Origin of facultative heterochromatin in the endosperm of *Gagea lutea* (Liliaceae)

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Dedicated to Professor Walter Gustav Url on the occasion of his 70th birthday

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Summary Facultative heterochromatin occurs not only in certain animals in connection with sex determination but also in at least one plant genus, *Gagea* (Liliaceae s. str.), but here in the course of embryo sac development, fertilization and endosperm formation. The present contribution intends to provide undebatable photographic and cytometric evidence, previously not available, for the events in the course of which three whole genomes in the pentaploid endosperm nuclei of *Gagea lutea* become heterochromatinized. In this plant, embryo sac formation usually follows the Fritillaria type, i.e., the embryo sac is tetrasporic, and a '1 + 3 position' of the spore nuclei is followed by a mitosis in which the three chalazal spindles fuse and two triploid nuclei are formed. A triploid chalazal polar nucleus is derived from one of these, which contributes to the pentaploid endosperm. These nuclei in the chalazal part of the embryo sac show stronger condensation compared with the micropylar ones. The pycnosis of the triploid polar nucleus is maintained and even enhanced during endosperm proliferation, while the micropylar polar nucleus and the sperm nucleus maintain their euchromatic condition. The origin of the heterochromatic masses in the endosperm nuclei from the three chalazal genomes of the central cell is unambiguously evident from the distribution of heterochromatic chromosomes in the first endosperm mitosis and the following interphase. DNA content measurements confirm a 3:2 relationship of heterochromatic and euchromatic chromosome sets, which is usually maintained up to the cellularized endosperm. Pycnotic nuclei in the chalazal part of megagametophytes are characteristic for several embryo sac types, but *Gagea* spp. it is documented that such nuclei can take part in fertilization and endosperm formation.

Key words DNA content, embryo sac development, endosperm, facultative heterochromatin, fertilization, *Gagea lutea*

Introduction

It has remained largely unknown for long time that facultative heterochromatin does occur in plants. According to the classical view, those chromosome segments or chromosomes that form chromocentres (condensed chromatin bodies) after telophase, in which they do not decondense as strongly as euchromatin does, are defined as heterochromatin (Heitz 1933). Brown (1966) made the important distinction between facultative and constitutive heterochromatin, the latter being characterized by a permanently condensated state, while the former is euchromatin that became inactivated during a certain developmental stage and may become re-activated later on. Facultative heterochromatin is well known from mammals, in which one of the

two X chromosomes in the female sex is inactivated and heterochromatinized (Barr and Bertram 1949; Lyon 1961), and from coccids, in which the paternal genome is heterochromatinized in males (Brown and Nur 1964; for review, see John 1988). The first observation relevant for the occurrence of facultative heterochromatin in plants can be ascribed to Geitler (1950) who noted in the liliaceous plant *Gagea lutea* a striking difference of nuclear structure between somatic and endosperm nuclei. The latter contained conspicuous masses of sticky heterochromatin that were completely absent in the former. Geitler (1950) following Darlington (1947) interpreted these masses as "excessive formation of thymonucleic acid in non-polymerized form". A quite different explanation was later provided by Romanov (1961; in English translation 1962), who had studied embryo sac development in *Gagea* spp. and presented the first correct description in *G. graminifolia*, *G. ova*, and *G. tenera* (Romanov 1936). In these species, the normal development proceeds according to the tetrasporic Fritillaria type. After meiosis (without cell-wall formation) the four megaspore nuclei assume '1 + 3 position' (one nucleus micropylar, three chalazal). During the following mitosis the three chalazal nuclei are united by a common spindle and a “secondary four-nucleate stage” (with nuclei in '2 + 2 position') is attained. The most chalazal nucleus becomes already pycnotic (relatively more dense) at this stage. One further mitosis results in an organized 7- to 8-nucleate embryo sac with 3-celled egg apparatus, haploid micropylar and triploid chalazal polar nucleus in the central cell, and two antipodal cells in linear arrangement. The micropylar one of these antipodal cells contains a triploid and noticeably condensed nucleus, the chalazal cell one or two heavily pycnotic nuclei. Romanov (1961; 1962) studied *G. parva*, *G. olgae*, and *G. chomutovae*. In the former two taxa he found a more vigorous "depression" (i.e., stronger pycnosis) in the chalazal part of the embryo sac than in the previously studied species (Romanov 1936), especially as this also involves the chalazal polar nucleus, which appears smaller than expected and more dense. The essential point now is that Romanov (1961; 1962) related the heterochromatin masses in the endosperm nuclei to the pycnotic condition of the chalazal polar nucleus, of which they are thought to be derived. This explanation was suggested by the aspect shortly after the first endosperm mitosis, in which heterochromatin was positioned laterally like a cap and mirror-symmetrically in the two sister-nuclei. In contrast, in *G. chomutovae* the chalazal polar nucleus was not pycnotic and accordingly of larger size than the haploid micropylar polar nucleus, and no heterochromatic masses were stated in the endosperm nuclei. Geitler (1963) quoted Romanov's hypothesis with approval.

