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The behavior of nuclei in germinating pollen grains of wheat, rice and maize

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Summary. 1. Three species of Gramineae were used for obeservations on the behavior of the tube nucleus and sperm nuclei in germinating pollen grains of *Triticum aestivum*, *Oryza officinalis* and *Zea mays*.

2. In general all three species follow the same pattern in the behavior of the three nuclei.

3. Normal pollen grains contain one tube nucleus and two sperm nuclei which usually lie on the side of the grain opposite to the one containing the germ pore.

4. Germination of the pollen grains on the stigma starts after 3—5 minutes.

5. Two sperms enter the pollen tube and the tube nucleus follows.

6. In wheat, the tube nucleus remains frequently in the pollen grain, while it almost always emigrates in *Oryza* and *Zea*.

7. Empty pollen grains could be observed in wheat 30 minutes after pollination.

8. Germination of the pollen grains is not uniform. Even after 24 hours we found pollen grains at five different stages of germination.

9. The regular sequence in the movement of male and vegetative nuclei is rarely disturbed.

10. It is likely that male gametes are transported passively by the cytoplasmic stream to the pollen tube during germination, since they lie nearer to the germ pore than to the tube nucleus.

11. It is suggested that autonomous movement of male gametes may act as auxiliary agent in transportation.

12. The tube nucleus seems to be intimately connected with the cytoplasm and is located far from the germ pore. This may be the main reason why the tube nucleus enters the pollen tube later than the sperm nuclei.

13. The movements of the vegetative and generative nuclei during pollen formation are given in detail based on an old investigation by the senior author.

14. The behavior of nuclei during gametogenesis follows a definite course, indicating autonomy in the movements of the vegetative as well as the generative nuclei.

Our paper deals mainly with the behavior of the tube nucleus and the two sperms of pollen grains germinating on the stigma. For our present investigations three representative species of cereals were used, namely, *Triticum aestivum, Oryza officinalis* and *Zea mays*.

As early as 1921, PERCIVAL observed the behavior of the vegetative and generative nuclei in germinating wheat pollen grains and he described it as follows: "The two curved male gametes travel into pollen-tube before the oval tube nucleus and very frequently the latter never leaves the pollen grain".

PERCIVAL's observation was confirmed by HIRAYOSHI (1938). According to PODDUBNAYA-ARNOLDI (1960), the behavior is the same in *Secale*. It was also confirmed for *Oryza* by HIRAYOSHI (1938) and CHO (1956). This procedure seems to be universal in all Gramineae. However, such a study had never been undertaken in maize in spite of unparalleled advances in the studies on its morphology, cytology and genetics. In a rather recent monograph of corn edited by SPRAGUE (1955), we cannot find a detailed description on this point. The knowledge seems not to have advanced beyond the reports of WEATHERWAX (1917, 1919). This shortcoming can be seen also in the figures showing the life cycle of maize in two textbooks on genetics (SRB and OWEN 1952, SINGLETON 1965). Here we see a germinated pollen grain whose tube nucleus precedes the two sperms.

This was one of the reasons why we have started our microscopical studies on the behavior of the tube nucleus and the two sperms.

In preparing this paper we have learned very much from two recent publications, MAHESHWARI's Recent Advances in the Embryology of Angiosperms (1963) and NAIR's Advances in Palynology (1964).

Method

Staining of the tube nucleus and male nuclei with acetocarmine is easy for wheat pollen grains, but very difficult for those of rice and maize. However if we strongly heat the pollen grains mounted in acetocarmine over the flame of an alcohol lamp, the trinucleate condition can be clearly seen in all three materials. The method was especially successful for wheat.

The germination of pollen grains was observed on self-pollinated stigmata. The grains were treated with acetocarmine at different intervals (5, 10, 20, . . . minutes) after artificial or natural pollination.

Results

1. Triticum aestivum

Pollen grains of common wheat are most suitable for the observation of the behavior of the tube nucleus as well as the two sperms.

