THE ANTIPODALS IN RELATION TO ABNORMAL ENDOSPERM BEHAVIOR IN HORDEUM JUBATUM×SECALE CEREALE HYBRID SEEDS[1]

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Received December 7, 1943

INTRODUCTION

GROSS mitotic irregularities in the endosperm are a characteristic feature of seed development following certain interspecific matings in the Gramineae. KIHARA and NISHIYAMA (1932) reported this behavior for the cross *Avena strigosa*×*A. fatua*, and it has been observed in other cereal hybrids since by several investigators. The seed resulting from mating *Hordeum jubatum* (squirrel-tail barley) with *Secale cereale* (rye), as described in the preceding paper, is a case in point. Abnormal chromosome distribution may occur at the first nuclear division following triple fusion; or the initial irregularity may appear at an early mitosis thereafter. A result of this behavior is that the endosperm nuclei come to vary widely in size and chromosome number; and the hybrid endosperm fails to become cellular as in normal *H. jubatum*. Eventually the ill-developed endosperm tissue ceases growth and the hybrid seed breaks down six to 13 days after fertilization.

The early and invariable occurrence of abnormal mitosis in the Hordeum ×Secale endosperms might lead one to think that the phenomenon is an immediate effect of the cross and the basic cause of the seed failure. This explanation rests on the assumption that the two diverse genomes, when combined, impair directly the capacity of the hybrid nucleus to divide in the usual manner. There are certain other clearly established facts, however, which are not reconcilable with such an interpretation. In the first place the embryo, which is likewise a hybrid structure, shows regular mitosis. Secondly, a *H. jubatum*×*S. cereale* plant has been reared to the adult stage from an embryo excised from the immature seed and cultivated on an artificial nutrient medium (BRINK, COOPER and AUSHERMAN, 1944). One would not expect the hybrid zygote to be capable of such extended development if the combined Hordeum and Secale genomes tend to upset the mitotic mechanism. A third fact casting doubt on this explanation is that mitotic irregularities eventually may appear also in the antipodals following fertilization of the squirrel-tail barley by rye. The antipodals are not of hybrid origin, of course, but carry a haploid complement of *H. jubatum* chromosomes only.

The above considerations make it probable that the mitotic abnormalities in the hybrid endosperm are of secondary origin. Since the nuclear disturbances

[1] Paper from the Department of Genetics, No. 327, Agricultural Experiment Station, UNIVERSITY of WISCONSIN. The writers desire to express their appreciation for support of the work from the ROCKEFELLER FOUNDATION. They are indebted to PERRY BUCKNER for technical aid.

are irreversible, however, development of the endosperm is permanently handicapped once the chromosome distribution is upset; and the impairment of this tissue leads ultimately to collapse of the seed, as described in the preceding article. On this view an explanation of seed failure following the cross is to be sought in the conditions underlying early growth of the hybrid endosperm. The primary endosperm nucleus may divide irregularly within an hour or so

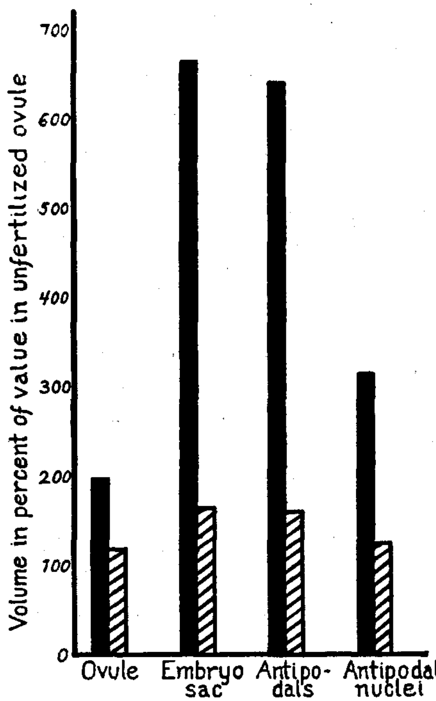

FIG. 1.—Relative volumes at fertilization of the ovule, embryo sac, antipodals and antipodal nuclei following the matings *Hordeum jubatum*, selfed (solid bar), and *H. jubatum*×*Secale cereale* (crosshatched bar). Volumes of the four respective tissues in the unfertilized ovule are taken as 100.

after fertilization. Accordingly, search for a possible cause of the aberrant endosperm behavior was directed to the embryo sac during the brief period between presentation of the two male gametes to the egg and polar nuclei, respectively, and the first endosperm division.

