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Haploidy and Plant Breeding [and Discussion]

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Haploidy and plant breeding

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[Plate 1]

A haploid is an organism that looks like a sporophyte, but has the chromosome complement of a reduced gamete. There are several ways in which haploids can occur or be induced *in vivo*: spontaneously, mostly associated with polyembryony, and through abnormal processes after crosses, like pseudogamy, semigamy, preferential elimination of the chromosomes of one parental species, and androgenesis. In the crops described, haploids are or are near to being used in basic research and plant breeding.

The application of haploids in breeding self-pollinated crops is based on their potential for producing fully homozygous lines in one generation, which can be assessed directly in the field. Early generation testing of segregating populations is possible through haploids, because doubled haploids (DH) possess additive variance only. Haploids can also be applied in classical breeding programmes to make these more efficient through improved reliability of selection.

The application of haploids in cross-pollinated crops is also based on a rapid production of DH-lines, which can be used as inbred lines for the production of hybrid varieties. By means of haploids all natural barriers to repeated selfing are bypassed.

In autotetraploid crops there are two types of haploid. One cycle of haploidization leads to dihaploids; a second cycle produces monohaploids. The significance of dihaploids is in their greatly simplified genetics and breeding and in the possibility of estimation of the breeding value of tetraploid cultivars by assessing their dihaploids. The main drawback of dihaploids is their restriction to two alleles per locus. Also, after doubling, it is impossible to achieve tetra-allelism at many loci, the requirement for maximal performance of autotetraploid cultivars. Tetra-allelism can be obtained when improved dihaploids have a genetically controlled mechanism of forming highly heterozygous restitution gametes with the unreduced number of chromosomes. Monohaploids, after doubling or twice doubling, may lead to fully homozygous diploids and tetraploids. These are important for basic research, but not yet for practical application. Meiotic data of potato homozygotes at three ploidy levels are presented.

1. Origin and induction of haploids

A haploid is an organism that looks like a sporophyte, but has the chromosome complement of a reduced gamete. Haploids can originate *in vivo* or *in vitro*.

(*a*) In vivo *origin and induction*

Haploids may arise from the embryo sac in the following ways.

(i) Spontaneously, where it is often associated with polyembryony (for review see Lacadena 1974). High frequencies are found in oilseed rape, *Brassica napus* (Thompson 1969, 1974), and flax, *Linum usitatissimum* (Plessers 1963; Rajhathy 1976; Thompson 1977).

(ii) After a normal double fertilization with subsequent preferential elimination of the chromosomes of a specific genome in the early stages of embryo development (for reviews see Kasha 1974; Jensen 1975). Main examples are: barley, *Hordeum vulgare*, from interspecific hybridization with *H. bulbosum* (Lange 1969; Symko 1969; Kasha & Kao 1970); and wheat, *Triticum aestivum*, from intergeneric crosses with *Hordeum bulbosum* (Barclay 1975).

(iii) Through pseudogamy, which is the development of an unfertilized female gamete or cell after stimulation by the male nucleus (for reviews see Rowe 1974; Chase 1969). Important examples are: maize, *Zea mays*, from intervarietal crosses (Chase 1949); potato, *Solanum tuberosum*, from interspecific hybridization with *S. phureja* (Hougas *et al.* 1958); lucerne, *Medicago sativa*, from interspecific hybridization with *M. falcata* (Bingham 1969); tobacco, *Nicotiana tabacum*, from interspecific hybridization with *N. africana* (Burk *et al.* 1979); poplar, *Populus* species, from interspecific hybridization with mentor pollen (Stettler *et al.* 1969) or toluidine blue pollen treatment (Illies 1974); wheat cultivar Salmon with *Aegilops* cytoplasm after pollination with other cultivars (Tsunewaki *et al.* 1968).

(iv) Through semigamy, whereby reduced male and female gametes participate in embryogenesis but nuclear fusion does not occur; this process results in chimeral plants with sectors of maternal and paternal origin (for reviews see Turcotte & Feaster 1974; Choudhari 1978). An example is cotton, *Gossypium hirsutum* and *G. barbadense* (Turcotte & Feaster 1967), where a high haploid frequency was found in the doubled haploid line 57–4.

(v) Through androgenesis, whereby the maternal nucleus is eliminated or inactivated before fertilization of the egg cell and the haploid individual contains in its cells the chromosome set of the male gamete only (for review see Chase 1963). The frequency of this type is generally extremely low, but a dramatic increase in the frequency of androgenetic haploids occurred in the recessive maize mutant 'indeterminant gametophyte' (Kermicle 1969).

(*b*) In vitro *induction*

Haploids *in vitro* may be obtained by culturing anthers (for reviews see Sunderland 1974, 1980) and ovaries (for review see San Noeum 1978). A great deal of work has been done on anther culture since the early 1950s (Tulecke 1953). The first haploid plants were obtained in 1964 from *Datura innoxia* (Guha & Maheshwari 1964), closely followed by *Nicotiana tabacum* (Bourgin & Nitsch 1967) and the monocotyledon *Oryza sativa* (Niizeki & Oono 1968). By 1974, haploids from *in vitro* culture had been obtained in as many as 58 species (Sunderland 1974); this number is expanding rapidly. On the other hand, the number of species in which workable numbers can be produced at reasonable costs is still rather limited. The Solanaceae family compares favourably with most other plant families. Among the Gramineae the occurrence of undesirable albino plantlets from anther culture is common. As far as we are aware the haploid frequency is sufficiently high for practical application only in some species of *Nicotiana* and *Datura*. It is expected that this stage will, in due time, be reached in other genera.

Concerning *in vitro* haploid induction, Sunderland (1974, 1980) put forward a hypothesis of pollen dimorphism. Pollen *in vivo* contains a fraction of grains with a natural potential for embryogenesis. The proportion of this embryogenic pollen is under genetic and environmental control and is positively correlated with the yield of embryos *in vitro*. The incipient state of embryogenesis may also be induced in non-embryogenic pollen by pre-culture treatments, e.g. temperature stress, thus increasing the yield of embryos. Genetic control has been recognized by many researchers: large differences are found in embryogenic ability within a species

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and even within one segregating population. The effect of culturing on embryogenesis is influenced by many variables: the composition of the medium, the solid or liquid state of the medium, the stages of the microspores, the incubation conditions (light, temperature) and the nature of the material put into culture. Microspore stages are important in determining morphogenetic pathways. Two pathways can roughly be distinguished (Sunderland 1980), one leading to haploids via divisions of either the generative or the vegetative nucleus or of both, and the other leading to homozygous diploids and polyploids owing to fusion of pollen nuclei.