The observations of Romanov (1961; 1962) clearly indicate that the heterochromatin masses in the endosperm of *Gagea* spp. are of the facultative category in classical cytological terms. Meanwhile immunostaining experiments have been done, demonstrating that this heterochromatin is not DNA-hypermethylated (Bužek et al. 1998a) but deacetylated at the N-terminal lysine residues 5, 8 and 12, but not 16 of the histone H4 (Bužek et al. 1998b). Deacetylation of histone H4 in absence of hypermethylation of cytosin also seems to be a characteristic of the human inactivated X chromosome (Belyaev et al. 1996; Jeppesen and Turner 1993).

As a matter of fact this hitherto unparalleled case of facultative heterochromatin in a plant has been largely ignored in the scientific literature. For instance, Romanov (1961; 1962) is not quoted in Johri et al. (1992) or, as far as we can state, in any other review on heterochromatin in general and in plants in particular, except by Geitler (1963) and Tschermak-Woess (1963). Reasons may be seen in the old-styled presentation of the observations (drawings of interphase nuclei) and poor availability of the publication. Our aim therefore is to present, for the first time and supplementing our previous immunostaining analyses (Bužek et al. 1998a; b), convincing photographic and cytometric evidence for the differential heterochromatinization of whole genomes in the endosperm of *G. lutea*.

Material and methods

Plant material was collected in the Prater meadows, Vienna, Austria. Ovules and young developing seeds were fixed in acetic methanol (3:1) or FPA_{50} (formalin, propionic acid, 50% ethanol; 5:5:90) and stored in 96% or 70% ethanol, respectively, at –20°C. Embryo sac development was analysed with cleared ovules (Herr 1971) and differential interference contrast

optics (data not shown) and dissected embryo sacs after Feulgen staining. If not stated otherwise, photographs were taken from nondried preparations. Cytoplasmic staining in FPA_{50}-fixed embryo sacs was intentional and obtained by less SO_2-water washing. Somatic nuclei were also stained with acetocarmine.

Feulgen densitometry was conducted on the Leitz MPV2 scanning cytophotometer (compare König et al. 1987) and on the video-based Kontron CIRES (Cell Image Retrieval and Evaluation System, version 3.1) (Temsch et al. 1998; Dimitrova et al. 1999). *Allium cepa* "Frühstamm" root tips (2C=33.5pg) were used as reference for DNA content determination (compare Greilhuber and Ebert 1994). The fixative was methanol-acetic acid (3:1). For the procedure see Greilhuber and Ebert (1994). Heterochromatin amount was determined first by taking the overall integrated absorbance of the nucleus at the empirically (MPV2) or automatically (CIRES) determined zero-background level, then raising the level until only the heterochromatin appeared segmented on the screen, and then again taking the integrated absorbance. Flow cytometry with ethidium bromide as stain was conducted as described in Baranyi and Greilhuber (1996) also using *A. cepa* for internal reference.

Results

The chromosome number of *G. lutea* is 2n=72 (6x; x=12). The haploid number n=36 was counted in the haploid metaphase of the first embryo sac mitosis and the diploid number 2n=72 in embryonic tissue. This agrees with most reports in the literature. The nuclear 2C content was determined from leaf tissue with flow cytometry as 40.46pg (SD, 0.028pg; n=9 runs; M. Baranyi, Institute of Botany, University of Vienna, pers. commun.) and confirmed with Feulgen densitometry from ovule tissue (39.50pg, n=30 nuclei). The structure of the somatic interphase nuclei is largely euchromatic-chromomeric with heterochromomeres and small chromocenters distributed more or less at random in the nucleus (Fig. 1a, b). Little constitutive heterochromatin therefore is present on the chromosomes.

With respect to the fertilization process the replication status of the nuclei in pollen grains and pollen tubes were measured quantitatively. At pollen dispersal, the vegetative nucleus is nonreplicated and diffuse in chromatin structure with few small chromocentres; the generative nucleus is replicated (Table 1 and Fig. 1c) and shows the prophase-like chromosome condensation which is characteristic for such nuclei (Schnarf 1941: p.137). During pollen tube growth the second pollen mitosis takes place, and the resulting sperm nuclei with a granular-fibrillar structure stay in G_1 until fertilization; also the vegetative nucleus remains unreplicated (Table 1 and Fig. 1d–h). This conforms to the "bicellular-G_1" pattern of microgametophyte development as classified by Friedman (1999). Apart from the evidence for the same pattern in *Tradescantia paludosa* (Woodard 1956), no other such cases in angiosperms have been recorded to date (compare Friedman 1999). Unreplicated sperm nuclei at fertilization have else been found only in *Ephedra trifurca* (Friedman 1991) and some grasses (see Friedman 1999). In *Gnetum gnemon* (Carmichael and Friedman 1995), in *Arabidopsis thaliana* (Friedman 1999), and some angiosperms with tricellular pollen (see Friedman 1999) sperm nuclei are already replicated at fertilization. Sperm cell contours could not be clearly traced with the method applied, but nuclear areas within one pollen tube did not show considerable size differences. Whether in our plant sample there are size differences between the sperm cells as observed by Zhang et al. (1995) in a polish accession must remain open.

