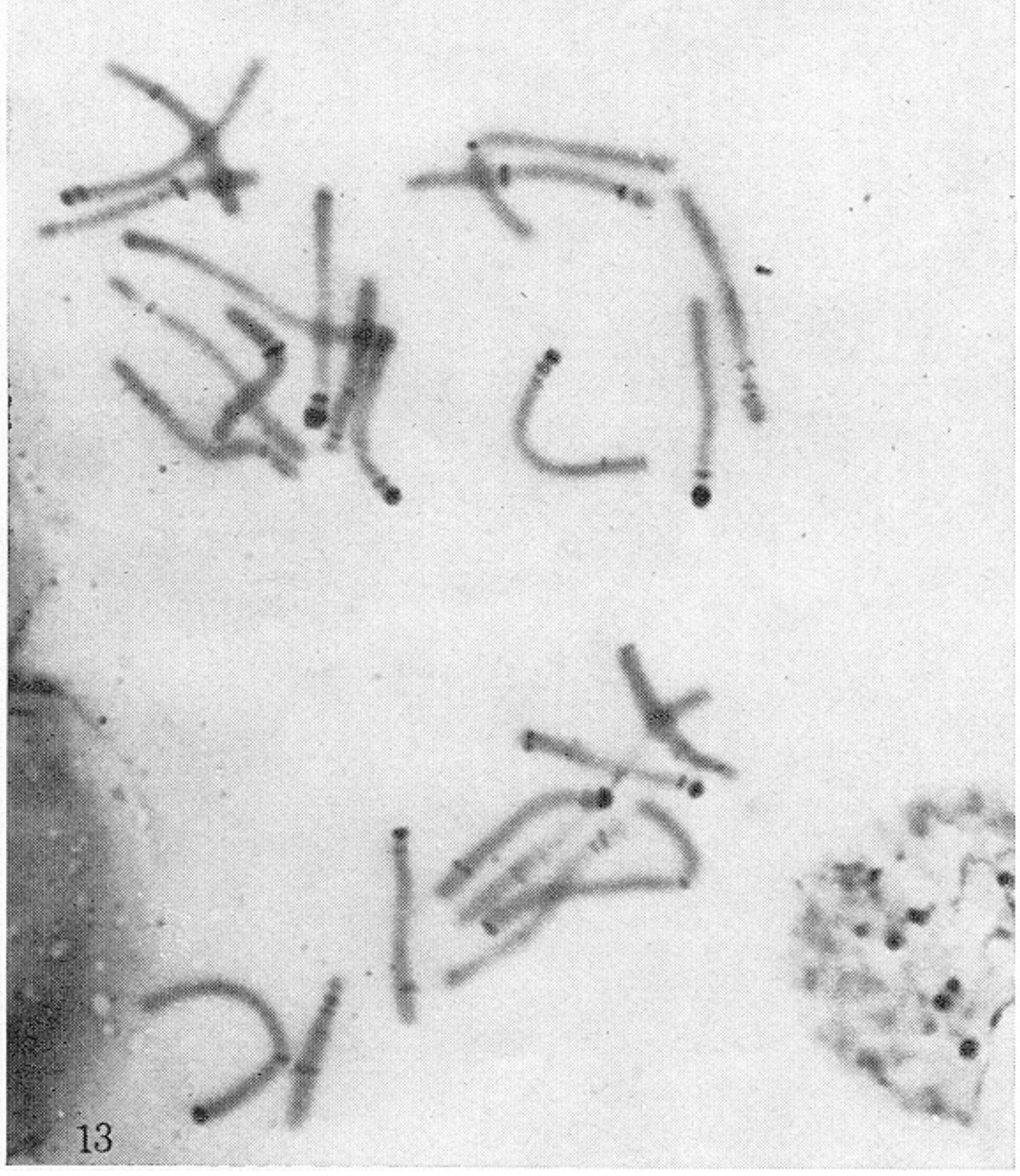
FIGURES 5–10. For description see opposite.