CHANGES IN THE OVULE AT FERTILIZATION

Since it was desired to determine whether significant effects on the ovule of the mating with rye could be observed before mitosis in the endosperm had begun, ovules were sought at two hours and four hours after pollination in which fertilization had occurred but in which division of the primary endosperm nucleus had not yet begun. Four such ovules from the *H. jubatum*×*S. cereale* mating and a like number from *H. jubatum* selfed were found in the material at hand. These eight ovules had been fixed at four hours after pollination. Three unfertilized *H. jubatum* ovules taken at two hours after pollination were available for comparison.

The volumes of these 11 ovules and of their contained embryo sacs, antipodals and antipodal nuclei were determined. These values were computed

TABLE 1

Volume of the Hordeum jubatum ovule and of certain contained structures at (1) the mature embryo sac stage, (2) after self-fertilization and (3) following hybridization with Secale cereale, the two latter classes of ovules being measured before the primary endosperm nucleus has divided.

TYPE OF OVULE	OVULE NO.	SIZE IN $MM^3 \times 10,000$			
		OVULE	EMBRYO SAC	ANTIPODALS	ANTIPODAL NUCLEI
1. *Hordeum jubatum* (unfertilized)	1	98.52	6.133	2.020	.4752
	2	87.92	6.568	1.695	.3766
	3	101.85	5.754	1.370	.2653
	Av.	96.10	6.152	1.695	.3724
2. *H. jubatum*, selfed	1	189.55	44.595	12.409	1.2498
	2	209.44	43.743	11.219	1.1257
	3	177.07	27.389	6.707	.9289
	4	183.62	48.873	13.205	1.4082
	Av.	189.92	41.150	10.885	1.1781
3. *H. jubatum*×*S. cereale*	1	102.10	9.767	2.752	.4623
	2	132.14	12.833	3.373	.6121
	3	120.97	9.180	2.641	.4579
	4	106.77	9.331	2.230	.4408
	Av.	115.49	10.278	2.749	.4933

from planimeter measurements of outline drawings of the tissues made with the aid of a camera lucida from successive serial sections cut at 15 microns thickness. An accurate estimation of volume is obtained in this way although the procedure is laborious. The measurements are presented in table 1 and illustrated diagrammatically in figure 1. The data are limited in extent but they are very instructive.

Large changes in size of certain tissues within the ovule accompany self-fertilization in *H. jubatum*. A comparison of the values in table 1 for *H. jubatum* selfed with those of the unfertilized ovules shows that total volume of the ovule doubles during the course of gametic union and that of the embryo sac increases over six-fold. The prominent antipodal tissue, occupying about one-quarter of the space in the embryo sac of the unfertilized ovule, likewise increases in size during this interval to over six times its former volume. The antipodal nuclei enlarge about three-fold. These observations reveal that fertilization of *H. jubatum* following selfing results in a very rapid and pronounced stimulation of the ovule, leading immediately to large increases in size of the embryo sac and antipodals. These changes become manifest before either the primary endosperm nucleus or the zygote divides.

A very different behavior is revealed in the ovules at the same stage when *S. cereale* is used as the staminate parent. The increase at fertilization in size

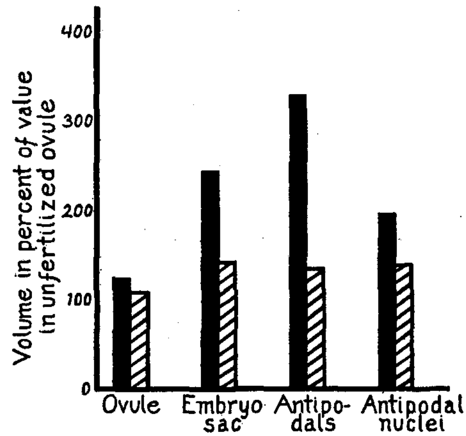

FIG. 2.—Relative volumes at fertilization of the ovule, embryo sac, antipodals and antipodal nuclei following the matings *Hordeum vulgare*, var. Olli, selfed (solid bar), and *H. vulgare* var. Olli×*Secale cereale* (crosshatched bar). Volumes of the four respective tissues in the unfertilized ovule are taken as 100.

of ovule, embryo sac, antipodals and antipodal nuclei is far less than that occurring following self-pollination. The expansion of the embryo sac associated with hybrid fertilization is only about one-eighth that in evidence following selfing and the enlargement of the antipodal tissue is correspondingly small. The antipodal nuclei, which increase in size about three-fold following self-fertilization, are only slightly enlarged following crossing. Evidently rye sperm evokes a much weaker response in the *H. jubatum* ovule than does *H. jubatum* sperm.