Both anther culture and haploid induction *in vivo* may give rise to heterozygous plants originating directly from so-called numerically unreduced ($=2n$) gametes. Different irregular meiotic events are known to lead to $2n$ gametes (see §2*c*).

2. Applications of haploids in plant breeding

The potential of haploids in plant breeding is dependent upon the nature of the crop: self-pollinated or cross-pollinated, diploid or autopolyploid.

(*a*) *Self-pollinated crops*

Self-pollinated crops are either diploids or allopolyploids. Pure allopolyploids are functional diploids and are often indicated as amphidiploids. They contain two genomes of each of two or three different species. The following crops are close to the use of haploids in breeding: the diploids barley, rice, *Datura* and flax, and the allopolyploids tobacco, cotton and wheat.

The basis of application is the so-called DH-line. A DH-line is a group of homozygous doubled haploids derived from a heterozygous diploid via monohaploids or amphimonohaploids. The potentials and advantages of using DH-lines in breeding may be summarized as follows.

(i) DH-lines enable the fastest possible production of homozygous lines, namely in one generation, whereas the conventional method of repeated selfings takes 6 or 7 generations.

(ii) DH-lines can be produced and reliably tested at any stage of a breeding programme from F_1, F_2 onwards to the purification of varieties.

(iii) DH-lines exhibit only additive and (additive × additive) variance, whereas early selfed generations contain a considerable proportion of non-selectable non-additive variance. Therefore DH-lines permit a more reliable selection in early generations and, in addition, an earlier and more reliable assessment of the breeding value of hybrid populations (Reinbergs *et al.* 1976). In view of possible undesirable linkages in the F_1, some breeders advocate the production of DH-lines in the F_2 or F_3.

(iv) DH-lines have the potential for improving the efficiency of existing breeding methods. So, in a recurrent selection method consisting of cycles of alternately intensive intercrossing and selfing of selected genotypes, selfing may well be replaced by DH-production because assessing DH-lines is much more reliable than assessing the selfed progeny of heterozygous plants.

(v) Whereas homozygous lines produced by repeated selfing are adapted to the climatic conditions of the region where they were produced, DH-lines have undergone no selection at all. This may be an advantage where breeding aims at producing varieties for regions with different climatic conditions (Kasha & Reinbergs 1975).

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It may be concluded that the main advantages of the haploid method in breeding self-pollinated crops are a saving of time through fast homozygosis and an improvement of the efficiency of classical breeding methods through the improved reliability of selection.

(*b*) *Cross-pollinated diploid crops*

Just as in self-pollinators, the application of haploidy in cross-pollinated diploid crops is based on the use of DH-lines. However, owing to inbreeding depression, these lines cannot be used directly, but only as parental inbred lines for the production of hybrid varieties. The haploid method is being applied or is approaching the stage of application in the following crops: maize, asparagus, cacao, sugar beet, turnip, cole crops and poplar.

When inbred lines are being developed via haploids, all barriers to repeated selfing, which are characteristic of natural cross-pollinators, are bypassed, e.g. dioecy, self-incompatibility and long juvenile periods. An additional advantage for self-incompatible crops is that selection for weak incompatibility alleles or genetic backgrounds inhibiting the activities of such alleles is avoided.

The time saving is particularly apparent in biennial crops (asparagus, which is also dioecious) and in crops with a long juvenile period (cacao, poplar). Only via haploidy can inbred lines be developed in these crops. The latter holds true also for developing all-male hybrid varieties of asparagus, because for this purpose supermale (YY) lines are needed, which can only be obtained through *in vitro* culture of anthers from normal male (XY) plants. Male asparagus hybrids are generally superior to female ones.

When DH-lines are produced, a large variation in vigour is normally observed. In this connection two aspects should be considered. First, when large numbers of DH-lines can easily be produced, selection of lines with a relatively high vigour may be carried out, so that an economical production of large amounts of single-cross seeds is feasible. Secondly, weak inbred lines may have valuable genotypes. Such genotypes could be saved when weak haploids can be hybridized somatically *in vitro* and the selection of F_1 hybrids is possible in the test tube on the basis of hybrid vigour. Such F_1 hybrids may then be used either as parents in three-way or double crosses, or directly if they can be propagated vegetatively on a large scale.

(*c*) *Autopolyploid crops* ($2n = 4x$)

We do not think that a practical application of haploidy is possible in autohexaploid crops like timothy grass (and maybe sweet potato), because halving their chromosome number leads to sterile trihaploids. The situation is different in autotetraploid lucerne and common potato. In autotetraploid crops, two successive cycles of haploidization are basically possible and have in fact been realized in the potato (Van Breukelen *et al.* 1977); first from tetraploid ($2n = 4x = 48$) to dihaploid ($2n = 2x = 24$), and secondly from dihaploid to monohaploid ($2n = x = 12$) (see figure 1). The first step is already a routine application; the second step is hampered by the generally very low frequencies of monohaploids as a consequence of (sub)-lethal genes, disharmonious genotypes in the 12-chromosome individuals, and the competition of the more vigorous plants from 2*n*-gametes. For both steps the dominant marker embryo-spot in the pollinator *Solanum phureja* (Hermsen & Verdenius 1973) is being widely used and is indispensable for the second step.

Some aspects of autotetraploid crops influence the exploitations of haploids in breeding. Thus, autotetraploids are highly heterozygous, and deleterious recessive genes are frequent in

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many varieties. As already mentioned, this reduces the frequency of monohaploids. Also, breeding of autotetraploid crops is complicated by tetrasomic inheritance. This is particularly so since the highest possible level of heterozygosity (four different alleles per locus = tetra-allelism) is desirable for quantitative traits, especially for yield (Mendoza & Haynes 1974; Busbice & Wilsie 1966; Dunbier & Bingham 1975).