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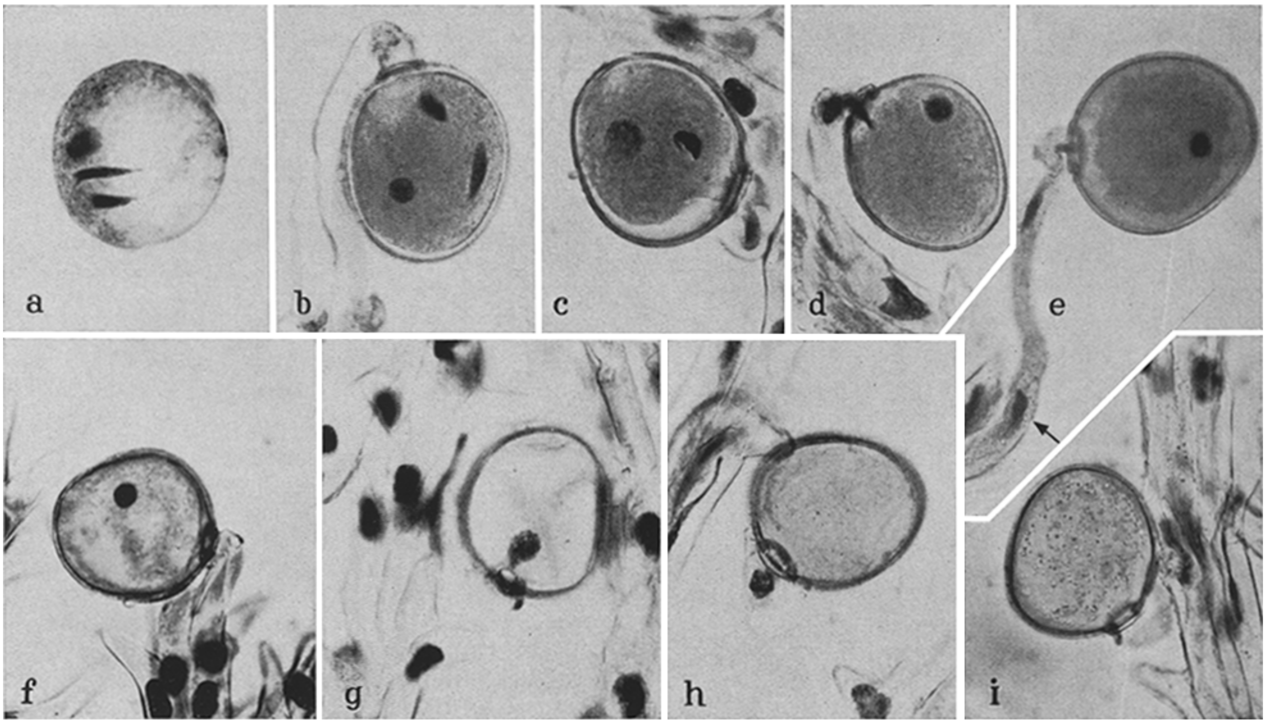

Fig. 1. Germination of pollen grains of *Triticum aestivum*. a) Normal pollen grain before pollination. It contains two wedge-shaped sperms and one tube nucleus (Stage I); b) Germinated pollen grain. Two sperms and one tube nucleus still inside the pollen grain (Stage II); c) One sperm entered into the pollen tube leaving the two other nuclei in the pollen grain (Stage III); d) Two sperms entering almost simultaneously the pollen tube. The tube nucleus round and compact (Stage III); e) Two sperm nuclei (shown by arrows) move forward in the pollen tube; one round tube nucleus remains in the pollen grain (Stage IV); f) Germinated pollen grain with one tube nucleus (Stage IV); g) Tube nucleus just entering into the pollen tube (Stage IV); h) Tube nucleus just entered into the tube (Stage V); i) Empty pollen grain after germination (Stage V). — Magnification is the same for all photomicrographs (Figs. 1—4). × ca. 500

Normal pollen grains contain one tube nucleus and two sperms. The sperms are usually wedge-shaped, but sometimes they are spindle-shaped (Fig. 1 a). Before the germination, the tube nucleus is roundish and has an irregular contour. But it becomes well rounded and compact after germination (Fig. 1 b).

The germination of pollen grains can be recognized three minutes after pollination from the appearance of a hyaline tip of the pollen tube protruding through the germ pore. However, not all pollen grains develop the pollen tube simultaneously. Therefore we can see pollen grains at various germination stages in the materials taken at the same time.