The embryological development in *G. lutea* on the female side has not been described previously except the somewhat incomplete observations by Stenar (1927), which have been corrected by Romanov (1936), however in the species *G. graminifolia*, *G. ova* and *G. tenera*. The ovule of *G. lutea* is bitegmic and tenuinucellat (Stenar 1927). The embryo sac development follows in the normal case the Fritillaria type (reviewed by Maheshwari 1946) (see "Introduction"). Aberrations seem to occur, but their frequency remains to be determined. For the present subject it is important to note that there is a gradient of "depression", i.e., progressive pycnosis, from mycropylar to chalazal in the chalazal half of the embryo sac. This is already evident when the lowermost nucleus of the coenomegaspore lags behind in the cell cycle (Fig. 2a), but at any rate in the secondary four-nucleate stage (Fig. 2b, c). The most chalazal triploid

nucleus generally cannot finish the second mitosis with the result of one heavily pycnotic restitution nucleus or two nuclei of mostly unequal size (Fig. 2e). The mitosis of the more micropylar triploid nucleus proceeds normally, the spindle axis is orientated longitudinally in the embryo sac. The present method did not allow a definite conclusion on the cell wall formation between antipodal cells and central cell. The upper antipodal nucleus is distinctly less strongly pycnotic than the lower one; nevertheless its chromatin structure is sticky-filamentous (Fig. 2e) and the DNA content seems to remain in 3C (Table 1). The triploid chalazal polar nucleus again is less pycnotic than the upper antipodal but of similar size as the micropylar polar nucleus and therefore of about 3-fold chromatin density. One exemplary egg apparatus shortly before fertilization consisted of an egg cell with an incompletely replicated nucleus, one synergid also with a partly replicated nucleus, and another synergid with a nonreplicated nucleus (Fig. 2d). The micropylar polar nucleus also seems to be partly replicated (Table 1, test I; and Fig. 2d). The DNA content of five measured zygotes was between 1.78C and 2.80C (Table 1). This means that the egg nucleus (and the sperm nucleus too, see above) may be unreplicated at fertilization.

Shortly after discharge of the pollen tube into one synergid a triple configuration in the central cell is seen (Fig. 2e, g), in which a distinctly more darkly Feulgen-stained chalazal polar nucleus lies tightly appressed to a haploid sperm nucleus and a haploid micropylar polar nucleus. It was not attempted to distinguish the latter nuclei. In the zygote the two gamete nuclei also lie tightly appressed and sometimes seem to be of unequal size (Fig. 2e, f). It was difficult to decide to what extent karyogamy is in progress before the first embryo and endosperm mitosis, respectively. Karyogamy is thus of the intermediate or postmitotic type (van Went and Willemse 1984). After fertilization, the vegetative nucleus of the pollen tube and the nucleus of the penetrated synergid progressively condense and finally are seen as heavily pycnotic X-bodies (Fig. 2e, f). DNA content measurements in few cells of egg apparatuses show that the surviving synergid may elevate its DNA content to 1.5C (Table 1). Also one of the X-bodies may contain 1.5 times the DNA content of the other one (Table 1, test T). However, the fully condensed X-bodies show reduced integrated absorbance compared with the values of vital nuclei (Table 1, test T).

Of special relevance for the proof of Romanov's hypothesis (1961) are the stages immediately before and after the endosperm metaphase. Figure 3a presents the prophase of the first endosperm mitosis clearly showing the dense chalazal polar nucleus entering mitosis together with the other two haploid nuclei with less strongly condensed chromosomes. No distinct borderline between the euchromatic prophase nuclei was seen. The following interphase exhibits two voluminous endosperm nuclei with highly dispersed euchromatin and a lateral caplike area of sticky-filamentous heterochromatin (Fig. 3b). As in all members of the family Liliaceae s. str. the endospermis of the nuclear type, i.e., without cell wall formation during the first mitosis (Tamura 1998). DNA content measurements in pairs of nuclei or measurable singles of such pairs, considered to be in G_1 because of shape or co-orientation in the same cytoplasmatic agglomeration, showed an overall 5C DNA content and a 2:3 ration of eu- and heterochromatic areas (Table 1; and Fig. 3b). One nucleus pair showed elevated DNA and euchromatin content and was presumably in S phase (Table 1, test Q; and Fig. 3d). Such nuclei clearly showed that both eu- and heterochromatic genomes form nucleoli (Fig. 3d). From somewhat later stages of endosperm development on for unknown reasons nucleoli are always associated with heterochromatin. In G_1 endosperm nuclei, considering the areas of eu- and heterochromatin and their DNA content an about 4.4-fold higher DNA density in heterochromatin than in euchromatin and zygotic chromatin are of similar density. (However, somatic interphase nuclei in the integument have an about 18-fold higher density that endosperm euchromatin and a 4.2-fold higher density than endosperm heterochromatin.) As Figure 3c shows, already the first endosperm mitosis can be disturbed and then results in a restitution nucleus. The central position of heterochromatin indicates that heterochromatin stickiness causes such failures of nuclear division. This, however, is not the regular case. The completely asymmetric distribution of the heterochromatin in the first endosperm nuclei is successively resolved in the subsequent mitoses and in interphase large sticky and irregularly shaped heterochromatic masses extend over large