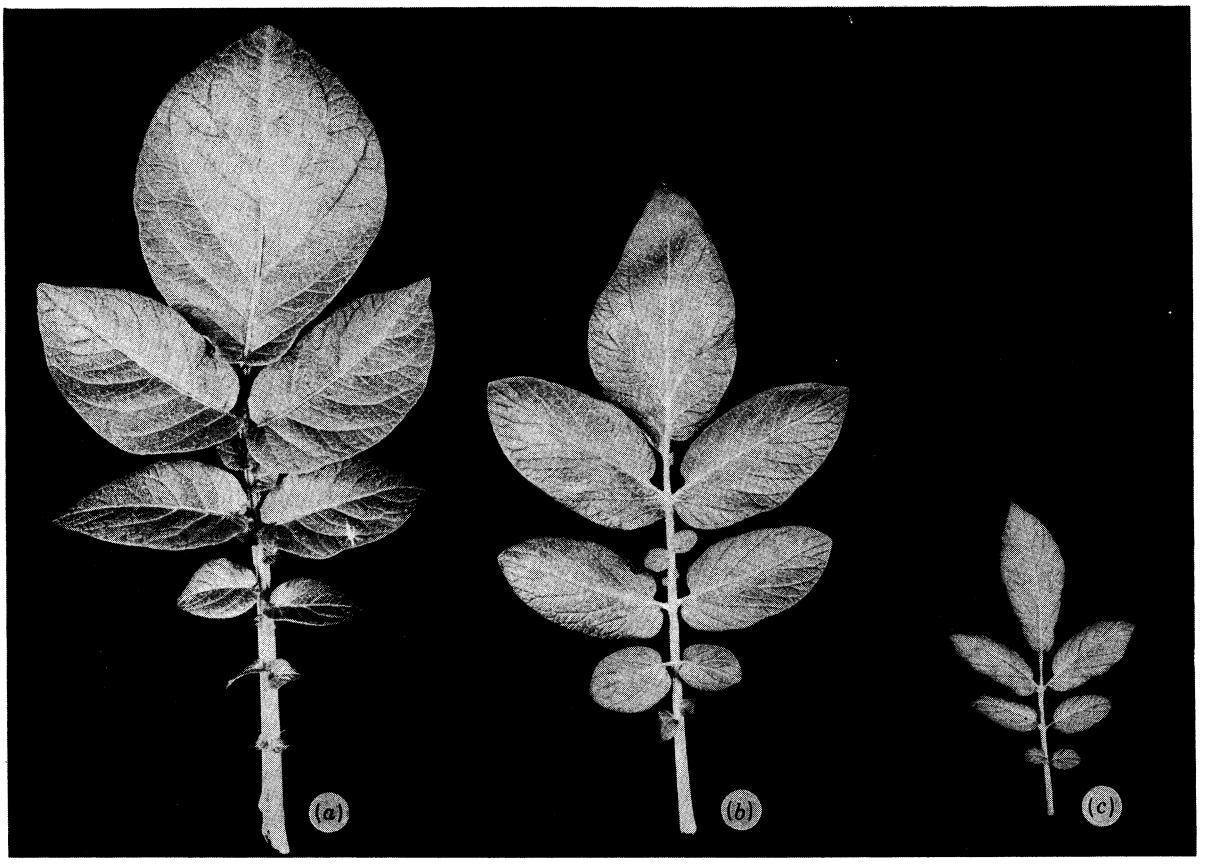

FIGURE 1. A representative leaf of potato cultivar Gineke (*a*) and of a dihaploid (*b*) and monohaploid (*c*) derived from that cultivar.

(i) *Dihaploids* ($2n = 2x$)

Obvious advantages of the application of dihaploids to breeding are the simplified genetics and breeding at the diploid level, the improved crossability with valuable diploid wild species which are indispensable, particularly in potato breeding, and finally the possibility of assessing dihaploids from cultivars (so-called 'gametic samples') to estimate the breeding value of each variety.

The great difficulties in working with dihaploids are a widespread occurrence of male sterility, a lowered female fertility and poor flowering of most dihaploids. In addition, self-incompatibility is introduced by haploidization, which hampers self-fertilization and may restrict crossability. A basic shortcoming of dihaploids is their restriction to two alleles per locus. For that reason we do not believe that diploid potatoes can ever compete with tetraploids.

If this is true, the breeder, after having obtained improved diploid genotypes, needs to have recourse to the tetraploid level in such a way that tetra-allelism is obtained at as many relevant

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loci as possible. Colchicine treatment or explant culture for doubling the chromosome number increase the coefficient of inbreeding and at best achieve di-allelism. A breeder therefore has to rely upon a genetically controlled meiotic abnormality leading to highly heterozygous restitution (or $2n$) gametes in the improved diploids. Such abnormalities result in the first instance from pseudohomotypic divisions (see figures 2 and 3, plate 1), in which there is no reduction division and no or hardly any genetic recombination, and secondly fused spindle formation at metaphase II of meiosis (see figures 4 and 5, plate 1), in which there is both reduction division and genetic recombination, the effect of which, however, largely disappears owing to nuclear restitution. These abnormalities, indicated as first division restitution (FDR), give rise to $2n$ gametes with a genotype nearly identical to that of the diploid parent.

The procedure in which these phenomena are used is called sexual or meiotic polyploidization. When a tetraploid cultivar is crossed with a diploid FDR genotype, unilateral sexual polyploidization occurs; if two diploid FDR genotypes are intermated, bilateral sexual polyploidization may take place. Both procedures may lead to highly heterozygous and vigorous tetraploid progeny.

The use of the FDR process in breeding is dependent on its inheritance. The fused spindle mechanism, which in some clones occurs in 70–80% of the pollen mother cells (see figure 4), is controlled by one recessive gene, according to Mok & Peloquin (1975). Iwanaga (1980) found the average frequency of that gene in 51 potato cultivars to be 70%. This high frequency suggests either a selective advantage of that gene or a role of that gene in the evolution of the potato. Similar genes may have played a role in the evolution of many other genera in which the restitution mechanism has been detected (Harlan & De Wet 1975).