11

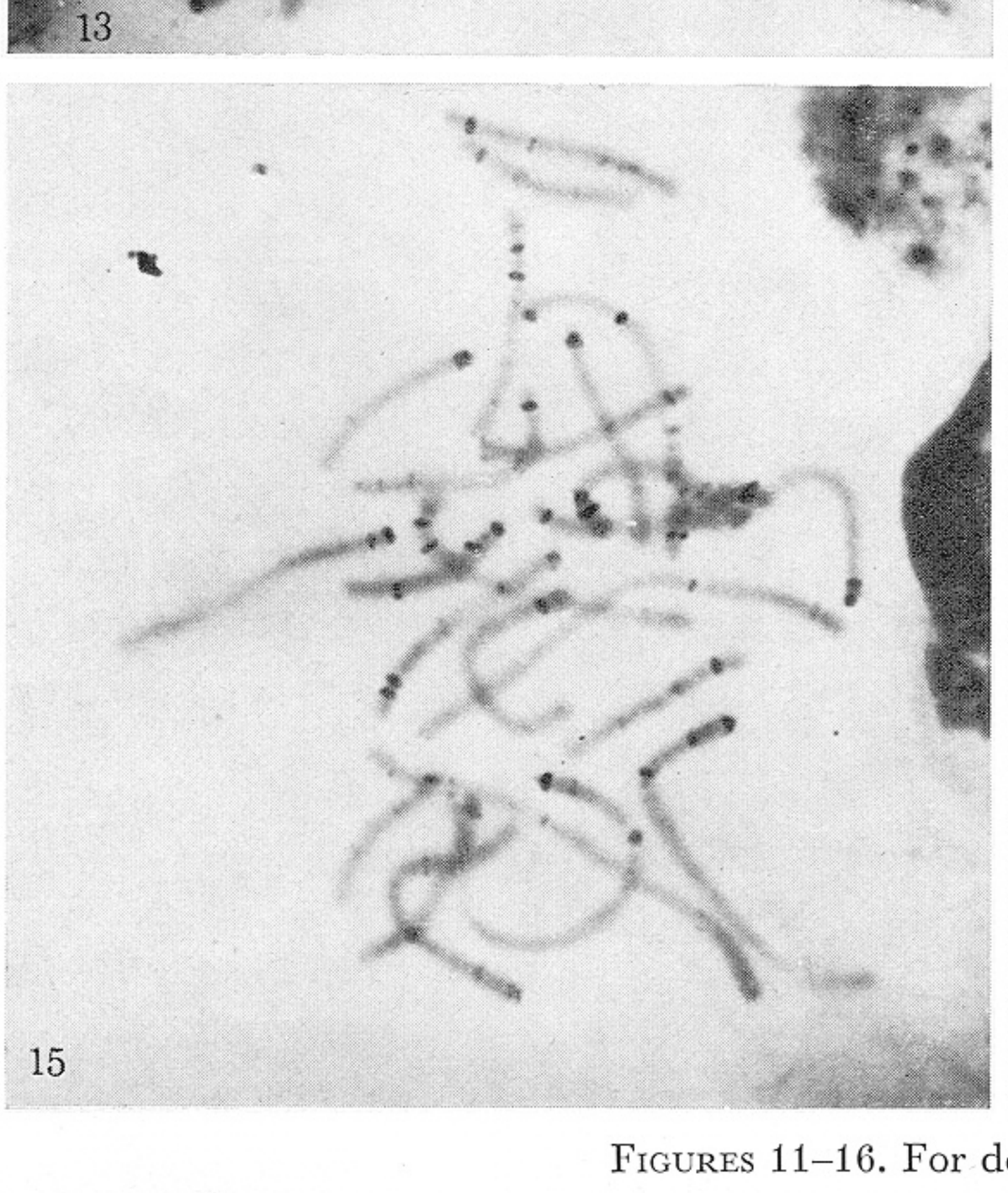


12

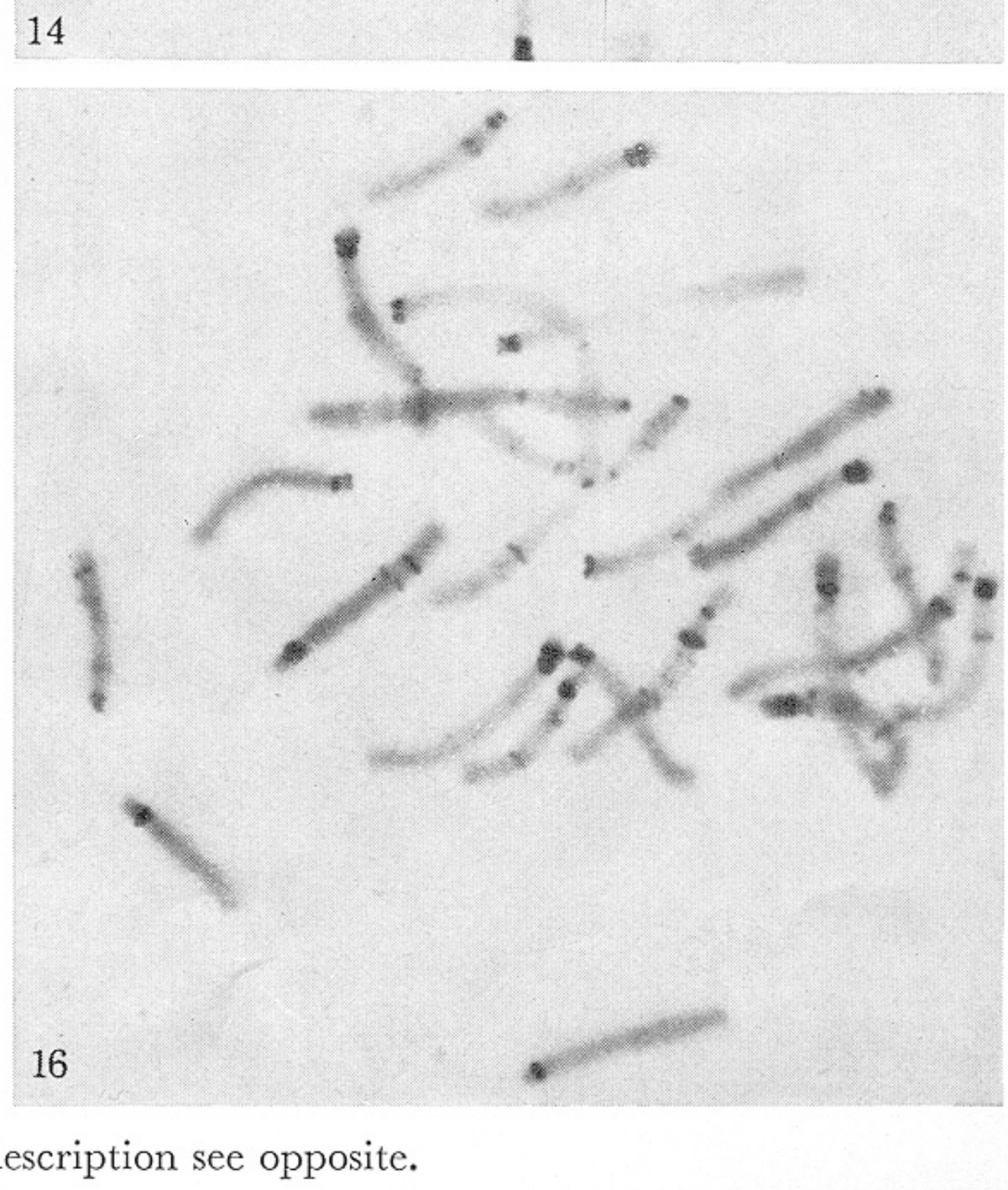


13

14

