ON A CASE OF POLYEMBRYONY IN PTEROCARYA FRAXINIFOLIA (JUGLANDACEAE) AND ON POLYEMBRYONY IN GENERAL*

F. BOUMAN AND F. D. BOESEWINKEL

Hugo de Vries-Laboratorium, Universiteit van Amsterdam

SUMMARY

A case of twin nucelli in an ovule of *Pterocarya fraxinifolia*, originated from a cleavage of the nucellus primordium and presumably representing a hitherto unrecorded type of polyembryony in *Juglandaceae*, is discussed. The qualification of this category of polyembryony as "false polyembryony" according to the conventional classification of Ernst is unsatisfactory. A new system of classification of polyembryony is proposed.

1. INTRODUCTION

Polyembryony (P.E.) is nowadays known to be of fairly common occurrence among *Spermatophyta* and is of considerable interest to the plant breeder and to the horticulturist (MAHESHWARI & SACHAR 1963). Since the first case of polyembryony (in an orange) was published by Antoni van Leeuwenhoek in 1719, many observations of this phenomenon in seed plants have been mentioned.

As early as 1859, BRAUN gave a survey of 58 cases recorded in the botanical literature, which he referred to four categories, *viz.*,

a. several embryo sacs in the nucellus of the ovule;
b. several cells of the embryo sac develop into germs;
c. a division of the pro-embryo; and
d. lateral coalescence of two ovules (inclusive of "such cases as those originating from an abnormal division of the ovule primordium").

Braun considered the last category to be "*unächte Polyembryonie*", *i.e.*, "false" or "spurious" polyembryony.

STRASBURGER (1878), after painstaking observations of the cytology of the embryo sac and the process of fertilisation, distinguished a fifth category, *viz.*, the relatively frequent adventitious embryo formation from nucellar tissue. The first proposal towards a hierarchic system of classification by ERNST (1901, 1918) has been almost universally adopted by prominent phytomorphologists and embryologists, though sometimes with minor modifications (see, *e.g.*, COULTER & CHAMBERLAIN 1903; SCHNARF 1929; MAHESHWARI 1950, 1952; LEBÈGUE 1952; MAHESHWARI & SACHAR 1963; DAVIS 1966). The most important classificatory criterion used by Ernst is the number of functional embryo sacs:

a. true P.E. *(vraie polyembryonie, polyembryonie réelle, echte Polyembryonie):*

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the development of two or more embryos within the same embryo sac of a single ovule (*i.e.*, unisaccally), subdivided in intra- and extra-saccal P.E.

b. false or spurious P.E. *(unechte Polyembryonie, pseudo-polyembryonie, fausse polyembryonie):* the development of embryos in different embryo sacs of the same ovule (*i.e.*, plurisaccally).

Proposals for a novel scheme of classification or for the employment of alternative classifactory criteria have been made by ARCHIBALD (1939), ROBIJNS & LOUIS (1942), LEROY (1947), YAKOVLEV (1957) and JOHRI (1965).

Archibald and Leroy consider the occurrence or the absence of a true fertilisation process and of the development of an endosperm the most important criterion for a classification. The difference between "spurious" and "true" intrasaccal P.E. being supposed to be smaller than that between "true" intrasaccal P.E. and "true" extrasaccal P.E. in the sense of Ernst, Robijns & Louis propose the following classification:

a. *Intrasaccal P.E.:* the germs develop in the same (single) ovule from cells situated in the same embryo sac or in several embryo sacs.
b. *Extrasaccal P.E.:* the germs develop within the same ovule from extrasaccal cells (cells situated outside of the embryo sac or sacs).

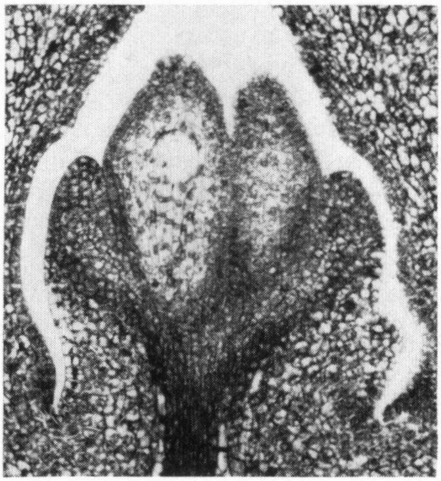
Yakovlev's classification is not a very satisfactory one by its inconsequent simultaneous application to different taxonomic categories (Gymnosperms and Angiosperms) and to different generations (*viz.*, gametophyte and sporophyte). The classification proposed by Johri chiefly concerns the artificially induced formation of embryoids out of cultures of vegetative plant tissues, and of embryos out of embryonal callus or callused nucellar tissue. Considering that such embryoids and embryos are not formed within an ovule or a developing seed, we do not think that Johri's classification has any bearing upon the classification of naturally occurring forms of P.E.