(ii) *Monohaploids* ($2n = x$)

Monohaploids from an autotetraploid crop are the only tool for the production of homozygous diploid and tetraploid genotypes of such a crop. Briefly, the procedure is twice haploidizing followed by twice doubling.

$$\underbrace{\text{cultivar} \rightarrow \text{dihaploid}}_{\text{heterozygous}} \rightarrow \underbrace{\text{monohaploid} \rightarrow \text{DH} \rightarrow \text{doubled DH}}_{\text{homozygous}}$$

In potato monohaploids, DH clones and doubled DH clones have been produced, and some could be studied cytologically. More than 90% of the 2247 pollen mother cells of 31 monohaploids studied had univalents only. In the 181 remaining pollen mother cells, the pairing configurations were 1, 2, 3 or even more bivalents, and in one cell a trivalent occurred. Clear differences in the amount of pairing were found between the monohaploids studied.

Description of plate 1

Figures 2 and 3. First division restitution through pseudohomotypic division in the potato clone Y 17. Bar is 10 μm

Figure 2. Upper cell mainly with univalents, lower cell with most univalents dividing mitotically.

Figure 3. A group of cells in different meiotic stages: metaphase I, anaphase and telophase I. Typical second division stages are lacking in this clone and dyads are formed at the end.

Figures 4 and 5. First division restitution through fused spindles in the potato clone IV P 10. Bar is 10 μm.

Figure 4. Metaphase II of meiosis showing cells with fused spindles (the majority) and cells with non-fused spindles that are either parallel (arrows) or at an angle (asterisk).

Figure 5. Dyads formed as a result of spindle fusion.

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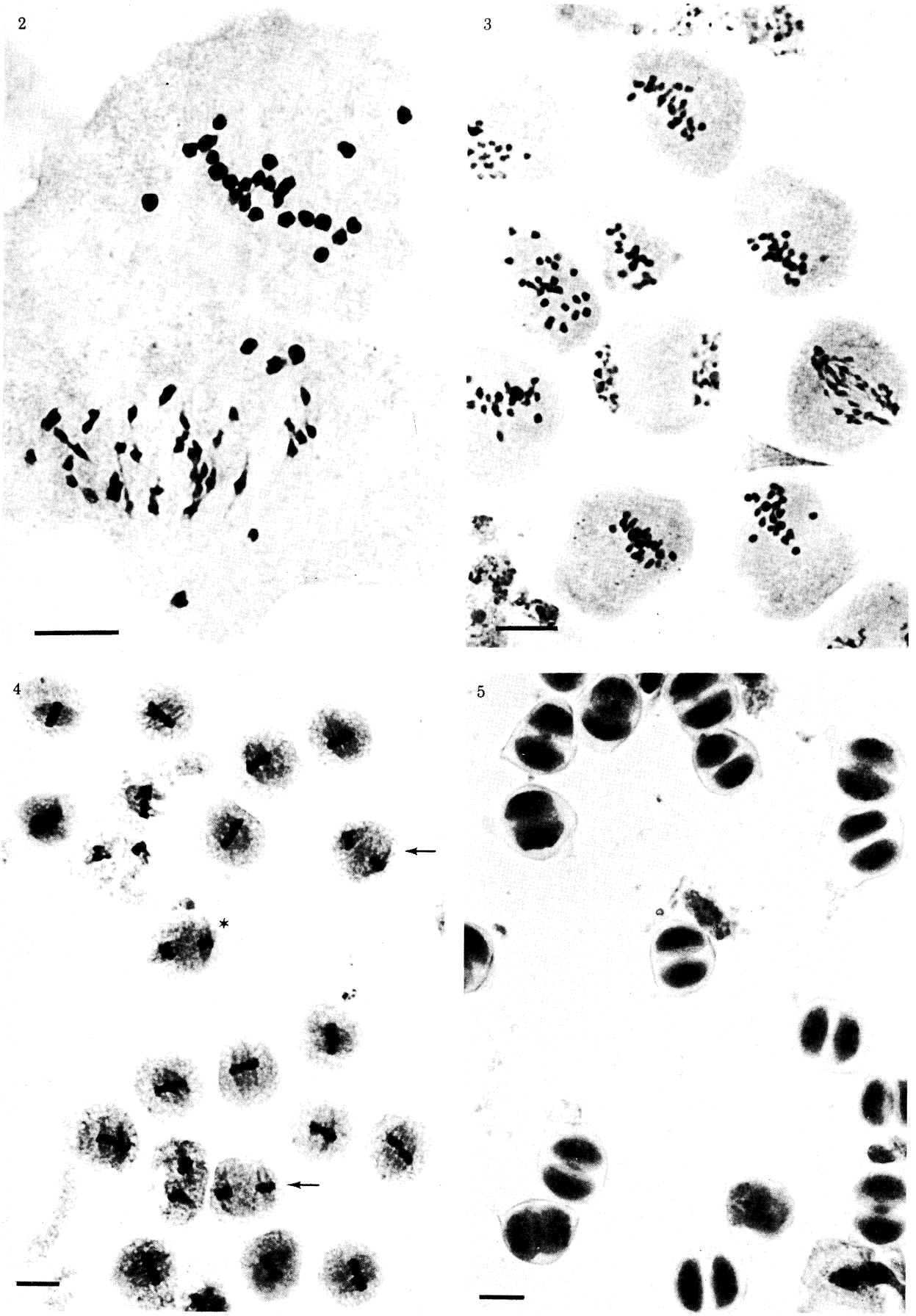

FIGURES 2–5. For description see opposite.

(*Facing p.* 504)

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Meiosis was also studied in eight double monohaploids. In six of them meiosis was nearly normal (in most cells 12 bivalents), but in two a high frequency of univalents was found, which may have been caused by desynapsis.