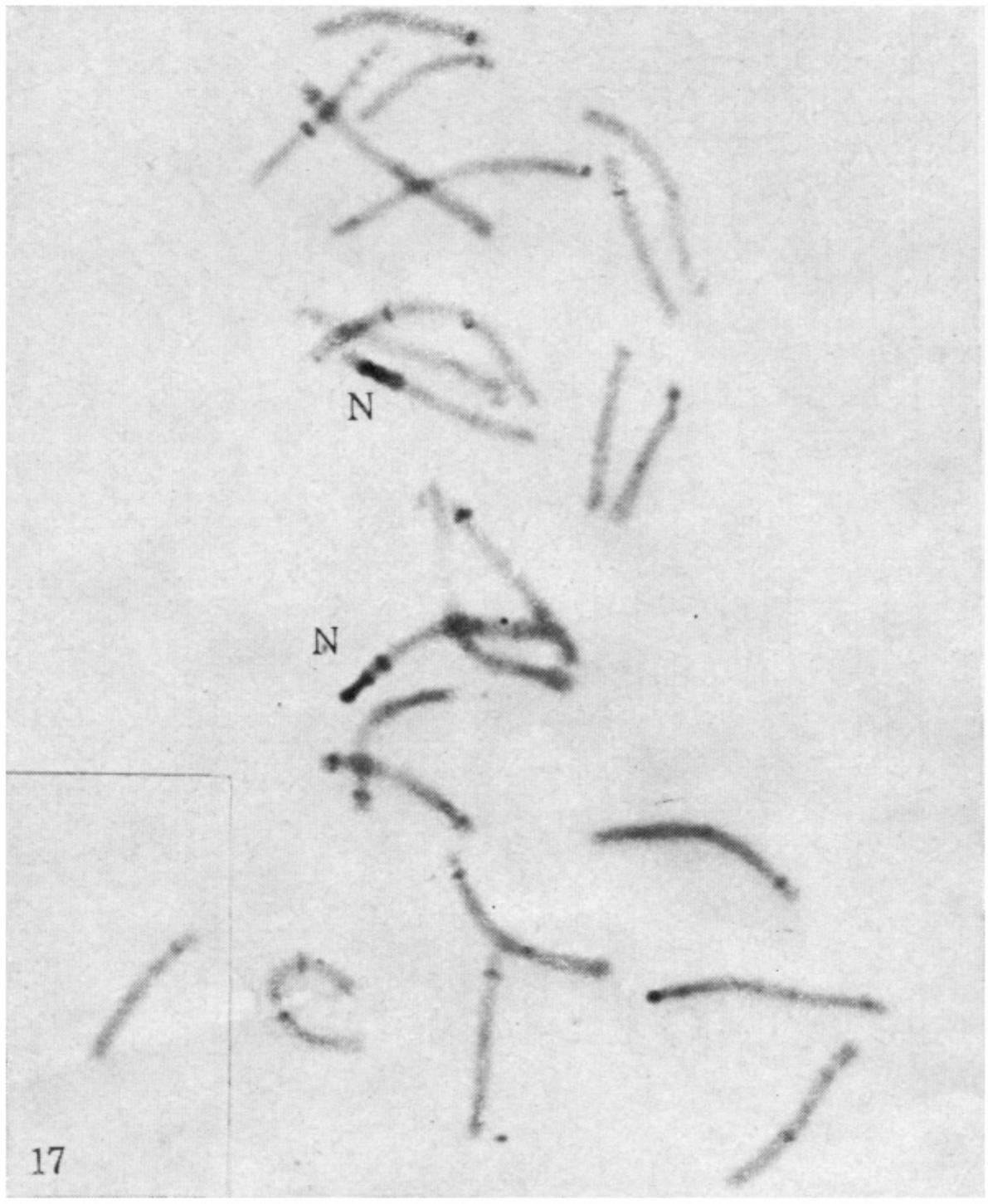


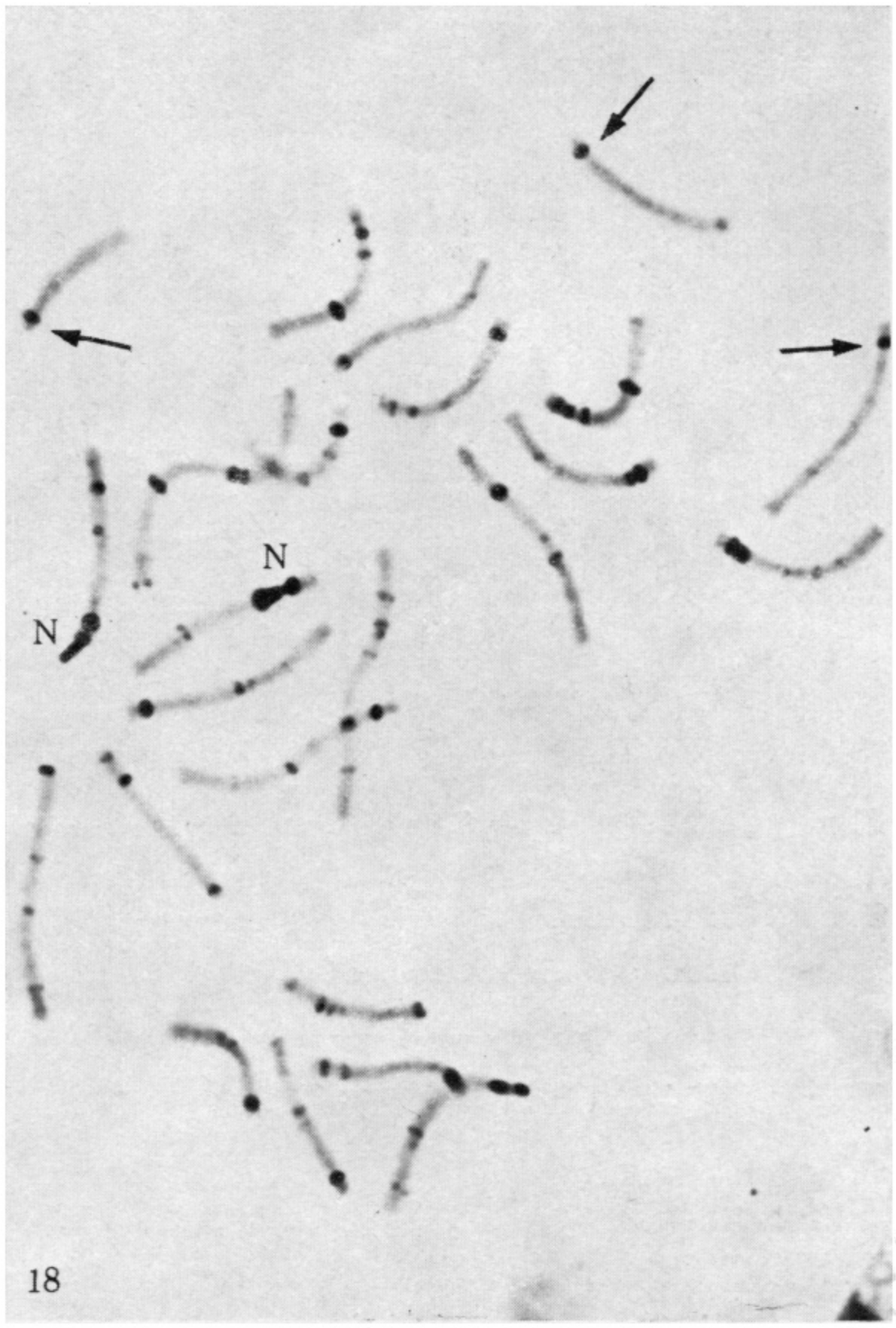
15

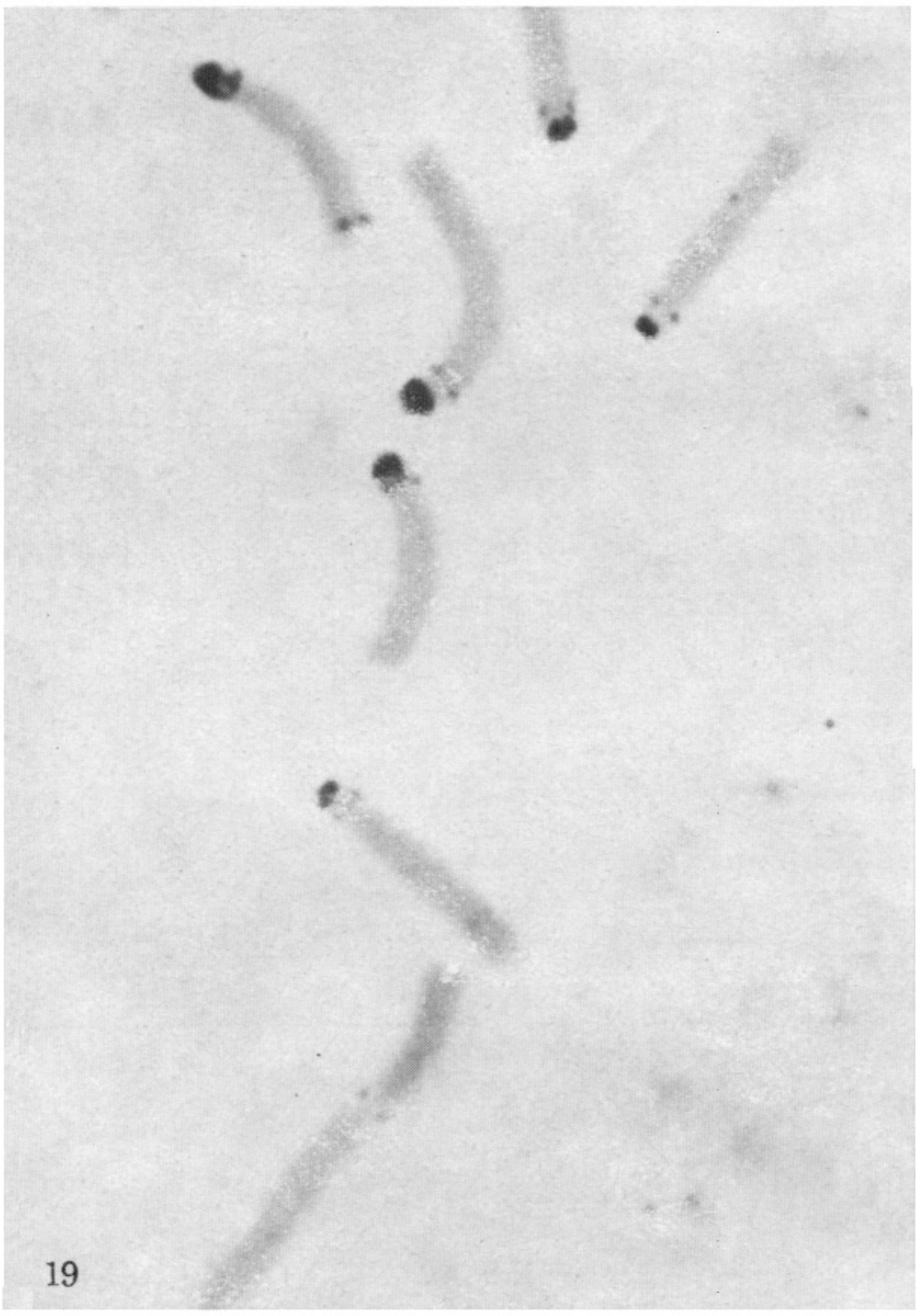


