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THE DISPOSITION OF GAMETE AND VEGETATIVE-CELL NUCLEI IN THE EXTENDING POLLEN TUBES OF A GRASS SPECIES, ALOPECURUS PRATENSIS L.

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RUSSELL & CASS (1981) have recently reported that in *Plumbago zeylandica* L. (Plumbaginaceae) the two male gametes and the vegetative nucleus establish a physical association in the pollen grain which is maintained in the extending pollen tube until the time of entry into the embryo sac. The division of the generative cell takes place in the maturing pollen grain in this species, and after the division the gamete cells remain in contact with each other through a common stretch of wall traversed by plasmodesmata, one gamete cell extending into a long, sinuous projection which wraps around, and occupies embayments in, the envelope of the much convoluted vegetative cell nucleus. A similar association of the male gametes with each other and the vegetative nucleus has been described by DUMAS & KNOX (1984) in another species with tricellular pollen, *Brassica oleracea* L. (Cruciferae), where one of the male gametes appears actually to be connected in some manner to the envelope of the vegetative nucleus. RUSSELL & CASS (1981) suggest that the connections between the gametes and the consistent association with the vegetative nucleus in *P. zeylandica* may have some functional importance during the passage through the pollen tube, perhaps in ensuring that the gametes are delivered more or less simultaneously into the embryo sac, which in this species lacks synergids. These observations are obviously of considerable significance, since they may indicate the need for a reassessment of the mechanism of double fertilisation in angiosperms. If something of the nature of a polarised fertilisation-unit is present on the male side, the long held view that the gamete fusions in the embryo sac are non-selective may have to be abandoned. The matter is of some importance for the interpretation of cytoplasmic inheritance through the male line, especially should the two male gametes not be identical one with the other but differ in their organelle content, as RUSSELL & CASS (1981) have shown to be the case in *P. zeylandica*.

Maintained connections between the male gametes during the passage through the tube have been described previously in several genera (e.g., in *Vallisneria:* WYLIE 1941), so this observation cannot be viewed as novel. However, the existence of a persistent linkage with the vegetative nucleus appears not previously to have been reported. Many accounts in the extensive earlier cytological

literature show that the vegetative nucleus is often widely separated from the gametes in the pollen tube, and that it may either lead or trail them during extension growth (e.g., WYLIE 1923; PODDUBNAYA-ARNOLDI 1936). It would seem, then, that the link between the gametes and the vegetative nucleus must be remarkably elastic, if it is indeed a universal feature.

In a few favourable cases we have been able to observe much of the course of gamete and vegetative-nucleus movement in *Secale cereale* L. (Gramineae; tricellular pollen) in individual extending tubes from the time of germination, and we have gained the impression that the gamete-pair and the vegetative nucleus move through the pollen tube independently, changing shape and separation as they do so. CASS (1973) described the pleomorphism of the gametes in another cereal, *Hordeum vulgare* L., and while noting that the gametes themselves tended to remain in association, did not advert to the existence of any connections between them and the vegetative nucleus.

Because of the congestion of organelles, storage products and membranes in the pollen tube it is often difficult to identify and locate the gametes in living tubes, and even in the most favourable conditions the limits of the attenuated and convoluted vegetative nucleus can rarely be established with the optical microscope. Furthermore, the evidence of fixed material may be open to question except where the material has been handled with great care. The tube is extremely sensitive to osmotic shock, and small disturbances can cause rapid changes in the shape and positions of the gametes and the vegetative nucleus. These complications, and the fact that many records relate to single observations or very small samples, lend an element of uncertainty to some of the earlier published accounts. In the present note we report on a statistical study of the distribution of the vegetative and gamete nuclei in the pollen tubes of *Alopecurus pratensis* L., stained *in vivo* with the DNA-specific fluorescent stain, 4-6-diamidino-2-phenylindole (DAPI). While this staining does not of course define the physical limits of the gamete cells, it precisely demarcates their nuclei in the actively growing tube, and often – although not invariably – allows the boundaries of the normally ill-defined vegetative nucleus to be located with reasonable certainty.