parts of the nucleus, while euchromatin assumes a more chromonematic structure (Fig. 4c). This particular aspect of heterochromatin is very unusual in comparison with any other type of constitutive heterochromatin as it is known from plants. During prophase the sticky condition of heterochromatin is retained very long (Fig. 4a), but in metaphase not any more distinctly expressed (Fig. 4b). Furtheron the nuclear volume shrinks (Fig. 4d), and consequently the density of eu- and heterochromatin increases but their proportion is more or less retained (Table 1). In cellularized endosperm the nuclei have conspicuously smaller size, 6 to 20% of the first interphase (G_1) (Fig. 4d). DNA measurements in such nuclei (standardized by embryo telophase nuclei from the same seed) basically confirm their pentaploid constitution and 2:3 relationship of eu- and heterochromatin (Table 1), the latter comprising mostly only one or two large chromocenters. Polyploid nuclei (10n or higher) remain rare also in cellularized endosperm. However, more irregular size distributions of nuclei in some endosperms were also observed. Mitotic irregularities therefore occur. It was noticed that Feulgen stainability of nuclei in cellularized endosperm and embryos at this stage is significantly reduced compared with ovule tissue before and shortly after fertilization. This points to some stoichiometric problem with quantitative DNA staining (polyphenolic impregnation of nuclei?) (Greilhuber 1988) in such seeds and requires a cautious interpretation if the measurement results (Table 1).

Discussion

Observations of critical stages before, at, and after fertilization clearly indicate the correctness of the interpretation of Romanov (1961; 1962) and suggestes that the heterochromatin masses in endosperm nuclei must be classified as facultative heterochromatin sensu Brown (1966), because they are derived from the three genomes of the chalazal polar nucleus, which themselves descend lastly from one diploid genome of the megaspore mother cell which is of course largely euchromatic. Chalazal pycnosis is of wide distribution in developmental types of embryo sacs in angiosperms (e.g., Maheshwari 1946), but the case of *Gagea* spp. is up to the present the only documented in which a pycnotic chalazal polar nucleus can take part in triple fusion and endosperm formation. It is of interest that *G. chomutovae* (Romanov 1961; 1962) and liliaceous plants as *Lilium* spp. and *Fritillaria* spp. with the same type of embryo sac development as *Gagea* spp. have pentaploid endosperm without differential heterochromatinization, while in the liliaceous genus *Tulipa* only diploid endosperm has become known (Rutishauser 1969: p.40; Pechenitsyn 1972a; b). The latter follows from a refusal of the chalazal polar nucleus to participate in triple fusion (Pechenitsyn 1972a; b), although in some species it can obviously enter a sticky metaphase (in *Tulipa affinis*, *T. butkovii*, and *T. vvedenskyi*; Pechenitsyn 1972b). Also in members of a quite distantly related plant genus, *Melampyrum* (Scrophulariaceae), with tetrasporic embryo sac (Drusa type), nonparticipation of a pycnotic chalazal polar nucleus in triple fusion results in a diploid endosperm (Greilhuber 1973).

Possibly the case of *Gagea* spp. is not the only case of differential heterochromatinization in endosperm. Romanov (1961; 1962) mentions the case of *Sagittaria graminea*, in which Johri (1936) described perhaps incorrectly an aberrant case of fertilization, namely. the fertilization of the first two endosperm nuclei by two sperm nuclei contributed by an accessory pollen tube. The drawing by Johri (1936) indeed reminds strongly of our figures of the first endosperm nuclei in *Gagea lutea*. Therefore we would suppose that a systematic search for differential heterochromatinization in endosperm could reveal further examples. Especially, embryo sacs with "vigorous depressions" in the chalazal part could be candidates as well as embryo sacs in which more than two maternal genomes are engaged in the "second fertilization" event. Moreover, it is not clear whether in endosperm certain taxa less conspicuous differential heterochromatinization occurs but has escaped notice.

Another conclusion from our observations is that generally the pycnotization phenomena in embryo sacs, antipodals and X-body formation included, are not mere degeneration in the course of dying, but represent genuine heterochromatinization as a mode of inactivation. Almost always this process is terminalizing the development of a cell or a nucleus, which is not determined to propagate itself furtheron. The endosperm nuclei of *Gagea* spp. represent an exception to the rule. It is probable, though not yet

proven, that the heterochromatinized genomes are reduced in their transcriptional activity, the ribosomal genes excepted (as concluded from the presence of nucleoli). The biological significance of this heterochromatinization and inactivation remains presently in the dark, however.