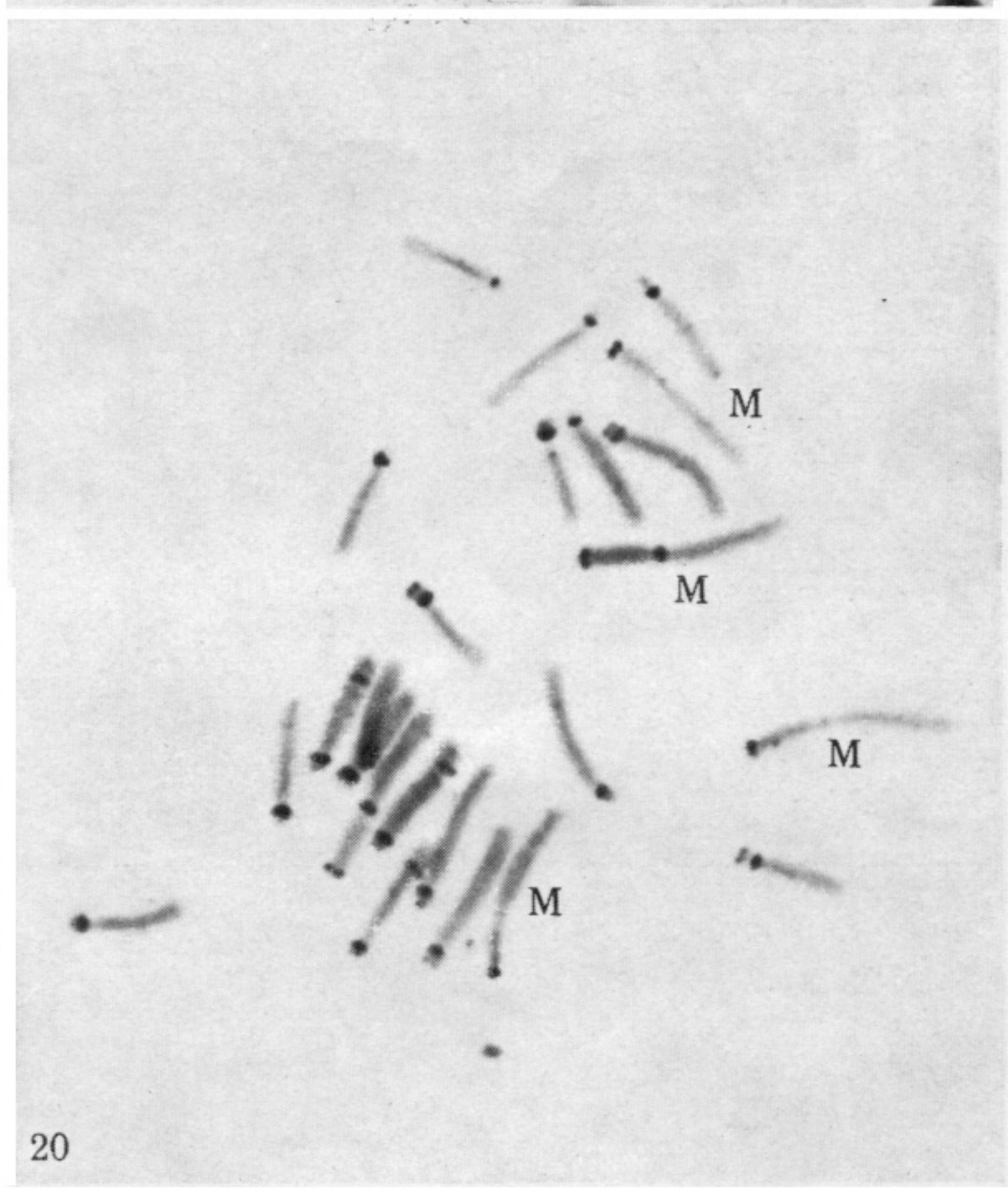
16

FIGURES 11–16. For description see opposite.









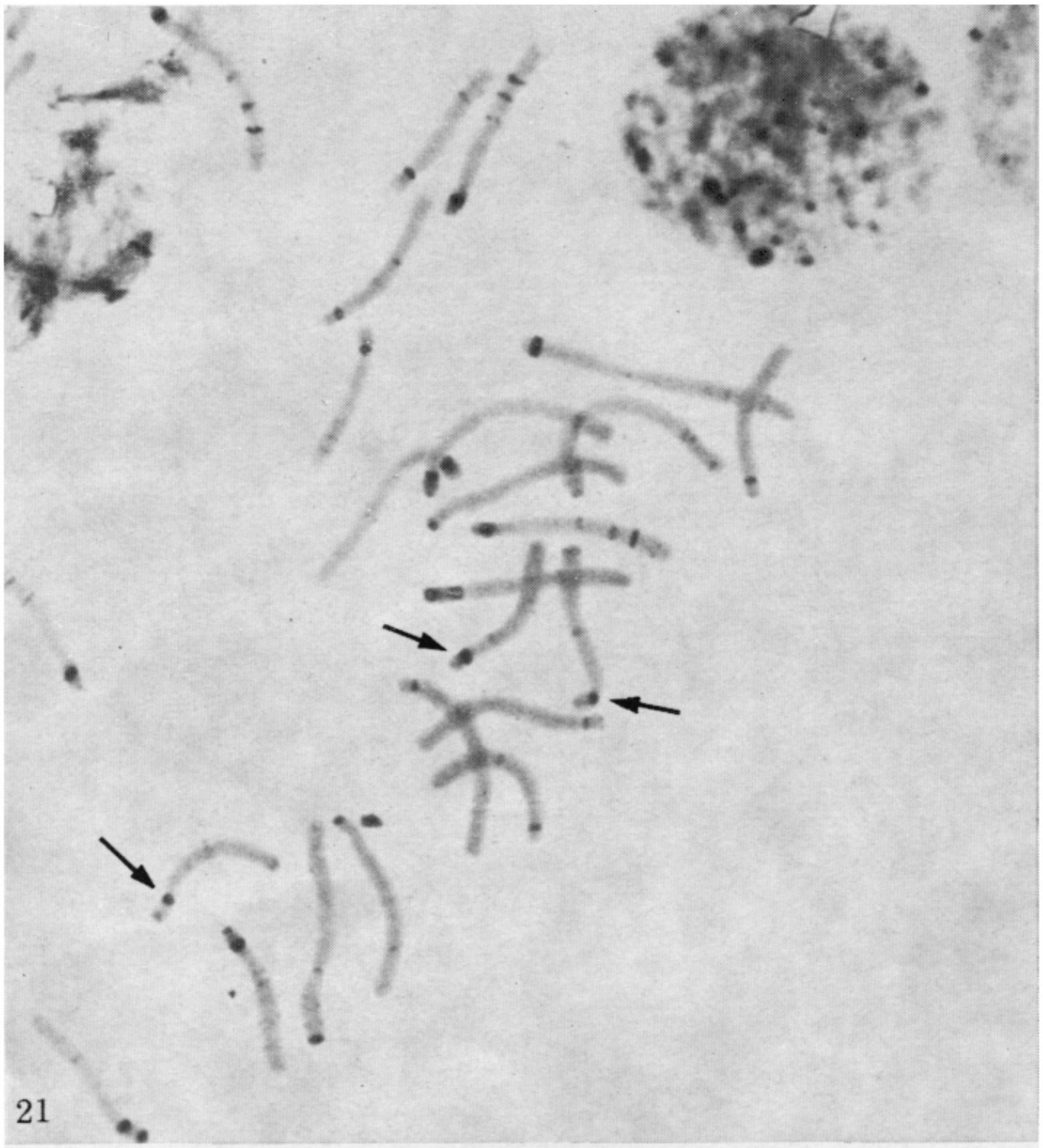
Figures 17–30 show metaphases from root tips of *Fritillaria*, differentially stained with Giemsa to show C-bands.