The process of germination of the pollen grains on the stigma can be divided into five stages according to the development of the pollen tube and the position of the three nuclei.

I. No pollen tube is formed (trinucleate) (Fig. 1 a).

II. Pollen tube elongates. The three nuclei are inside the pollen grain (Fig. 1 b).

III. Two sperms move into the pollen tube one by one or two together through the germ pore (Fig. 1 c—1 e).

IV. Only the tube nucleus remains in the pollen grain (Fig. 1 f—1 g).

V. Empty pollen grains (Fig. 1 h—1 i).

The results of our observations are given in Table 1.

Table 1. *Frequency of five stages (I—V) of germinating pollen grains observed at different intervals after pollination.*

Minutes	I	II	III	IV	V	Total	Burst pollen grains
5	66 (98.5)	1 (1.5)	0 (0.0)	0 (0.0)	0 (0.0)	67	many
10	42 (56.0)	13 (17.3)	17 (22.7)	3 (4.0)	0 (0.0)	75	many
20	32 (52.4)	4 (6.7)	9 (14.7)	9 (14.7)	7 (11.5)	61	a few
30	13 (38.2)	2 (5.9)	13 (38.2)	6 (17.7)	0 (0.0)	34	a few
45	14 (20.0)	7 (10.0)	25 (35.7)	21 (30.0)	3 (4.3)	70	a few
60	19 (17.1)	7 (6.3)	12 (10.8)	63 (56.8)	10 (9.0)	111	seldom
24×60	12 (7.5)	55 (34.6)	13 (8.2)	63 (39.6)	16 (10.1)	159	seldom

As shown in the last column of this table, exploded pollen grains were very often found when they were stained with acetocarmine shortly after pollination (5—10 minutes). Such pollen grains might have been fixed by acetocarmine treatment just before or after germination. This might be the reason why germinated pollen grains at stage II are found rarely (1.5%) when observed five minutes after pollination.

Burst pollen grains decreased in number with the time elapsed from pollination. They were rather rare 30 minutes after pollination, and $^1/_3$ of pollen grains reached to the advanced stages

IV and V after 45 minutes. After 60 minutes $^2/_3$ of pollen grains were at stages IV and V.

The plants (two Chinese varieties) used for our observations were cultivated in our greenhouse and the ears were brought to our laboratory room (18 °C). As the preparations were made uniformly, the results should be comparable.

The results given in the last line at the lower end of Table 1 were obtained from a pistil one day after pollination. This material was, like the others, a hexaploid wheat, a commercial variety cultivated near Misima. The preparations were very clear. Therefore the determination of the germination stages was accurate. The microphotographs (Fig. 1b—1i) are taken from this material. From the table we see that after 24 hours 10% of all pollen grains observed were at stage V, while about 40% were at stage IV.

It is interesting to note that we find still many pollen grains (34.5%) at stage II in this material, even after 24 hours. There are two possible interpretations of this finding.

(1) The pollen grains dusted on the stigma were still alive after about one day and started to germinate just before fixation.

(2) The pollen grains have germinated within a few minutes after pollination, but stopped to develop further and remained in this condition (Stage II).

Our observations seem to support the second alternative as no protoplasmic flow was observed in the pollen grains 2—4 hours after pollination.

Abnormalities were observed, for instance, the tube nucleus entering into the pollen tube leaving

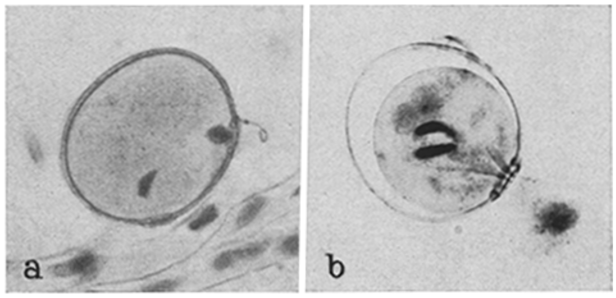

Fig. 2. Abnormalities in the sequence of nuclear migration. — a) A germinated pollen grain; tube nucleus just passes through the germ pore leaving one sperm behind; b) A burst pollen grain. Two sperm nuclei remain in the pollen and the tube nucleus is outside.

one sperm behind inside the pollen grain (Fig. 2a). HIRAYOSHI reported in his paper on *Oryza* that sometimes the tube nucleus moves into the pollen tube prior to the sperms. In wheat such anomalous behavior of the tube nucleus was mostly met with when the bursting pollen grains were discharging the tube nucleus together with a cytoplasmic mass, while one or two sperm nuclei were left behind (Fig. 2b).