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Table 1. Measurements of DNA content in various classes of nuclei and estimation of the amount of facultative heterochromatin in embryo sac and endosperm nuclei of *Gagea lutea*

Nucleus class	No. of nuclei	DNA content (C; mean ± SD or range)[a]	% Heterochromatin[b]
Male gametophyte (pollen at shedding stage)			
A[c] Generative (CIRES)	52	2.02 ± 0.07	n.d.
A Vegetative[d] (CIRES)	52	1.00 ± 0.06	n.d.
Male gametophyte (in style)			
B Vegetative (CIRES)	22	1.11. ± 0.08	n.d.
B Sperm (CIRES)	50	1.16 ± 0.14	n.d.
Female gametophyte before fertilization			
C Egg	6	1.40–2.30	n.d.
D Synergid (CIRES)	18	0.91–1.95	n.d.
E Micropylar polar (CIRES)	6	1.00–2.59	n.d.
F Chalazal polar (CIRES)	6	2.90–3.59	n.d.
G Chalazal polar (CIRES)	1	6.53	n.d.
H Upper antipodal (MPV2)	1	3.11	100.00
I Egg (MPV2)	1	1.50	n.d.
I Micropylar polar (MPV2)	1	1.66	n.d.
I 1st Synergid (MPV2)	1	1.27	n.d.
I 2nd Synergid (MPV2)	1	1.05	n.d.
Female gametophyte after fertilization			
J Zygote (CIRES)	5	1.78–2.80	n.d.
K X-body, early (CIRES)	1	0.95	n.d.
L Triple configuration (CIRES)	4	6.25–10.65	n.d.
M Zygote (MPV2)	1	1.84	n.d.
M Endosperm, after 1st mitosis (MPV2)	1	5.51	59.06
M Endosperm, after 1st mitosis (MPV2)	1	5.71	60.99
N Endosperm, after 1st mitosis (MPV2)	1	5.56	59.58
O Endosperm, after 1st mitosis (MPV2)	1	5.95	56.35
P Endosperm, restitution after 1st mitosis (MPV2)	1	10.26	60.06
Q Endosperm, after 1st mitosis (MPV2)	1	7.9	50.35
Q Endosperm, after 1st mitosis (MPV2)	1	7.5	54.05
R Endosperm, cellular, pro- and telophases (CIRES)	53	n.d.	58.41 ± 4.24[e]
S Endosperm, cellular, interphases presumably G_1 (MPV2)	20	4.68[f]	69.01 ± 9.99[e]
T Zygote (MPV2)	1	3.19	n.d.
T Synergid (MPV2)	1	1.54	n.d.
T 1st X-body (MPV2)	1	0.59	100.00
T 2nd X-body (MPV2)	1	0.76	100.00

[a]If not stated otherwise, for C level determination 2C and 4C nuclei from the integuments were used

[b]The amount of constitutive heterochromatin is small and was not determined (n.d.) photometrically

[c]Nuclei preceded by the same capital letter were from one embryo sac or from the same test

[d]Reference nuclei for the generative nuclei of the same test

[e]Mean with standard deviation (SD); includes some euchromatin over and below heterochromatin