FIGURE 17. *F. reuteri*. Heteromorphy is evident in the nucleolar pair (N). (Magn. × 1650.)

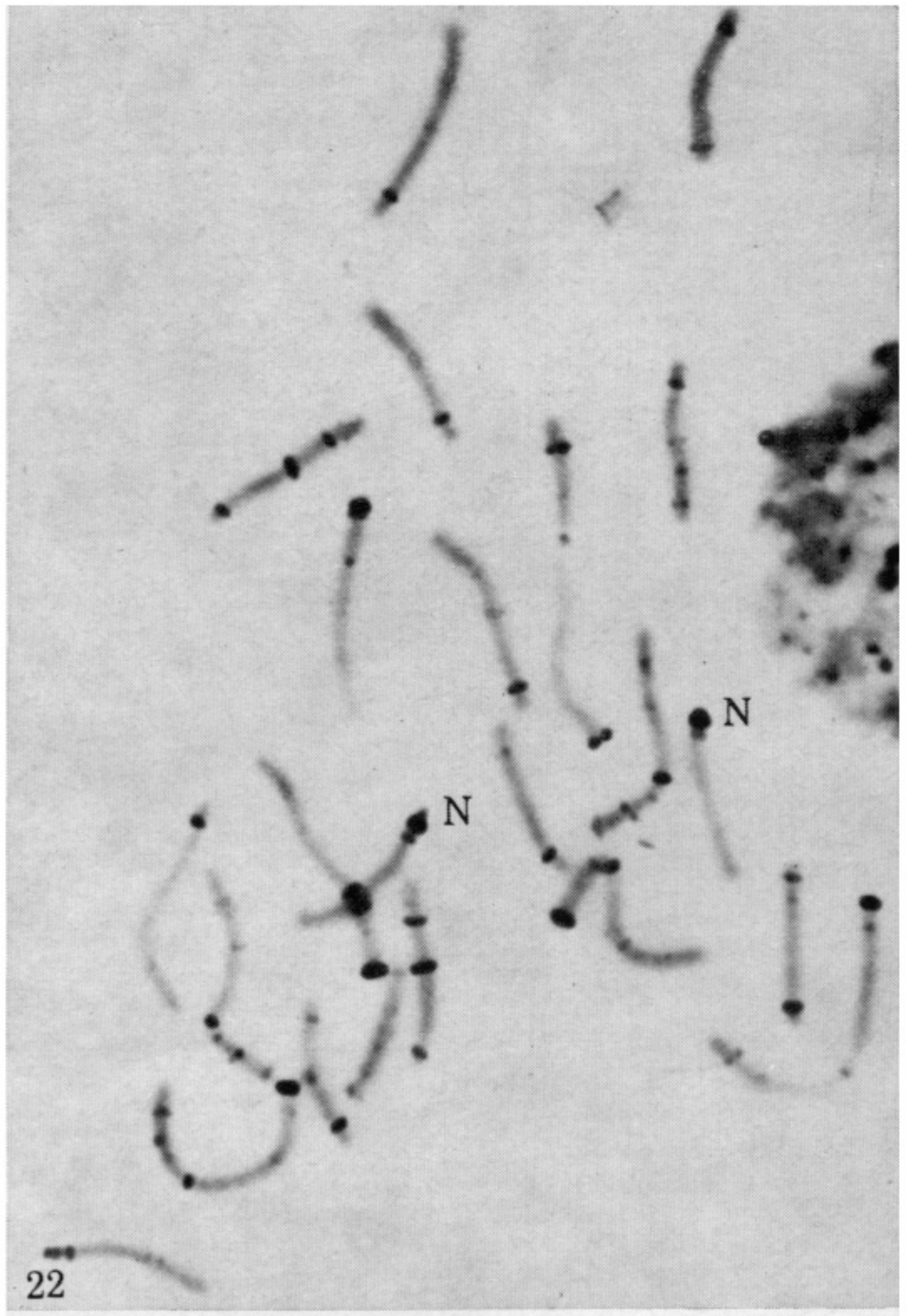
FIGURE 18. *F. rhodocanakis*. The centromeres are masked in many chromosomes by heterochromatin additional to that of the centromeres, as indicated in some ST-chromosomes by arrows. Heteromorphy is evident in the nucleolar pair (N). (Magn. × 1650.)

FIGURE 19. Some chromosomes of *F. acmopetala*, taken at high magnification to show the centromeric dots, representing the centromeres. (Magn. × 3000.)

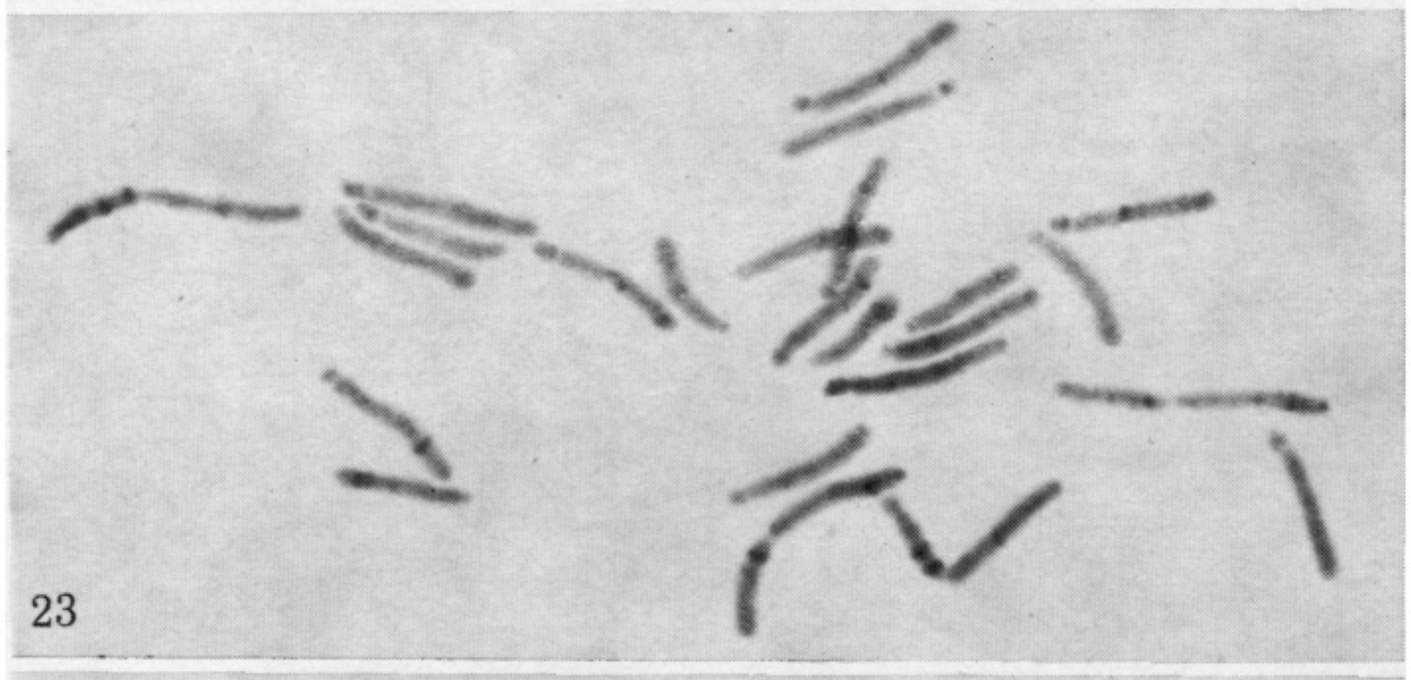
FIGURE 20. From *F. latifolia*. The M-chromosomes (M) show differences at the centromere; in 3 of them only one of the two centromeric dots is in view and in the fourth the centromere is masked by a contiguous band. (Magn. × 1650.)



