

hexoside. Thus, it should be noted that anthocyanins of Japanese wild iris consist of the glycosides of malvidin, petunidin and delphinidin. Of the plants in the Apogon section, *I. chrysographes* and *I. delavayi* have also been shown to contain a malvidin derivative in addition to

delphinin⁹. Differing from those plants, *Iris* species belonging to the section Xiphium, e.g. *I. hollandica* cv. 'Wedgwood'¹⁰ and *I. tingitana* cv. 'Prof. Blaauw'¹¹, and to the section Eupogon, e.g. tall bearded garden iris cv. 'Floridor' (Cayeux 1929)¹², contain only delphinidin glycoside.

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Some aspects of ovular development during megasporogenesis in *Pisum sativum* L.¹

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Summary. This work reports some findings which will aid us in our understanding of the female phase of *Pisum sativum* embryology. We have observed that the central planes between one ovule and the next form an angle of 60°. We suggest that this ovule arrangement may make it easier for the pollen tube to reach the ovular micropyle in the fertilization process. On the other hand, ovary and ovule growth do not depend on the megasporogenesis stage but have individual characteristics for each plant, every one depending on its own phenotype.

The main aspects of ovule and ovary structure in *Pisum sativum* have been understood for a long time. Cooper² reported the existence of a single ovary and the pattern of the ovular growth, the early arching of the ovules and the existence of only 1 megaspore mother cell (MMC) in each ovule. Recently, Rembert³ has described the ovule and the ovary on *Trifolium repens*, a species belonging to the same family as *Pisum* (Papilionaceae) by means of scanning electron microscopy; his results confirm the earlier studies of Cooper and add some details about ovular development. Nevertheless, nothing has been said either about the relative disposition of the ovules within the ovary or whether the ovule and ovary growth are related to the megasporogenesis progress or not.

Material and methods. Floral buds of *Pisum sativum* L. were collected from plants growing in a greenhouse. Ovaries were dissected from the floral buds and fixed in 3% glutaraldehyde in 0.025 M cacodylate buffer for 2 h, then postfixed in 1% osmium tetroxide in the same buffer for 1 h, and after dehydrated in alcohol series and embedded in Epon 812. Semithin sections of 2 µm were made with a LKB pyramitome and observed under the light microscope, either directly by phase-contrast or stained with toluidine blue.

Results. *Pisum sativum* L. ovules are clearly orientated to the stylar pole of the ovary from the time that differentiation of the MMC begins. Their relative position is not random, because once a central, well-oriented section of an ovule has been obtained, it is possible to get another section, also well-orientated, of the contiguous ovule, by turning the section plane 60° approximately (figures 1, 2

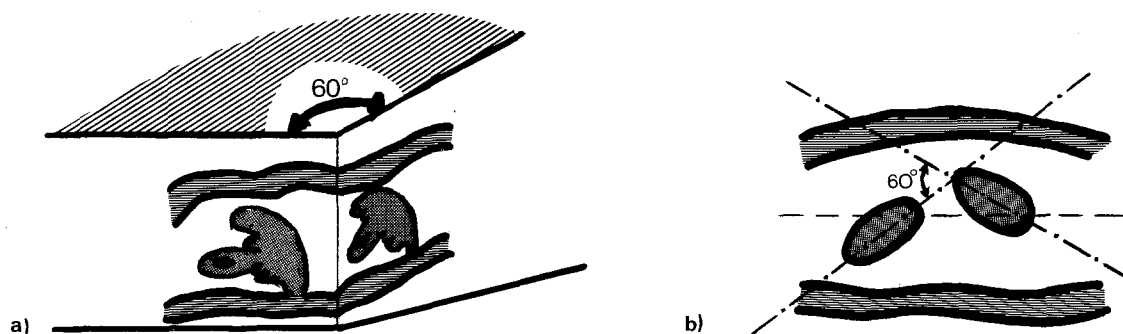
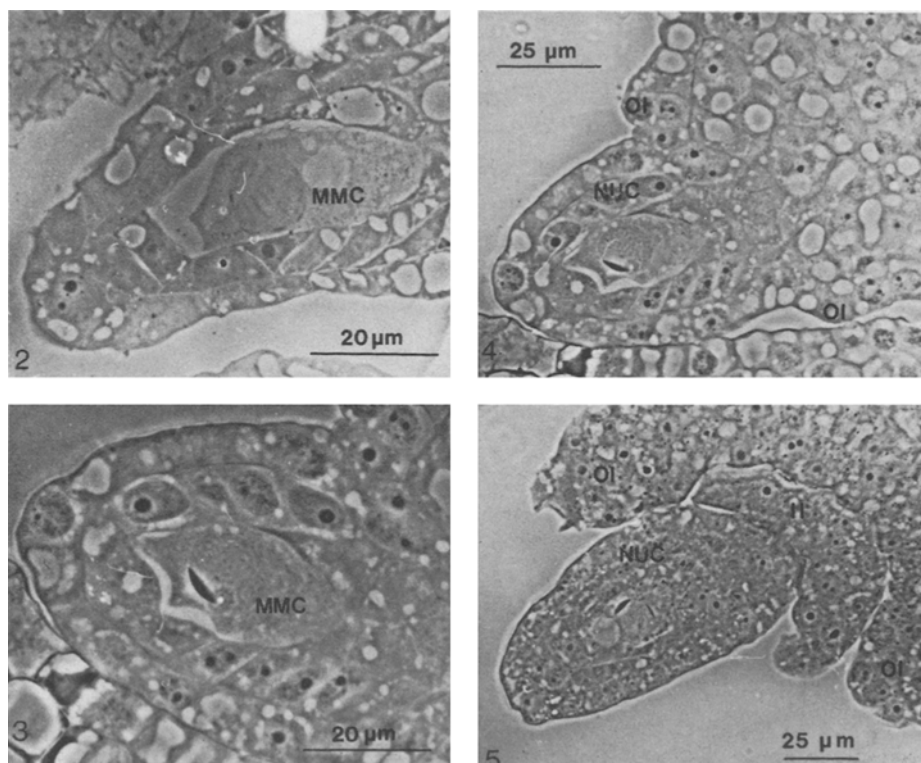