2. *Oryza officinalis*

The behavior of the tube nucleus and two elliptic sperms seems to follow a similar pattern as in common wheat (Fig. 3a—3f). However it seems that the tube nucleus of *Oryza officinalis* travels always to the pollen tube soon after the two sperms had entered the tube, as shown by CHO (1956) for *O. sativa*. HIRAYOSHI (1938) found only one exceptional pollen grain having a remaining tube nucleus among several hundred germinated pollen grains in *O. sativa*.

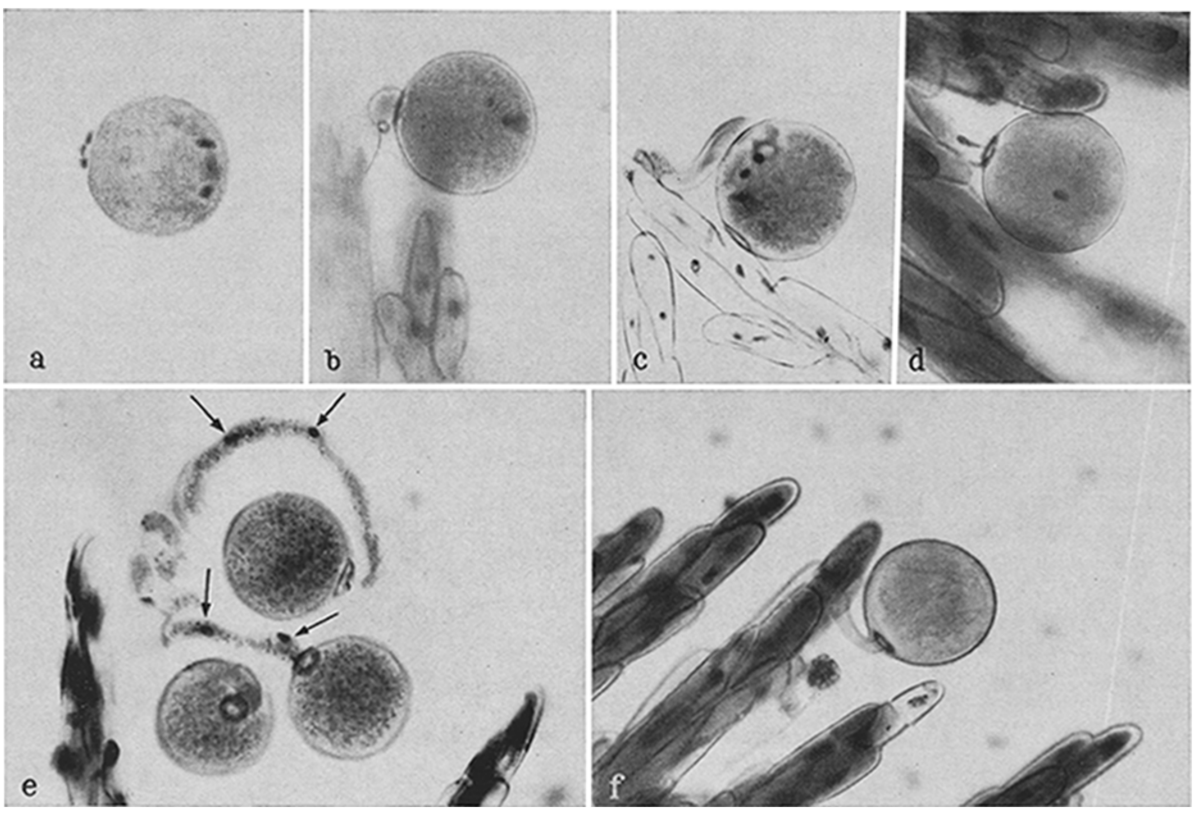

Fig. 3. Germination of pollen grains of *Oryza officinalis*. — a) Normal pollen grain with two dark elliptic sperms and one round tube nucleus. All three nuclei are found on the opposite side of the grain in relation to the germ pore (Stage I); b) Germinated pollen grain with three nuclei still far from the germ pore (Stage II); c) Germinated pollen grain with three nuclei inside the pollen grain (Stage II). All three nuclei near the germ pore; d) One sperm passing through the germ pore and the other still inside the pollen grain. The tube nucleus is not well stained (Stage III); e) Two sperm nuclei (indicated by arrows) enter the pollen tubes of two germinated pollen grains (Stage IV); f) Empty pollen grain after germination (Stage V).