DH clones were doubled to the tetraploid level, and four such homozygous tetraploids were studied meiotically. The number of quadrivalents in three of the tetraploidized monohaploids was very high (6.1–10.5 per cell) compared with that in cultivar Gineke (2.4 per cell). One tetraploid clone behaved similarly to its diploid parent in showing desynapsis. The high average number of quadrivalents in most homozygous autotetraploids compared with cultivars might indicate that potato cultivars are not fully autotetraploid and display a decreased homology between their genomes.

The potential of monohaploids for potato breeding is very limited. Unless monohaploids can be produced in large numbers and somatic hybridization of monohaploids becomes routine, the use of monohaploids for breeding hybrid varieties does not make sense.

The new idea of growing potatoes from true seeds, which despite disadvantages has a number of striking advantages, especially for use in developing countries, has been associated with the production of homozygous genotypes and F_1 hybrids to improve the uniformity of the crop. However, with the knowledge and material available, the idea may at present only be realized by exploiting the FDR mechanisms mentioned previously.

3. Conclusion

The lack of methods for an efficient and cheap production of large numbers of haploids has limited the number of crops in which haploids can be used for breeding purposes. There are worldwide interests and activities in the field of induction and use of haploids in basic research and breeding. It may be expected that in the decades ahead rapid progress will be made.

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Discussion

J. Heslop-Harrison, F.R.S. (*Welsh Plant Breeding Station, Aberystwyth, U.K.*). Professor Hermsen has described an interesting mechanism for the formation of diploid gametes. As I understand his account, diploidization occurs by the fusion of metaphase plates after meiosis I, an event observed as an occasional aberration in many dicotyledons. However, his micrographs show an unusually high proportion of meiocytes with parallel meiosis II spindles. In dicotyledons in general, the spindles of the second division tend always to be at right angles – this is a pre-requisite, indeed, for the cleavage that gives the tetrahedral disposition of the spores in the

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tetrad. Would one be right in supposing that the genetic stocks that he is dealing with have an abnormally high incidence of second-division cleavage anomalies?

J. G. T. Hermsen. In this particular genotype high frequencies of fused spindles and 2*n* pollen are found. There are other plants, however, with far fewer 2*n* pollen grains. This indicates that the expressivity of the responsible gene varies according to the genotype. This is quite common with meiotic mutants. The gene appears to be present in many genotypes of many species.

A. P. M. den Nijs (*Institute for Horticultural Plant Breeding, Wageningen, The Netherlands*). I should like to underline Professor Hermsen's statement that these gametes are of frequent occurrence. Searches for 2*n* pollen and 2*n* eggs in many diploid species of the *Solanum* polyploid complex by several workers have revealed substantial numbers of plants producing a moderate to high percentage of 2*n* pollen or 2*n* eggs in addition to normal gametes. One single recessive character, *parallel spindles* (*ps*), has been found to be responsible for the production of FDR 2*n* pollen. Genetic control of 2*n* egg production has been indicated. The *ps* allele frequently occurs in many accessions of various diploid species, suggesting a role for this gene in sexual polyploidization during the evolution of the polyploid complex of the tuber-bearing Solanaceae. If so, the gene should also be encountered in polyploid species and it has indeed often been found in *Solanum tuberosum* ($2n = 4x$), for example, and in dihaploids derived from it. The occurrence of 2*n* gametes facilitates gene flow from the diploid level to the tetraploid and even the hexaploid level by unilateral (e.g. $4x \times 2x \rightarrow 6x$) and bilateral (e.g. $2x \times 2x \rightarrow 4x$) sexual polyploidization.

J. G. T. Hermsen. The role of 2*n* gametes in the evolution of polyploid complexes has been suggested by several authors and reviewed by Harlan & De Wet (1975). Regarding the genus *Solanum*, Dr den Nijs referred to the recessive gene controlling parallel spindles (*ps*). Dr Ramanna and I believe that only fused spindles invariably lead to FDR gametes, whereas parallel spindles are incidental orientations of the two metaphase II spindles, which may or may not lead to FDR gametes (see figure 4). Professor Heslop-Harrison made the interesting suggestion that the *ps* gene might well cause a random orientation of second metaphase spindles and this might lead to a greater chance of fused spindles compared with the normal orientation at an angle of 60° in *Solanum* genotypes not having *ps*. However, this hypothesis can hardly explain the high frequency of pollen mother cells with fused spindles.

To return to the original question of Professor Heslop-Harrison about cleavage anomalies, Dr M. S. Ramanna has found that the parallel orientation of metaphase II spindles, although theoretically unexpected, may occur at very high frequencies in normal tetrad-forming genotypes. Ramanna's interpretation is that, regardless of their orientation at metaphase II, the spindle axes are reoriented through the formation of the secondary phragmoplasts at the end of telophase II. This phenomenon is well known in dicotyledonous angiosperms with simultaneous cytokinesis during the second division of meiosis. We have diploid potato clones that produce nearly 100% dyads instead of tetrads. If we suppose that dyad formation is a consequence of abnormal cleavage, these clones must have a high incidence of cleavage abnormalities.