Fig. 1. Schematic drawings of the relative arrangement of the ovules within the ovary. *a* Front view of the Epon block showing 2 well-orientated sections of 2 contiguous ovules. We can get these sections by turning the section plane an angle of 60°. *b* Top view of 2 contiguous ovules; ---, central suture of the ovary, -.-.-, micropyle - MMC - chalaza plane.



Figures 2 and 3. Light micrographs showing 2 well orientated sections of 2 contiguous ovules which have been obtained by turning the section plane, 60°. MMC: megaspore mother cell.

Figures 4 and 5. In both ovules shown in these pictures, the MMC passes through the same meiotic stage while the integument development is very different. NUC, nucellus, OI, outer integument.

and 3). A central, well-oriented section is that which has the maximum possible MMC surface on the micropyle-chalazal axis. When this is so, we can observe 2 rows of nucellar cells on both sides of the MMC on the section. The integument section is asymmetric, due to the campylotropous character of the ovule. The part containing integumentary tissue located in the opposite side to the carpel wall, after the central ovular axis, shows a greater degree of development (figure 5).

In general, as megasporogenesis progresses, the ovule grows, but it is difficult to establish a close morphological correlation between the two processes in such a way that one could attribute a stage of ovule development to each phase of meiosis. 2 conditions are always observed: on the one hand, just before the beginning of meiosis, the ovule is a mass of nucellar cells without integument differentiation; and on the other hand, at the time of megaspore maturation, the outer integument entirely envelopes the nucellus. Nevertheless, it is possible to observe ovules with their MMC in the same meiotic phase, which show a very different integumentary development (figures 4 and 5).

Something similar occurs with the relationship between the ovary size and the MMC meiotic phase of the ovules enclosed in it. In selected ovaries between 0.8 and 1.5 mm long, we found ovules with MMC undergoing meiotic prophase I, without any correlation between the ovary size and the prophase stage.

Discussion. According to our observations the situation of the ovules in the ovary is such that there is an angle of 60° between the central planes of one ovule and the next. We suggest that this ovular arrangement in the ovary may make it easier for the pollen tube to reach the ovular micropyle in the fertilization process. If all ovular central planes were concurrent, the next ovule might be an obstacle for the pollen tube in reaching the ovule micropyle.

In previous papers dealing with mechanisms of fertilization this arrangement phenomenon of the ovules has not been described. Maheswari⁴ reported many cases in several species in which fertilization is made easier, but *Pisum* is not mentioned. Cooper⁵, in his early embryological study on this species, only described the progress of the pollen tube through the ovary, which takes place along its ventral suture; he did not describe the relative disposition of ovular micropyles.

According to our findings, there is no correlation between the development of the ovary and ovule during megasporogenesis and the meiotic stage. We think that this is due to the monocarpellar character of the *Pisum* flower², so the growth speed of the ovule and of the ovary may be considered as individual characteristics for each flower. The MMC differentiation process must induce the commencement of integument growth, while the megaspore differentiation induces the end of the integumentary development. The speed of this process will depend, therefore, on both the individual genetic complement of the plant and its environmental conditions but not on MMC. The relationship between the ovary size and its developmental stage must be something like this. Furthermore, though the ovary ontogeny involves growth, the individual size of each ovary in each megasporogenesis stage is a phenotypic (genotypic plus environmental) character.

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