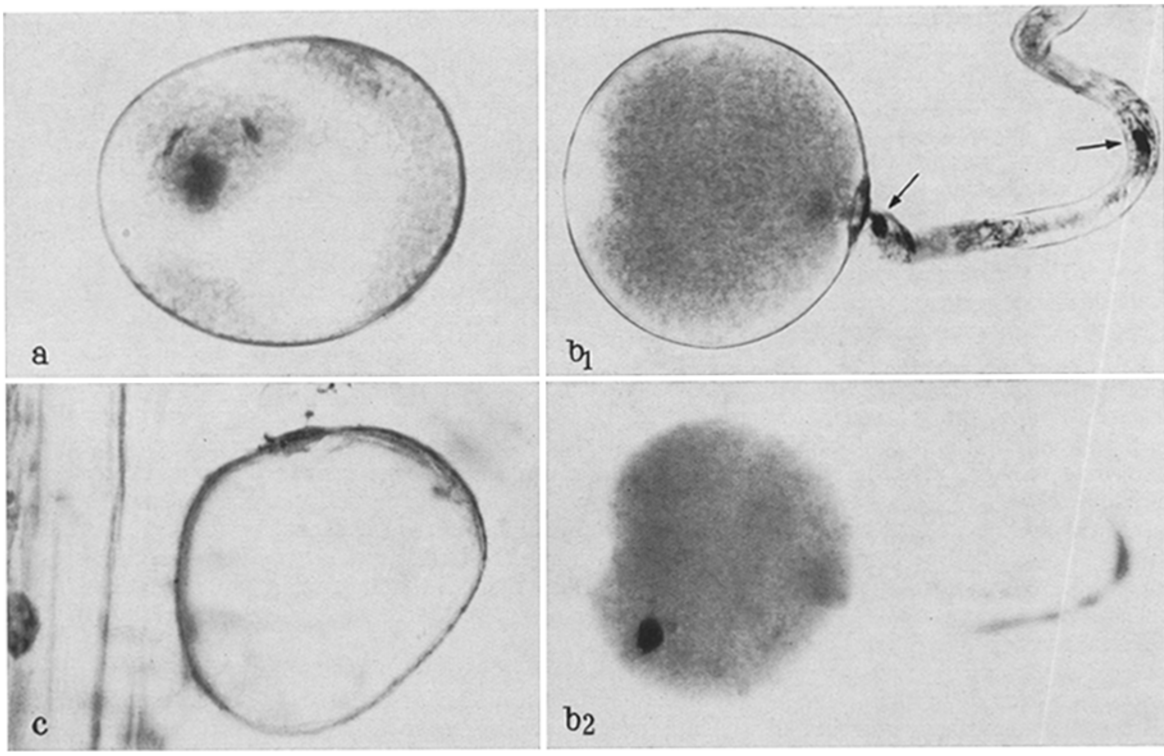

Fig. 4. Germination of pollen grains of *Zea mays*. — a) Normal pollen grain before pollination (Stage I). Three nuclei lie closely together; b1) Germinated pollen grain; two sperms in the tube (Stage III); b2) Same pollen grain as b1 focussed to show the tube nucleus; c) Empty pollen grain after germination (Stage V).

After CHO (1956), the pollen tube begins to appear 1.5—3 minutes after pollination at room temperature of 27 °C. The fertilization takes place 1.5—4 hours after pollination. This is very quick compared with wheat and corn in which the time interval from pollination to fertilization was found to be approximately 15 hours (WAKAKUWA 1934) for wheat and 16 hours for corn with short silks (RANDOLPH 1936).

3. *Zea mays*

Maize has a large pollen grain. Therefore it seemed at first glance that the observation might be simple. However it was very difficult owing to the occurrence of dense starch grains.