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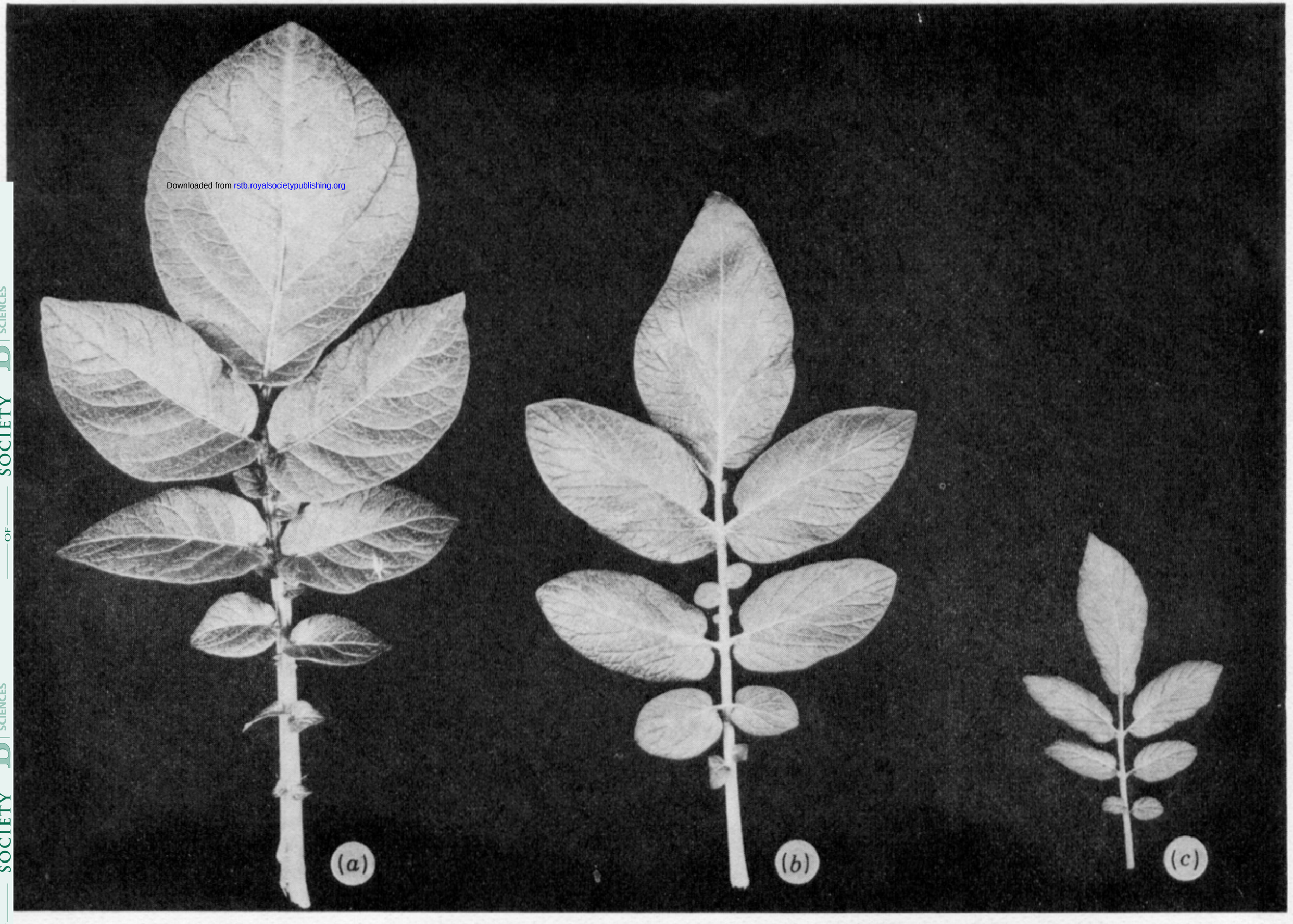

FIGURE 1. A representative leaf of potato cultivar Gineke (*a*) and of a dihaploid (*b*) and monohaploid (*c*) derived from that cultivar.

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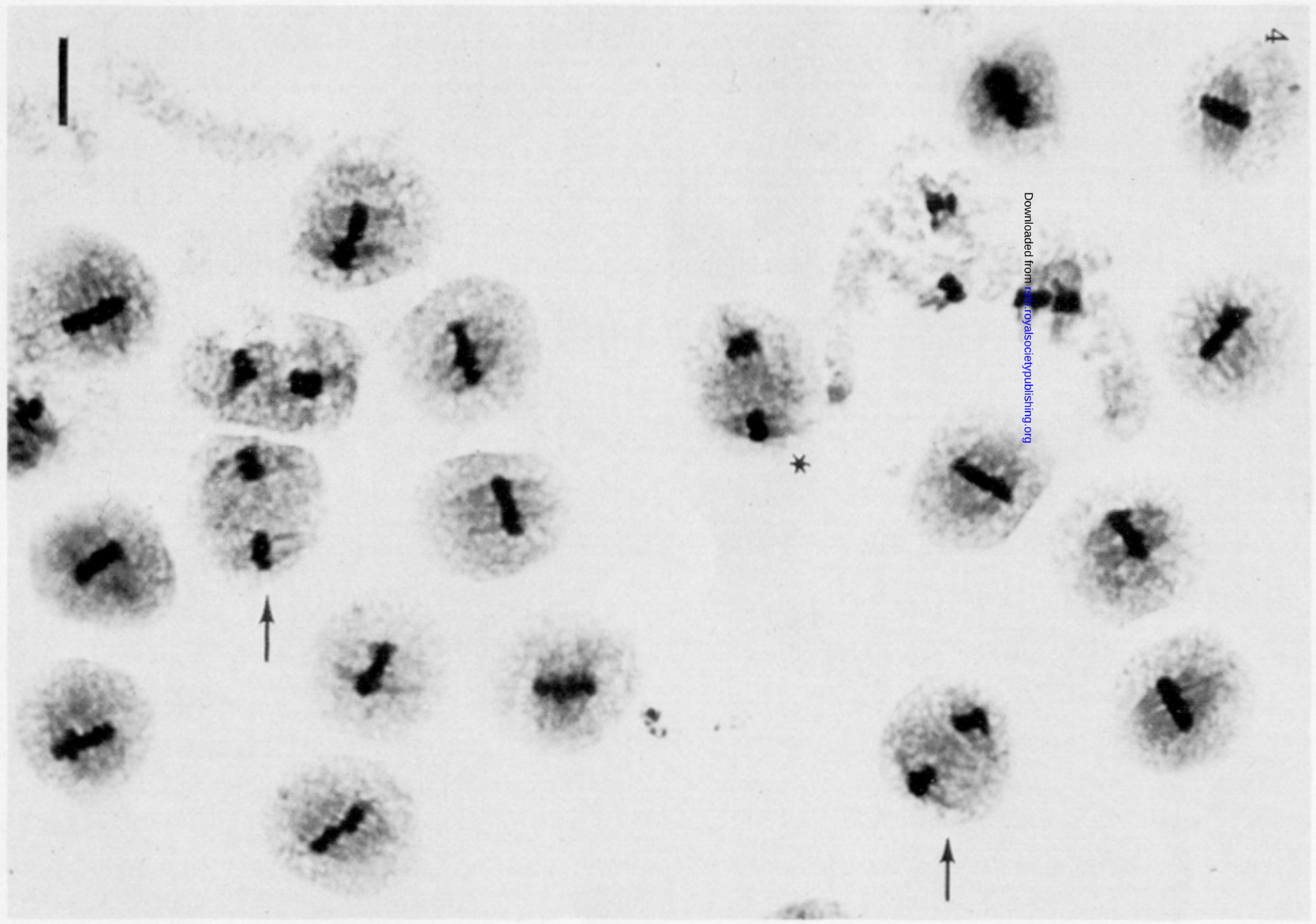