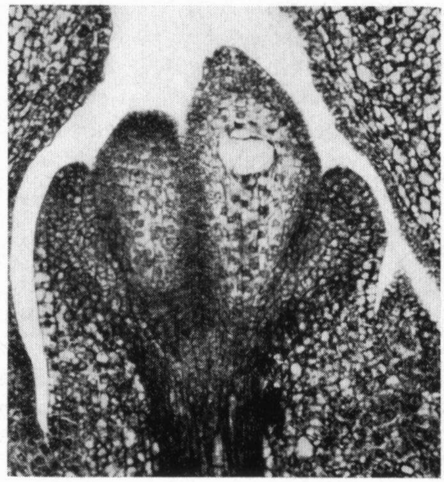
Within the family of the *Juglandaceae*, the occurrence of more than one embryo sac in a single nucellus has been reported by NAVASHIN (1897), KARSTENS (1902), NICOLOFF (1904, 1905), NAVASHIN & FINN (1913), and NAST (1935). According to Karstens and to Nast, the supernumerary embryo sac(s) are either derivatives of different macrosporal mother cells or derivatives of different megaspores of the same tetrad. ROBIJNS (1938, 1942) mentions the occurrence of 2 or 3 young viable seedlings within one common testa. On the basis of the relative positions of the embryos in respect of one another he believed that the case described in 1938 can be satisfactorily explained by assuming unisaccal P.E. (by the development of additional embryos out of one or both of the synergids), and the example described in 1942 by assuming plurisaccal P.E. Such causal explanations based on the conditions observed in mature seeds are of course highly speculative.

2. OBSERVATIONS

During a histogenetic study of integument development in two juglandaceous genera (BOESEWINKEL & BOUMAN 1967), in 9 gynoecia of *Pterocarya fraxinifolia*

(Lam.) Spach twin nucelli, originated from a cleavage of the nucellus primordium after the initiation of the integument, were noted *(figs. 1, 2* and *3)*. In all cases the double nucelli were provided with a completely developed embryo sac and both had the characteristic crassinucellate appearance, *viz.*, a number of parietal cell layers formed by periclinal divisions from the primary parietal cell.





Figs. 1 and 2. Longitudinal sections of the same ovule with twin nucelli of *Pterocarya fraxinifolia*, each section more or less median through one of the two nucelli. Distance between the two sections 21μ. Each nucellus with an embryo sac (the white area with a few marginally situated nuclei).

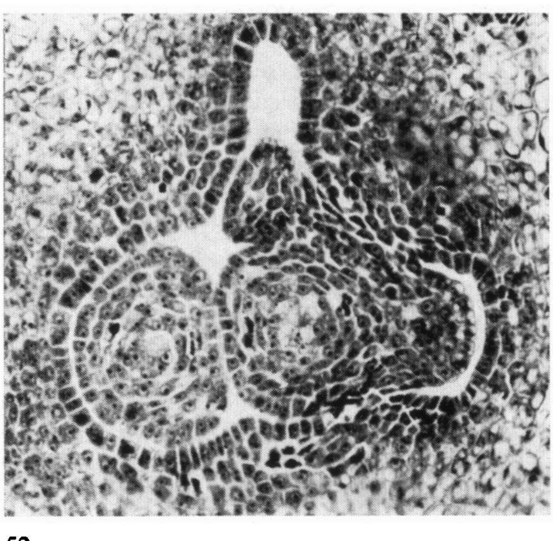


Fig. 3.
Transverse section through a young gynoecium of *Pterocarya fraxinifolia*, showing twin nucelli and on the one side (at the right) the integument primordium (this ovule represents a somewhat younger stage of development than the one shown in *figs. 1* and *2*).