We found no difference in the behavior of vegetative and generative nuclei in maize in comparison with the other two species described above, as can be seen from Fig. 4a—4c. The tube nucleus enters into the pollen tube after the two sperms and does not remain in the pollen grains just like in *Oryza*.

The tube nucleus is irregular in outline as in the other two species. But it becomes round or elliptic and compact after germination. The two male nuclei are spear-shaped, pointed at one end. Sometimes they are bent in the middle (Fig. 4a). After WEATHERWAX (1917), however, the sperms are long and slender, pointed at the ends and usually crescent-shaped. The difference seems to depend on the staining technique.

4. *Movement of the vegetative and generative nuclei in wheat pollen grains*

NAVASHIN et al. (1959) proposed a theory of passive transportation of the vegetative nucleus and sperms through the streaming cytoplasm of the pollen tube (cf. STEFFEN 1963). This view would appear very convincing in sight of the strong current of protoplasm running into the pollen tube. If so, why are the sperms the first to enter into the pollen tube?

The situation of three nuclei in the mature pollen grains just after shedding can answer this question. As shown in Diagram 1, the tube nucleus is found almost always (97.4%) at the opposite side of the pollen grain in respect to the germ pore (A—C, cf. Figs. 1a and 3a), while only seldom (2.6%) could we see it near the germ pore (D—F). So it may be expected that the sperms would enter the pollen tube before the tube nucleus. This interpretation is also in accord with the finding that there are irregularities in the sequence of nuclear transportation.

IWANAMI (1956) observed in *Lilium auratum* a protoplasmic movement in the pollen grains just

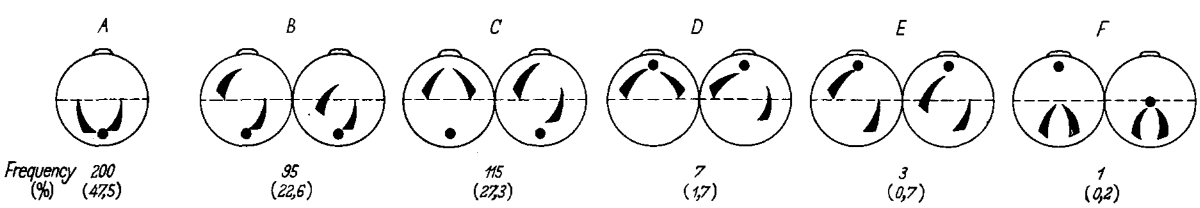

Diagram 1. Position of the tube nucleus and the two sperms in mature wheat pollen grains in relation to the germ pore. The diagram shows the frequency of various configurations (A—F).

before germination. This movement was called "agitation", which with the formation of the pollen tube was transformed into streaming movements of "rotation" and "circulation".

Such movements were clearly observed in wheat pollen grains. They could change the position of the three nuclei found in nongerminating pollen grains (Fig. 1a). Factually, when we observed the position of the nuclei at stages II and III, we found all three scattered over the whole cytoplasm (Fig. 1b—1c). Thus, the protoplasmic stream might not always carry the two sperms prior to the tube nucleus. However, it is rather the rule that both sperms enter into the pollen tube before the tube nucleus.

Therefore, in addition to the cytoplasmic movement we should assume one more agent taking part in the movement of the sperms, namely their own amoeboid movement. STEFFEN (1963) and POLUNINA et al. (1959) have confirmed an independent movement of the sperms from the observation on living pollen tubes (after STEFFEN 1963). Above all we should keep in mind that the male gametes are free from the cytoplasmic network of the pollen grain. Consequently the transportation of sperms through the cytoplasmic stream could be easy. Thus, we may assume that an autonomous movement would help the sperms as an auxiliary agent to join the cytoplasmic stream, even if they were located far from the stream.