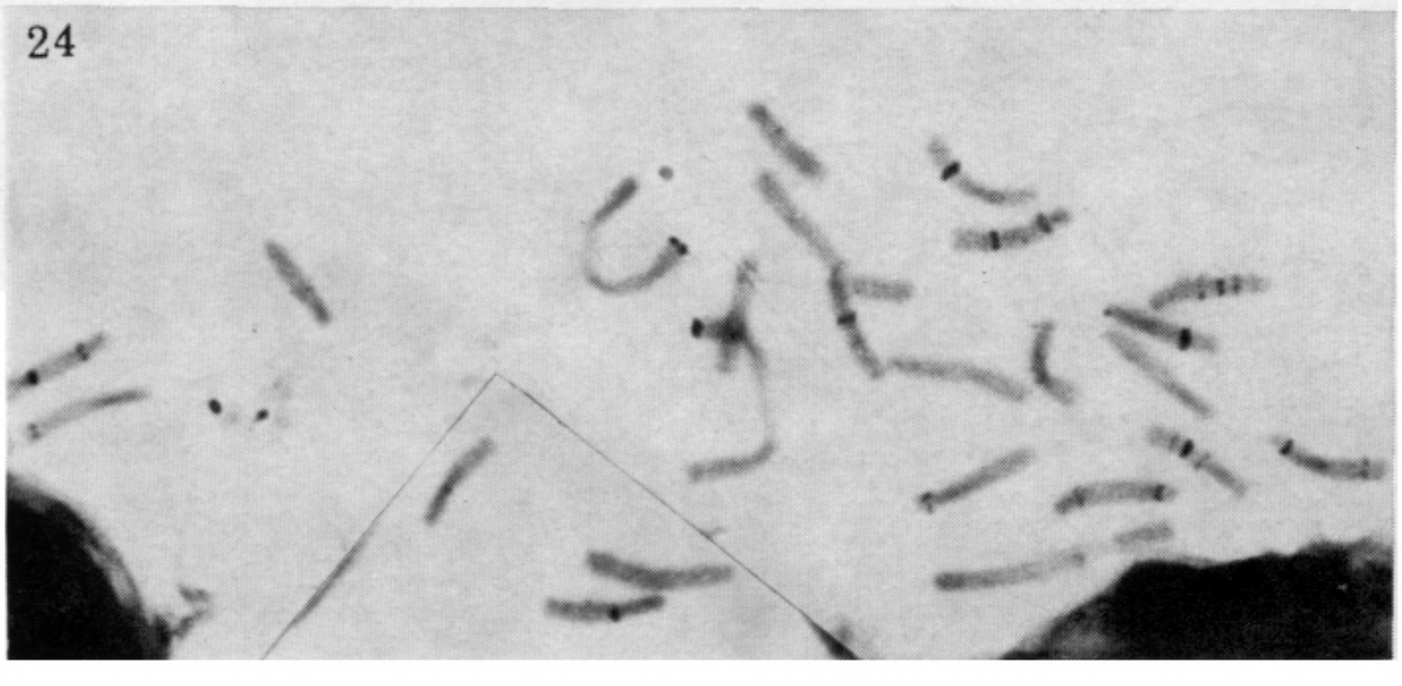
21



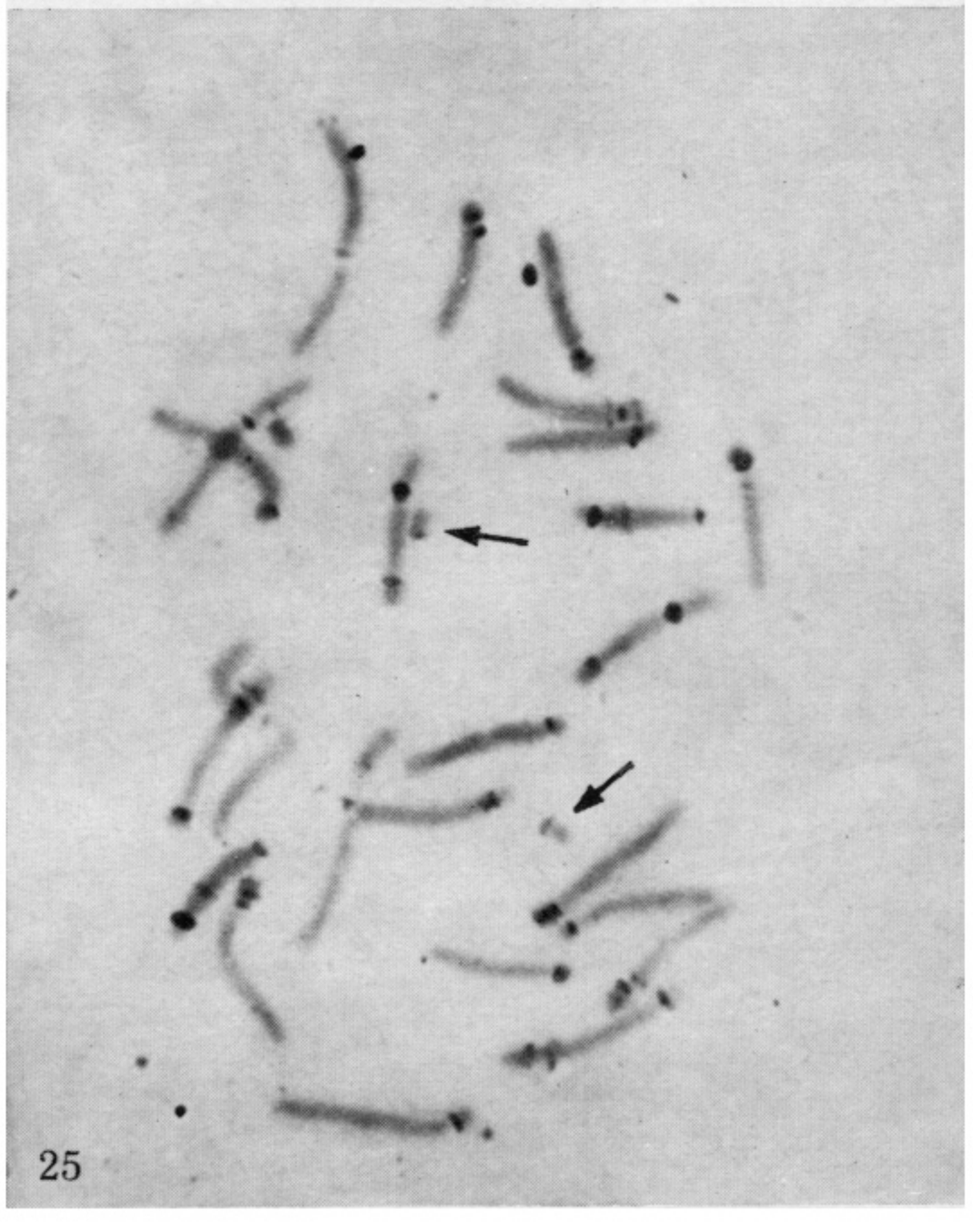
22



23

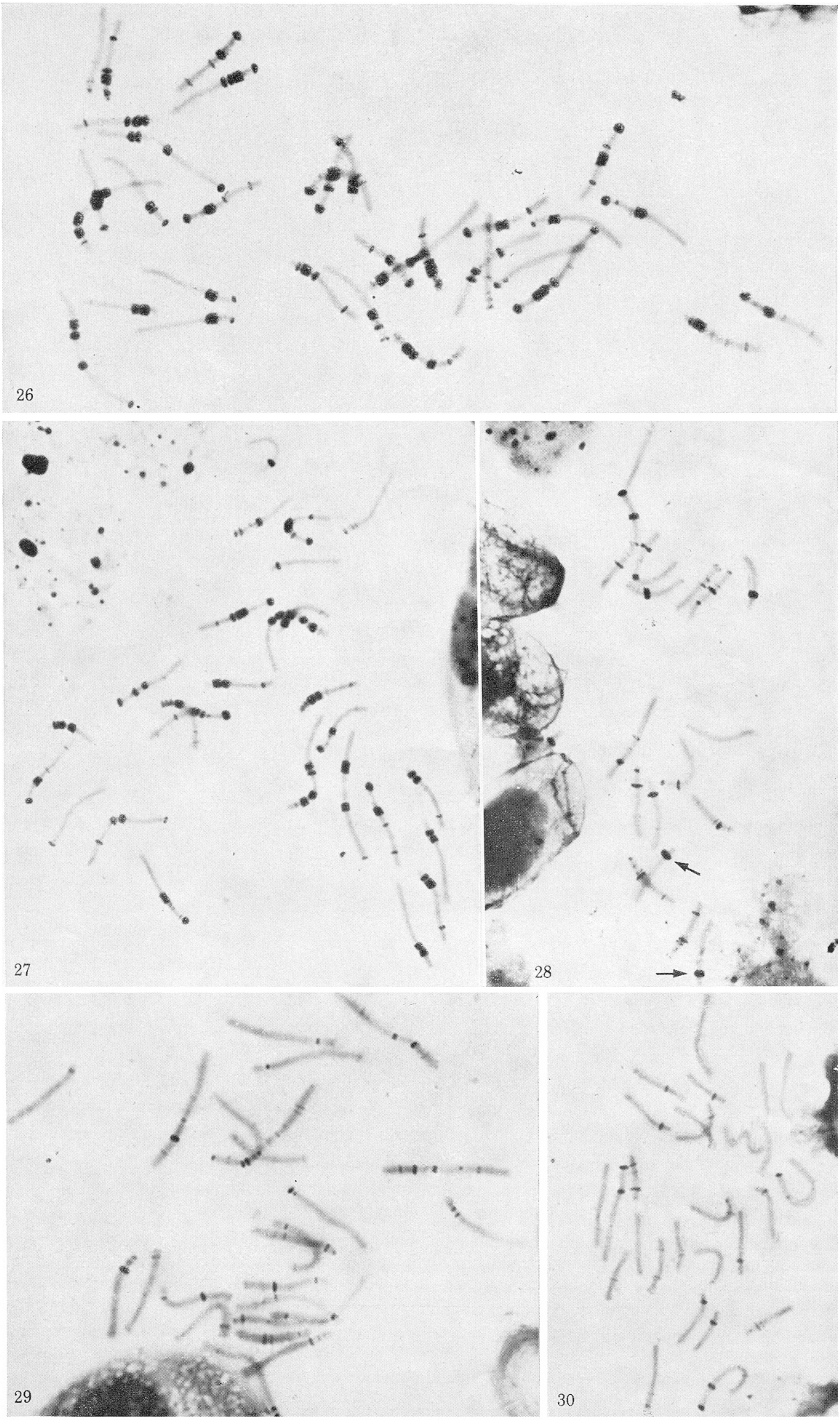


24



25

FIGURES 21–25. For description see opposite.



FIGURES 26–30. For description see opposite.