THE HORDEUM VULGARE×SECALE CEREALE OVULE

The great enlargement of the antipodals and of the embryo sac in self-fertilized *H. jubatum* normally occurring before the primary endosperm nucleus divides and the failure of rye sperm to stimulate the tissues in like degree appeared to be of basic significance in understanding the cause of failure in the hybrid seeds. The presumed facts were based on such limited data, however, that an independent confirmation of the relation was desirable. Additional material was available for histological study from the mating of common barley, *H. vulgare*, with rye by which the broader validity of the earlier observations could be tested. Data were taken accordingly on volume of ovule, embryo sac, antipodals and antipodal nuclei on five seeds each at the fertilization stage following the matings, *H. vulgare* (var. Olli) selfed, and *H. vulgare* var. Olli×*S. cereale*, and on four unfertilized barley ovules of the same variety used in the two matings above. The results are presented in table 2 and figure 2.

It is apparent from the data in table 2 that the relationship observed following the cross *H. jubatum*×*S. cereale* holds also in a similar cross when

TABLE 2

Volume of the Hordeum vulgare ovule and of certain contained structures at (1) the mature embryo sac stage, (2) after self-fertilization and (3) following hybridization with Secale cereale, the two latter classes of ovules being measured before the primary endosperm nucleus has divided.

TYPE OF OVULE	OVULE NO.	SIZE IN MM³×10,000			
		OVULE	EMBRYO SAC	ANTIPODALS	ANTIPODAL NUCLEI
1. *Hordeum vulgare* (unfertilized)	1	286.0	18.221	1.942	.3105
	2	293.6	40.336	5.328	.6832
	3	337.5	45.222	6.284	.7077
	4	368.3	17.759	2.504	.2196
	Av.	321.3	30.384	4.014	.4802
2. *H. vulgare*, selfed	1	360.8	72.951	12.205	.8055
	2	407.4	74.400	13.277	1.0774
	3	457.9	100.148	15.943	1.0705
	4	436.0	74.271	15.127	1.0287
	5	351.9	51.163	9.325	.7636
	Av.	402.8	74.587	13.175	.9491
3. *H. vulgare*×*S. cereale*	1	315.4	28.189	3.703	.6171
	2	250.5	23.965	3.009	.3172
	3	431.3	59.351	7.171	.7392
	4	383.5	54.596	6.319	.8997
	5	376.8	53.672	6.842	.7878
	Av.	351.5	43.955	5.409	.6722

H. vulgare is used as the pistillate parent. With the advent of fertilization there is a striking increase in size of the embryo sac, antipodals and antipodal nuclei in the common barley ovule following selfing and much smaller increases in the corresponding parts of the ovule following crossing with rye.

Mitotic irregularities in the endosperm are a conspicuous feature of development following the *H. vulgare*×*S. cereale* mating; and collapse of this seed occurs earlier than in the other hybrid mating. The responses of the embryo sacs and antipodals to the rye sperm in *H. jubatum* and *H. vulgare* also are similar. Both the immediate results of hybridization and the subsequent gross behavior of the seeds, therefore, are found to be much alike in the two cases.

Hence one may conclude with some confidence that the early effects of crossing described for the few *H. jubatum*×*S. cereale* seeds observed are actually characteristic of the hybrid condition.

SUBSEQUENT BEHAVIOR OF THE ANTIPODALS

The differential stimulation of the *H. jubatum* antipodals at the fertilization stage, depending upon whether the sperm is derived from the same species or