Samples of pollen of *A. pratensis* were collected directly from dehiscing anthers and transferred immediately into 100 µl liquid media in 1.5 ml capsules for culture on a rotator (20 rev/min) at 24°C. The basal medium contained 25 per cent sucrose, 10^{-3}M $Ca(NO_3)_2$ and 10^{-3} M H_3BO_3; pollen cultured in this served as a control for that in the staining medium, of the same composition, with the addition of 0.0005% DAPI. A maximum tube growth rate of 0.38µm sec^{-1} was observed during the first 10 min from the onset of hydration. Samples of the cultured tubes were withdrawn from the capsules and observed with a Vickers M17 system with a X60 apochromatic objective and UV epi-illumination using excitation filter MUG 2 and barrier GG400+420. Measurements were made directly with a grid-graticule eyepiece.

Seventy-five tubes of a suitable straightness for measurement and with reasonably well stained nuclei were selected for detailed survey. Amongst these, one

Table 1. *Alopecurus pratensis:* pollen-tube length, maximum vegetative nucleus dimension and maximum gamete nucleus dimension after 10 min growth.

	N	Range (μm)	Mean (μm)
Tube length	75	22.5–225.0	97.47 ± 4.19
Maximum vegetative nucleus dimension	42	5.6– 35.0	13.58 ± 1.12
Maximum gamete nucleus dimension	42	1.9– 27.5	7.75 ± 0.85

Table 2. *Alopecurus pratensis:* positions of vegetative and gamete nuclei in tubes in which the nuclei had emerged from the grain after 10 min growth.

	N	Maximum lead (μm)	Mean lead (μm)
Vegetative nucleus leading	19	44	22.84 ± 4.13
Gamete nuclei leading	24	64	29.24 ± 3.75

or more nuclei had emerged from the grain and entered the tube in 10 min in a total of 45, while in the remaining 30 all three nuclei were still retained in the grain. Statistics for pollen-tube length for the whole population are given in *table 1*, together with the maximum dimensions of vegetative and the larger of the gamete nuclei, as observed in the plane of the microscope image, for the 42 tubes with emergent nuclei in which the measurements could be made with reasonable certainty. The extremely wide range for the maximum dimensions of both gamete and vegetative nuclei (more than 6:1 for the former and 14:1 for the latter) reflects the continuous changes of shape both types of nuclei undergo during their passage through the tube. In *table 2*, the relative positions of the vegetative and the nearest gamete nucleus are shown for 43 of the surveyed tubes in which measurement could be made unambiguously along the axis. Where the vegetative nucleus was ahead, the measurement was between the trailing edge of this nucleus and the leading edge of the nearest gamete nucleus, and where the gametes were ahead, from the trailing edge of the rearmost to the leading edge of the vegetative nucleus. The ratio of the number of tubes with vegetative nucleus lead to the number with gamete nucleus lead is not significantly different from 1:1. In one instance only did it seem that the male gamete nuclei were separated in the tube by the vegetative nucleus, and this case was dubious since the leading nucleus was extremely attentuated. The separation of the male gamete nuclei from each other was examined in 39 tubes. In 17 of these, the nuclei were either overlapping along the axis of the tube, parallel to each other, or intertwined. The mean separation along the length of the tube in 22 others was 2.59 ± 0.61 μm.

It is evident, then, that in *A. pratensis* the type of nucleus – whether vegetative or gamete – leading through the tube is a matter of chance, as has been suggested for other species (WYLIE 1923); the determining circumstance is no doubt simply which happens to be first in leaving the grain. However, the male gamete nuclei

themselves clearly do stay in close spatial proximity both in their exit from the grain and during the early growth of the tube, strongly suggesting that the gamete cells containing them remain linked to each other throughout germination and at least for a considerable period thereafter. On the other hand, the variation in the relative positions of the gamete and vegetative nuclei and their wide average separation in the tube show that if there is a persistent linkage between the vegetative nucleus and the gamete cells in this grass species it must indeed be very adaptable, capable of extending in either direction in the tube, and of being stretched certainly to lengths of greater than 50 μm. Detecting and tracing such a connection, which must surely be tenuous should it indeed exist, will be difficult technically. However, it will obviously be informative now to combine *in vivo* DAPI staining for the nuclei as employed here with differential interference microscopy to aid in the identification of the limits of the gamete cells in actively growing tubes where gamete and vegetative nuclei are widely separated.

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