[f]Calibrated against 6 embryonic telophases from same ovule

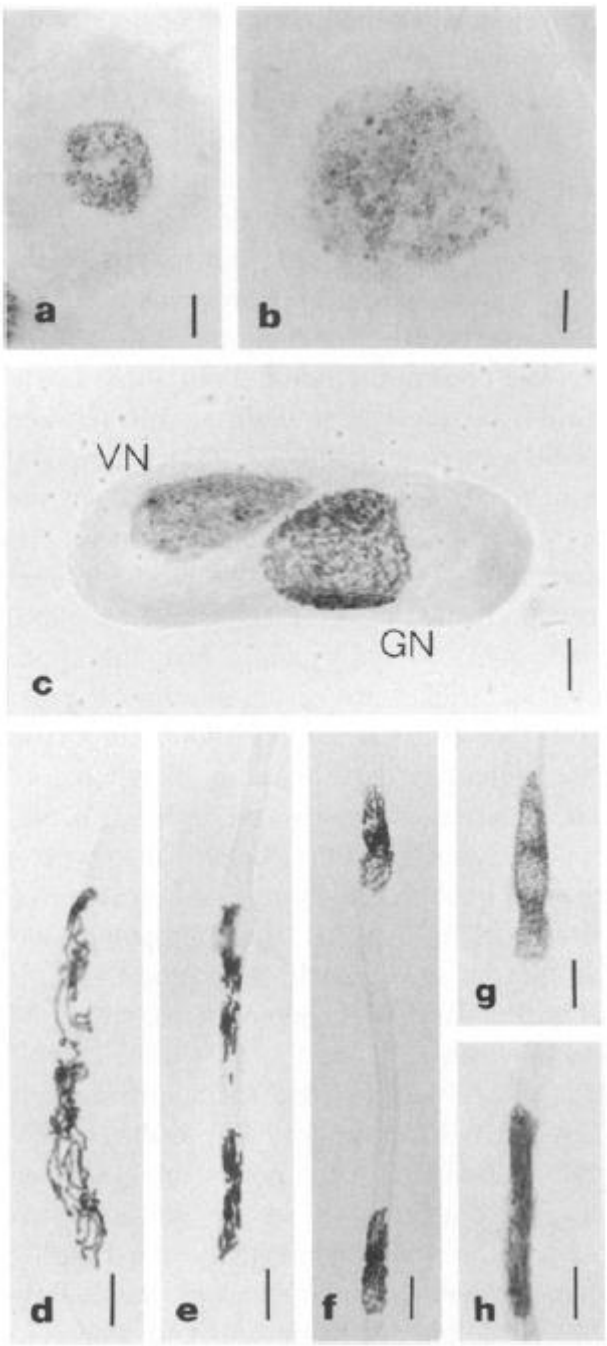

Figure 1. *Gagea lutea*, nuclei from somatic ovular tissue (**a** and **b**) and male gametophyte (**c**–**h**). **a** Diploid nucleus from the integument. **b** Endoreduplicated nucleus from young elaiosome. **c** Pollen grain at shedding stage showing vegetative nucleus (VN) and generative nucleus (GN). **d** Prophase of second pollen mitosis. **e** Anaphase of second pollen mitosis. **f** Telophase of second pollen mitosis. **g** One of two sperm nuclei. **h** Vegetative nucleus of pollen tube. **a** and b Acetocarmine. **c**–**h** Feulgen; nuclei from air-dried slides. Bars: 10µm