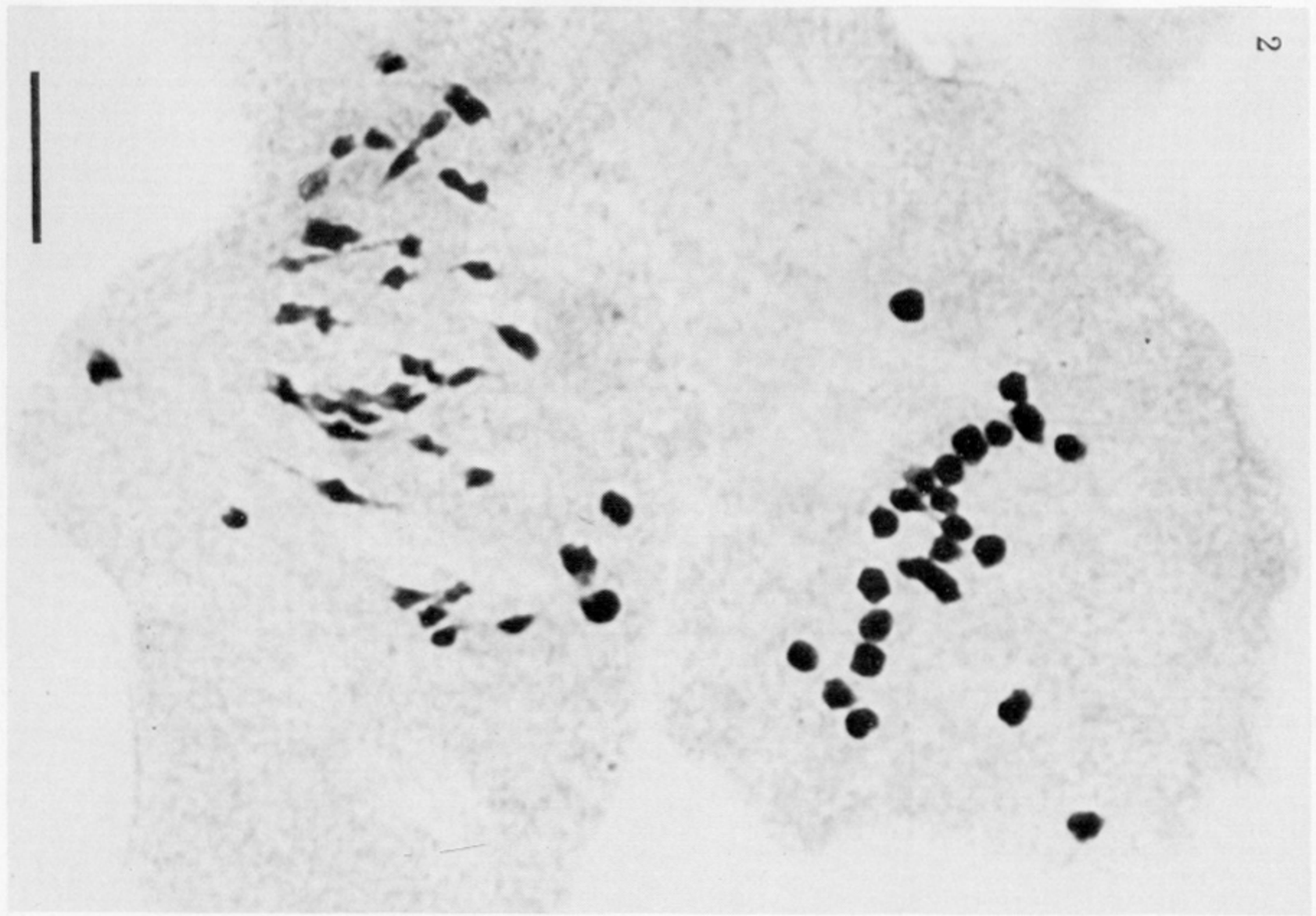

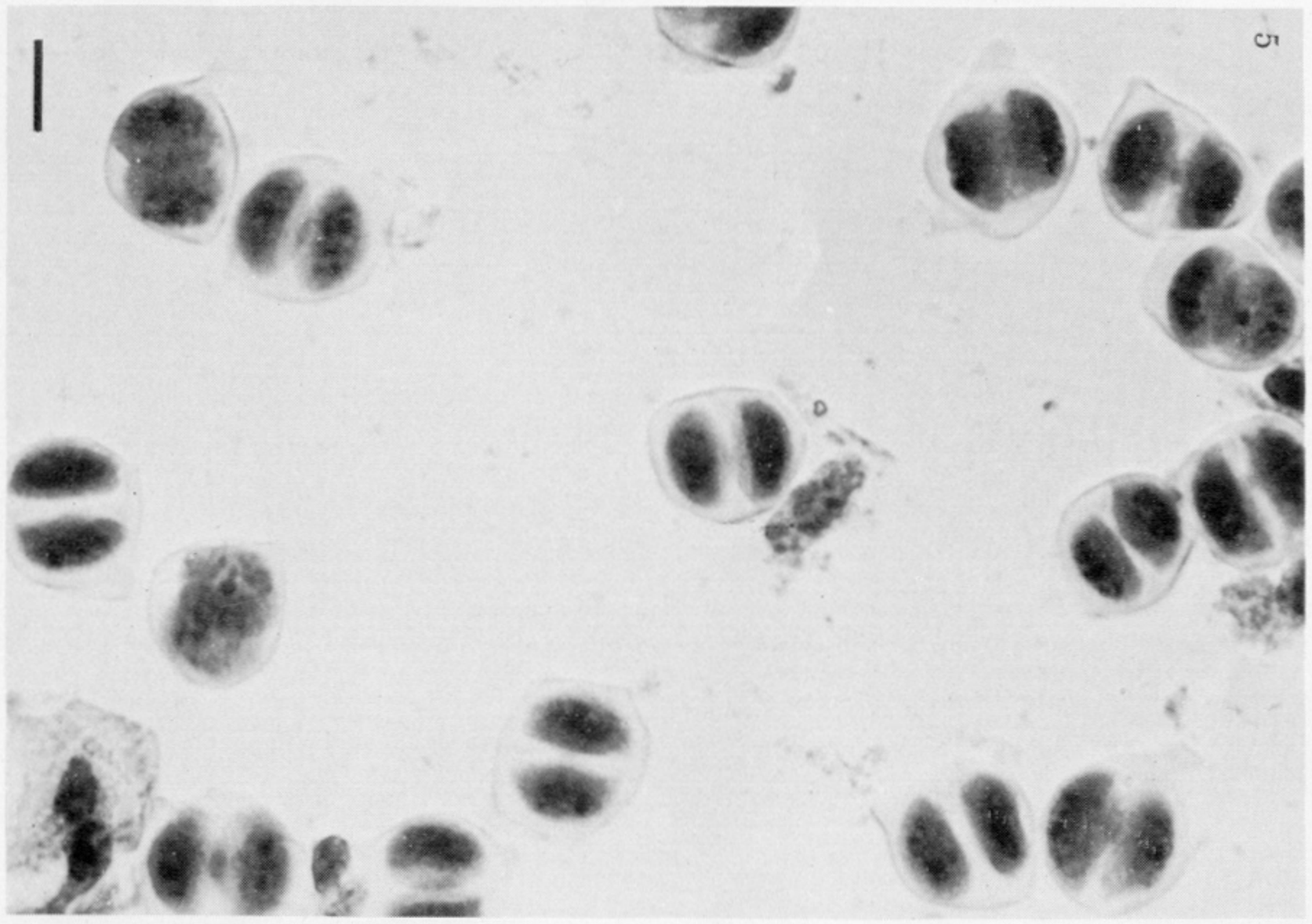