3. DISCUSSION

Polyembryony has up to now always been defined in the broadest sense: "*die Entwicklung von zwei oder mehreren Embryonen in demselben Samen*" (ERNST 1918); "*die Bildung mehrerer Embryonen in einem Samen...*" (SCHNARF 1929); "... the production of two or more embryos within an ovule" (WEBBER 1940); "... the occurrence of more than one embryo in a seed, ..." (MAHESHWARI 1950), etc.

The study of P.E. meets with many practical limitations. When polyembryonic seeds are examined, the origin of the P.E. is a matter of speculation because it can not unambiguously be deduced from the topography of the seed contents and from the degree of ploidy of the embryos. Conversely, when during a microscopical examination of embryo sacs clear indications of the incidence of P.E. had been observed, in many instances one failed to ascertain whether the ripe seeds indeed contain several viable embryos. For practical reasons the definition of P.E. *sensu lato* appears to be recommendable.

Furthermore, the examples of plants whose embryogenesis is completely known show that, owing to some mutual influence or developmental competition (MAHESHWARI 1952), as a rule only one of the pro-embryos develops into a viable germ, *e.g.*, in the majority of the cases of habitual P.E. in Gymnosperms; in the phrasing of ERNST (1901): "*Polyembryonie führt bald zu der Erzeugung mehrerer keimungsfähigen Embryonen, bald fallen die in Mehrzahl angelegten Embryonen von einem gewissen Stadium an der stärkeren Entwicklung eines einzigen zum Opfer*". SCHNARF (1929) expresses it as follows: "*Nicht immer führt die Polyembryonie zur Ausbildung mehrerer keimfähigen Embryonen. Im Gegenteil ist es viel häufiger, dass aus der Konkurrenz mehrerer Keimlinge schliesslich einer als Sieger hervorgeht und nur eine Keimpflanze entsteht*".

For all these reasons one should define P.E. in its widest sense as every incidence of clear indications of potential P.E. (such as the presence of several embryo sacs or archegonia in a single ovule), as well as every case in which two or more young embryos occur in a single developing seed, irrespective of the question if these young embryos may eventually become viable germs or not.

It is striking that neither Ernst nor one of his followers have explained the essence of the criterion on the basis of which the distinction between false and true is made. Ernst's definition of false P.E. falls completely within his own definition of P.E. The fundamental criticism by FISHER (1914), WEBBER (1940), ROBIJNS & LOUIS (1942), and GUSTAFSSON (1946) is, therefore, justified: the essence of P.E. being the development of more than one embryo in an ovule (or seed), no good reason is apparent to regard those cases in which embryos are formed in two or more embryo sacs within a single ovule as "false" P.E. (Fisher). Webber's conclusion: "*False P.E. would be limited to those cases in which multiple embryos are derived from different nucelli*" is not quite correct either because the definition of P.E. refers to ovules and (or) seeds and not to nucelli. In Webber's circumscription the kind of P.E. caused by a cleavage of the ovule primordium *after* the initiation of the integument(s) would fall between the ship and the quay. The incidence of this type of P.E. was altogether overlooked by MAHESHWARI & SACHAR (1963). PENZIG (1922; see also SCHNARF 1929) explains older reports of the occurrence of twin nucelli in, *e.g.*, *Orchis morio* (SCHACHT 1850) and *Gymnadenia conopsea* (STRASBURGER 1878) by a secondary fusion of two ovules instead of by a splitting of a primordium. Their reasoning does not hold for twin nucelli in gynoecia which are, possibly, primarily uni-ovulate such as those of *Morus alba* (HOFMEISTER 1858) and *Peperomia pellucida* (JOHNSON 1900). The occurrence of twin nucelli in *Pterocarya fraxinifolia* reported in the present paper is indubitably caused by a splitting of the ovular primordium *after* integument initiation had taken place, not only because the juglandaceous gynoecium is primarily "monomerous", and contains only a single ovule according to MEEUSE (1964), but also because, as far as we could ascertain, no anomalous gynoecia with two or more ovules have ever been recorded in this family.

For the lack of a satisfactory alternative system of classification a proposal is made here for a very simple and logical classification, based on the most fundamental processes taking place during normal sexual reproduction in spermatophytic plants and thus providing indications for the chronological sequence, the situation (topography) and the degree of ploidy of the supernumerous embryos or (in the cases of an incomplete or altogether wanting development of the normal zygote) of all germs in polyembryonic ovules (or seeds).