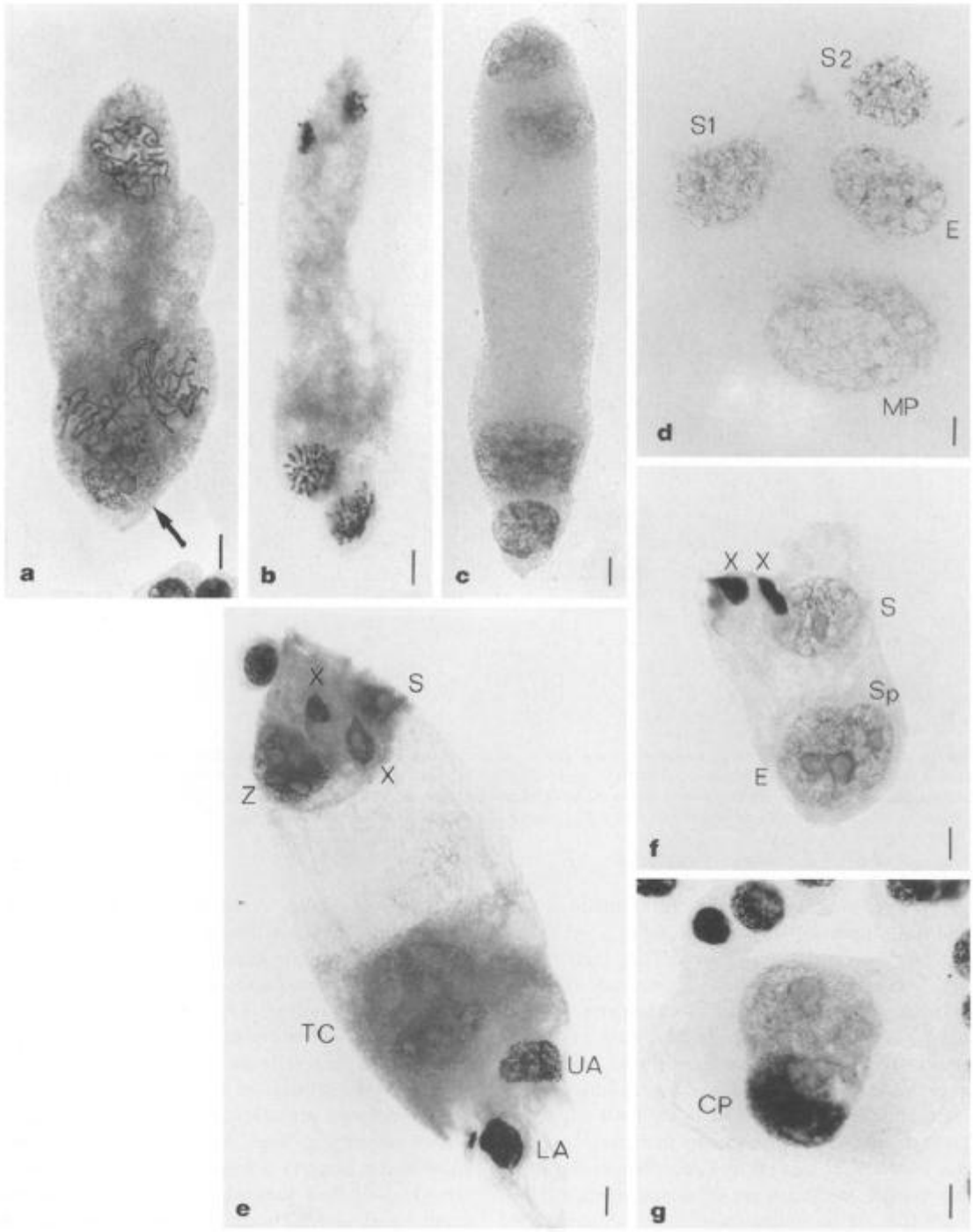

Figure 2. *Gagea lutea*, stages of embryo sac development before and after fertilization (orientation, micropylar at top). **a** Prophase of first embryo sac mitosis, '1 + 3 position', lowermost chalazal nucleus still in interphase (arrow). **b** Anaphase of first embryo sac mitosis showing haploid configurations micropylar and triploid configurations chalazal. **c** Secondary four-nucleate stage, note stronger pycnosis and a smaller size of the most chalazal nucleus. **d** Egg apparatus before fertilization from pollinated flower. One synergid (S1) has a partly replicated nucleus, the other (S2) has a nonreplicated nucleus. The nucleus of the egg cell (E) is partly replicated, the micropylar polar nucleus (MP) also seems partly replicated. **e** Complete fertilized embryo sac, X-bodies (X) not yet fully condensed, triple configuration (TC) in the chalazal part of the central cell, antipodals strongly pycnotic, lower antipodal nuclei (LA) of very unusual size due to a defective mitosis; the nucleus close to the embryo sac apex is from the nucellus. (S) Surviving synergid, (UA) upper antipodal nucleus, (Z) zygote. **f** Fertilized egg apparatus, note obviously unequal size of gamete nuclei and strongly condensed X-bodies. (E) Egg cell, (S) surviving synergid, (Sp) sperm nucleus, (X) X-bodies. **g** Triple configuration, note higher density of chalazal polar nucleus (CP); sperm nucleus and micropylar polar nucleus not individually distinguishable. **a–c** and **e–g** FPA_{50} fixative. **a–g** Feulgen. **d** Nuclei from air-dried slides. Bars: 10μm

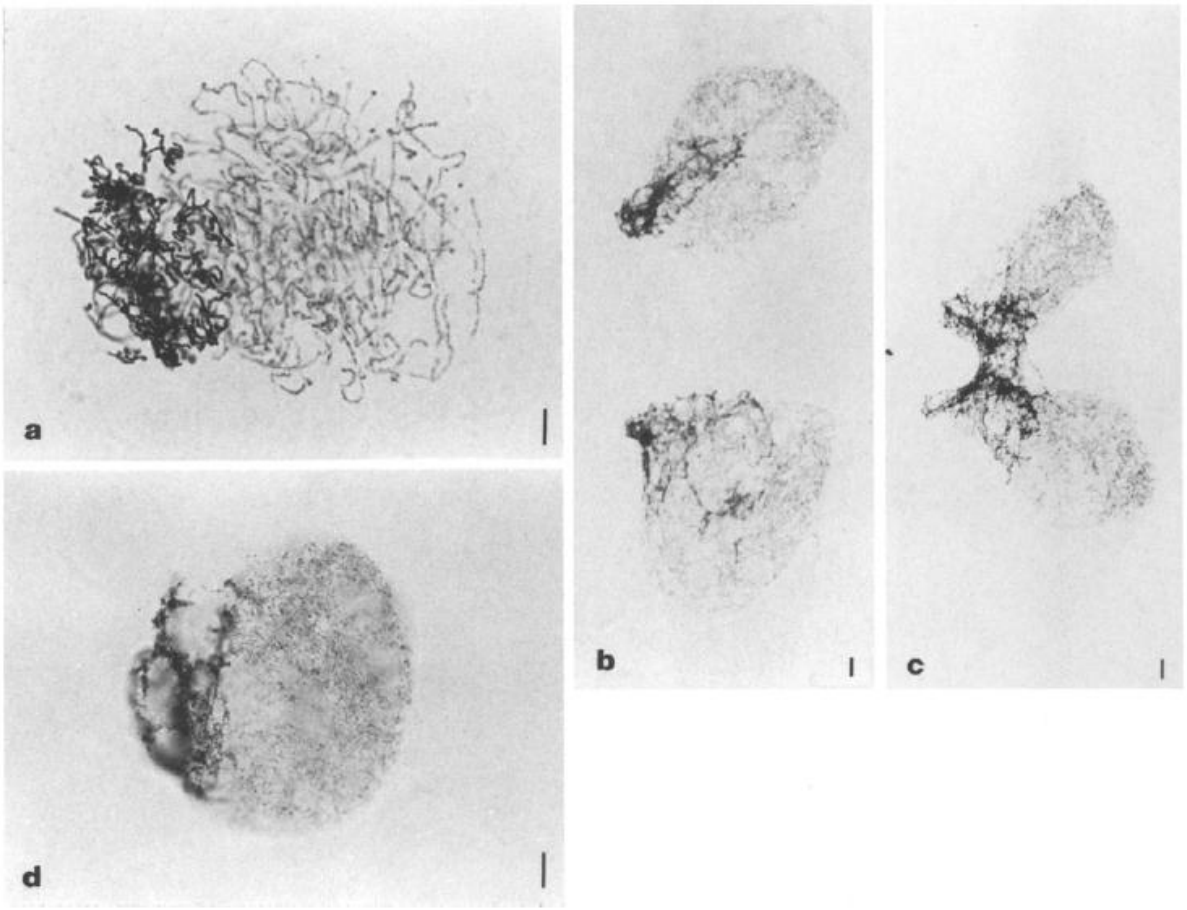

Figure 3. *Gagea lutea*, nuclei during and after first endosperm mitosis. **a** Pentaploid prophase of triple configuration, note heterochromatinized chromosomes laterally positioned (left). **b** G_1 nuclei after first mitosis. **c** G_1 restitution nucleus resulting from an incomplete first mitosis due to heterochromatin stickiness. **d** One of the first two endosperm nuclei, in S phase, note nucleoli formed by nucleolus organizer regions in both eu- and heterochromatic genomes. **a**–**d** Feulgen. **b** and **c** Nuclei from air-dried slides. Bars: 10μm