This might not be applicable to the same extent to the large tube nucleus which is usually considered to be ephemeral or degenerating and takes a poor stain. But it also undergoes changes in morphology and size and is not free from protoplasmic network, judging from its irregular and indistinct outlines which give place to round shape and compact appearance. After germination a hyaline ring around the nucleus is sometimes observed (Fig. 1d). These transformations could be favorable to a free movement also of the tube nucleus. It could be transported through the

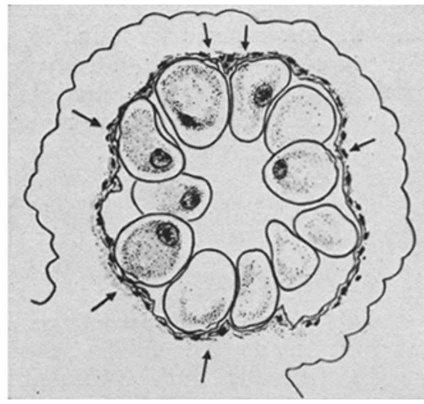

Fig. 5. Cross section through an anther of *Triticum monococcum*. Pollen grains at the stage shown in Fig. 6b (arrow shows the germ pore).

cytoplasmic current to the pollen tube together with the cytoplasm surrounding it or would remain within the pollen grain in naked condition.

After STEFFEN (1963), the position of the nucleus and that of the germ pores and furrows show a relationship which is generally constant for a species. The pollen grains of the three species used for our observation are identical concerning the relative position of the three nuclei and the germ pore. Therefore, the same or almost the same behavior of the three pollen nuclei could have been expected.

In this connection, we want to mention an old observation reported by the senior author on the behavior of the vegetative and generative nuclei during the development of pollen grains in *Triticum monococcum* (KIHARA 1937)[1]. The following is his description supplemented by our present study (see footnote) (Figs. 5 and 6a—6h).

After the separation of the pollen tetrads the four microspores grow very rapidly (Fig. 6a). They lie

[1] Similar results were obtained earlier by TAKAG (1936) who used *Secale cereale* for her study.

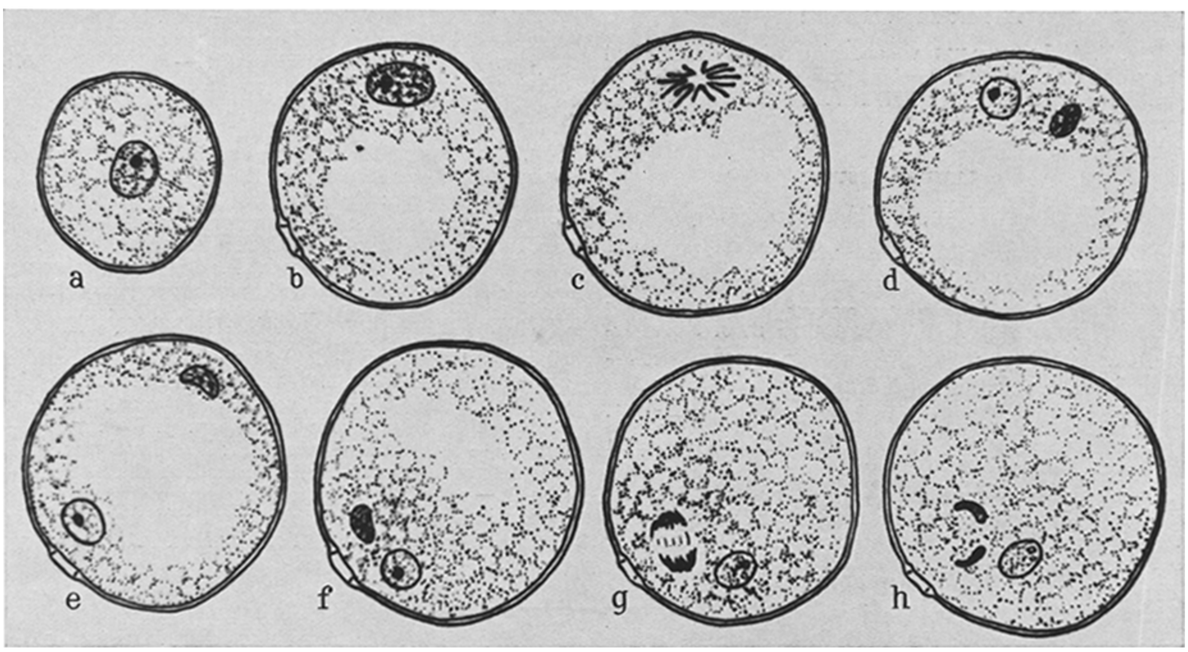

Fig. 6. Development of pollen grains of *T. monococcum* (schematized). — a) Young pollen grain immediately after the separation of a tetrad; b) Germ pore and vacuole are formed (cf. Fig. 5); c) First division; d) Two daughter nuclei from first division; e) Vegetative nucleus moves away from the generative nucleus to the opposite side; f) Two nuclei lie closely together; g) Division of the generative nucleus; h) Just completed pollen grain.