A fertilised ovule and the issuing seed normally consist of cells derived from three generations, *viz.*:

1. the testa formed out of the remains of the integuments and the nucellus, hailing from the mother plant, *i.e.*, from *the sporophyte of the parental generation;*
2. the embryo sac (the primary endosperm with an archegonium or archegonia in Gymnosperms), derivative(s) of cells of *the gametophyte;* and
3. the pro-embryo, embryo (or ultimately, young seedling), the early developmental stage of *the new sporophyte of the second (filial) generation*, normally developed from a zygote formed by the fusion of the egg cell with a spermatic nucleus.

When two or more embryos occur in the ovule or in the seed, the most logical question is: from which of these three generations has (have) the supernumerary embryo(s) originated? This forms the basis of the new classification proposed below.

The phenomenon of P.E. is of course closely associated with the problem of apomixis (see BATTAGLIA 1963, for details).

The concept of *spurious polyembryony* (false P.E., pseudo-P.E.) must be restricted to those cases in which the two or more embryos present in the same structure originally belonged to two or more secondarily fused (concrescent) ovules or seeds, *e.g.*, twin ovules with fused (outer) integument ultimately developing into an apparently single seed containing at least two embryos.

Polyembryony:

Includes all cases in which there are clear indications of the potential or actual occurrence of two or several pro-embryos or embryos in a single seed, irrespective of the question whether they may ultimately give rise to viable seedlings, to be divided into three categories:

I. Supernumerary embryos originate from cells of *the sporophyte of the parental generation* (= adventitious embryony) without the normal alteration of sporophytic and gametophytic phases (= homophasic reproduction) and from one of two sources, *viz.;*
1. From nucellar cells or nucellar tissue (= nucellar P.E.), or
2. From cells of the (inner) integument (=tegumentary P.E.).

II. Supernumerary embryos originate from cells of *the gametophyte* in various ways, *viz.:*
1. By the formation of two or more (multiple) embryo sacs in one ovule, *i.e.*, plurisaccal P.E., in two possible ways:
   A. By the development of two or more embryo sacs by the secondary multiplication of a single ovular primordium after integument initiation has commenced;
   B. By the development of several sporal mother cells (multicellular archespore), resulting in
      a. sporic embryo sacs and reduced gametophytes (*i.e.*, euspory); or
      b. sporic and asporic embryo sacs, with reduced gametophytes and "unreduced gametophytes" (euspory and apospory);
   C. By the development of two or more macrospores of the same sporal tetrad (derived from a single macrospore mother cell).
2. By the formation of embryos from cells of a single embryo sac, *i.e.*, unisaccal P.E., in one of the following ways:
   A. By the initiation of several archegonia in the primary endosperm (megagametophyte), as in Gymnosperms;
   B. By the development of two or more potential egg cells within a single archegonium as in Gymnosperms;
   C. By the development of more potential egg cells within a single embryo sac in Angiosperms;
   D. By the development of one or of both synergids, whether or not after having been fertilised (amphi- or apomictic embryos);
   E. By the development of one or more antipodal cells, whether or not after previous fertilisation (similar to D); or
   F. By the development of polar nuclei or cells of the secondary endosperm (still a disputed case!).

III. Supernumerary embryos originate from *the new (filial) sporophyte*, from three possible sources in a derivative of a fertilised or "unreduced" unfertilised egg cell, and are genetically identical by being twins to multiplets:

1. By a mitotic division of the zygote into daughter cells each developing into a (pro-)embryo;
2. By a splitting or cleavage of the pro-embryo; or
3. By a division of the embryo.

IV. Supernumerary embryos originate from the *male gametophyte* by a development of the spermatic nucleus (or nuclei), *i.e.*, androgenesis (still a disputed case: see LEWIS 1964).

Complications may arise by the possibility of the incidence of P.E. in one individual according to more than one of the possible pathways.

Our knowledge concerning the homology relations between the various components of the megagametophyte (primary endosperm with the archegonia) of the Gymnosperms and the cells of the angiospermous embryo sac is still so limited that theoretical considerations on this topic (such as those of FAVRE-DUCHARTRE 1965, 1966) are too speculative to permit a solid basis for a reduction of the number of cases here distinguished under unisaccal P.E..

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