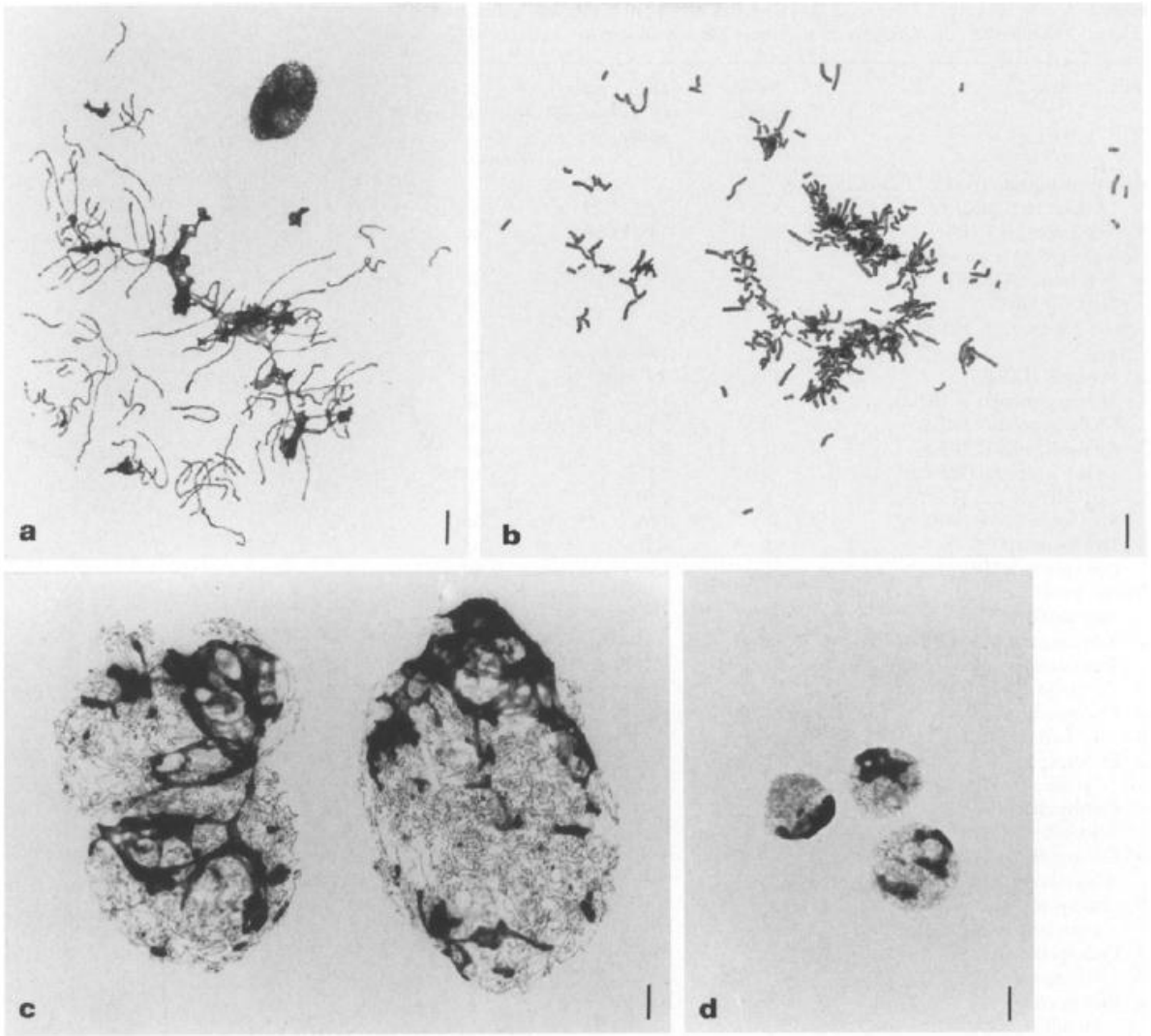

Figure 4. *Gagea lutea*, endosperm nuclei. **a** Pentaploid prophase of young endosperm showing about 72 euchromatic chromosomes and sticky chromosome assemblages; the compact nucleus at the upper right is from the somatic ovular tissue. **b** Pentaploid metaphase. **c** Interphase nuclei from young endosperm, note chromonematic euchromatin structure and sticky heterochromatin. **d** Isolated interphase nuclei from cellularized endosperm, note reduced size and low number of chromocenters. **a**–**d** Feulgen. **d** Nuclei from air-dried slides. Bars: 10µm