scattered in the cavity of the anther. With the growth of the anther the microspores are found to adhere at one end to the tapetum cells (Fig. 5). They form a ring in cross section with a hollow space in the middle. An oval-shaped vacuole develops in the middle of the cell (Fig. 6b). The microspores stick to the tapetum at the end containing the germ pore, as if indicating the passage of nourishment from the tapetum to the young grains, while their nucleus lies near the other, free end (Fig. 5).

In this position the first nuclear division takes place simultaneously (Fig. 6c—6d) and one larger daughter nucleus moves to the opposite side, while the other, smaller one (the generative nucleus)[1] remains in place (Fig. 6e). Consequently, two nuclei are found on the opposite sides of the vacuole which occupies a large area in the middle of the cell. Then the generative nucleus moves toward the larger tube nucleus and the two nuclei lie closely together (Fig. 6f). The generative nucleus divides here (Fig. 6g) and two sperms are produced. The vacuole disappears at this stage. The formation of the pollen grain is completed (Fig. 6h).

The three nuclei lie close to the germ pore at the beginning. As mentioned above, we have observed that before pollination they are found mostly at the opposite side, facing the germ pore. Thus, the nuclei move frequently from one place to another and their movement follows a definite course. This indicates that the nuclei can move autonomously.

Many authors favor the view that the male gametes have their own cytoplasm. VASIL (1962) thinks that the male cytoplasm is reduced to its barest minimum in the pollen grain or in the pollen tube. If so, the fate of the cytoplasm surrounding the male gametes at the time of or after fertilization is of a considerable interest. We cannot yet answer this question from our present studies. With regard to cytoplasmic male sterility, a study of the cytoplasm of the sperms may have important implications.

Acknowledgement

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Zusammenfassung

Bei 3 Gramineenarten, *Triticum aestivum*, *Oryza officinalis* und *Zea mays*, wurde das Verhalten der Zellkerne im keimenden Pollenkorn untersucht.

In allen 3 Fällen enthalten die Pollenkörner normalerweise einen vegetativen Kern und zwei Spermakerne, die meist an der der Keimpore gegenüberliegenden Seite des Pollenkorns liegen.

Die Keimung auf der Narbe beginnt nach 3—5 Minuten. In den Pollenschlauch treten zuerst die beiden Spermakerne ein. Bei *Triticum* bleibt der vegetative Kern häufig im Pollenkorn, bei *Oryza* und *Zea* dagegen folgt er fast immer den Spermakernen. Leere Pollenkörner wurden 30 Minuten nach der Bestäubung beobachtet. Die Keimung verläuft nicht einheitlich; nach 24 Stunden konnten noch Pollenkörner 5 verschiedener Keimungsstadien gefunden werden.

Wahrscheinlich werden die Spermakerne während der Keimung passiv durch die Plasmaströmung zum Pollenschlauch bewegt, da sie der Keimpore näher liegen als der vegetative Kern. Der vegetative Kern scheint eng mit dem Zytoplasma verbunden zu sein und liegt von der Keimpore weit entfernt. Vielleicht sind dies die Hauptgründe dafür, daß der vegetative Kern später als die Spermakerne in den Pollenschlauch eintritt.

Auf Grund einer früheren Untersuchung des älteren Verfassers wird eine ausführliche Beschreibung der Bewegungen der Kerne während der Pollenbildung gegeben. Das Verhalten der Kerne während der Gametogenese folgt einer bestimmten Richtung, die auf ein autonomes Agens in den Bewegungen der vegetativen und, besonders, der generativen Kerne schließen läßt.

[1] Smear preparations of our present material showed clearly that the generative nucleus is surrounded by its own cytoplasm. This is also true for *Oryza* (KIHARA and HIRAYOSHI 1942).

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