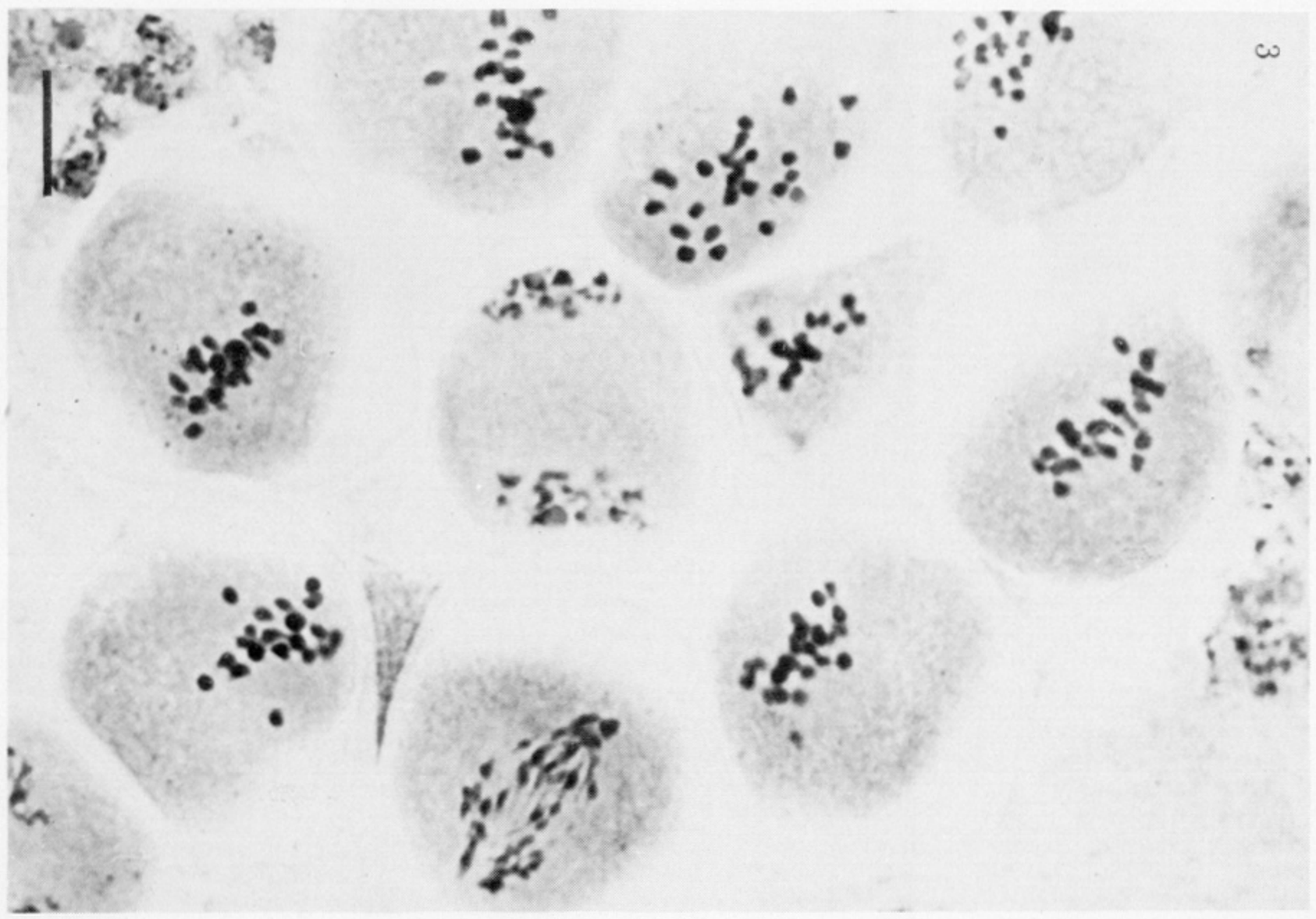

FIGURES 2–5. For description see opposite.