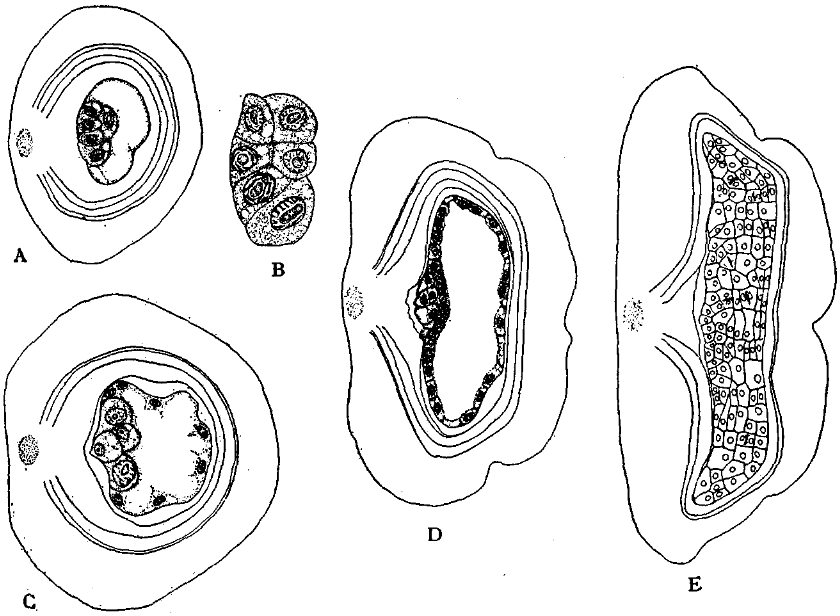

FIG. 3.—Transverse sections of developing grains of *Hordeum jubatum* through the mid-region of the antipodal tissue at stated intervals following self pollination.

A. 10 hours. The antipodal cells and nuclei have greatly increased following fertilization. The cytoplasm has a spongy appearance and vacuoles are forming in the apical portions of the cells. ×69.

B. 10 hours. A group of antipodal cells showing the vacuolate condition of the cytoplasm and the enlarged nuclei. ×142.

C. 28 hours. The antipodal cells are fully extended and the cytoplasm is highly vacuolate. ×69.

D. 48 hours. The antipodal cells are highly vacuolate and shrinking in size. ×69.

E. 72 hours. The antipodal tissue is no longer present. ×69.

from rye, foreshadows striking divergencies in the subsequent behavior of the tissue. The major developmental changes occurring in embryo, endosperm and antipodals of the two classes of seed up to 72 hours after pollination are summarized in table 3, which is based upon the detailed results reported in the preceding paper. We are especially concerned here with the extraordinary contrast in antipodal behavior.

The increase in size of antipodal cells and nuclei in normal *H. jubatum* which, as shown above, is detectable even at the fertilization stage, continues to about 28 hours. Just prior to fertilization these cells are small and the nuclei are embedded in very dense cytoplasm. Both cells and nuclei have increased greatly in size at ten hours following pollination and many small vacuoles are appearing in the cytoplasm (fig. 3, A and B). At 28 hours this tissue is most

TABLE 3

A summary of development between 10 and 72 hours of normal Hordeum jubatum seeds in contrast with H. jubatum×Secale cereale seeds.

TIME IN HOURS	MATING	EMBRYO	ENDOSPERM	ANTIPODALS
10	*H. jubatum*, selfed	zygote	4- to 8-nucleate	Cells and nuclei much enlarged; prominent vacuoles in cell apices
	H. jubatum×*S. cereale*	zygote	2- to 4-nucleate	Cells and nuclei variable in size, mostly small; cytoplasm, mostly dense
28	*H. jubatum*, selfed	4 to 6 cells	Typically 64-nucleate	Cells fully extended, highly vacuolate, no increase in number
	H. jubatum×*S. cereale*	zygote to 4-celled	2 to 16 nuclei varying in sizes; mitosis often aberrant	Cytoplasm mostly dense; some increase in cell size
48	*H. jubatum*, selfed	20 to 30 cells	Cellular near embryo; central cavity enclosed by layer of cells	Shrunken and flattened between endosperm and remnant of nucellus
	H. jubatum×*S. cereale*	3 to 10 cells	Varying from 2 giant nuclei to 32 or more of variable size	Cells actively dividing; 32 mitotic figures found in one seed
72	*H. jubatum*, selfed	120 cells	Central cavity filled with cells	Have disintegrated, only fragment remaining
	H. jubatum×*S. cereale*	50 to 80 cells	Very variable in number and size of nuclei; no cell formation	Cells still increasing in number; mitosis is irregular

fully extended (fig. 3 C). There is no increase in number of antipodal cells and nuclei beyond that characteristic of the mature embryo sac stage. After 28 hours the antipodals begin to regress (fig. 3 D); and by 72 hours they are no longer present as an organized structure (fig. 3 E). The antipodals, therefore, are a prominent and presumably active tissue in normal *H. jubatum* throughout the period when the endosperm is free nucleate. They quickly decline when the rapidly developing young endosperm becomes cellular.

The antipodals in the hybrid seeds, on the other hand, change slowly during the period in which their normal counterparts are enlarging rapidly. Furthermore, the early changes in cell and nuclear size are not only small but also uneven and the cytoplasm remains dense with a very few small vacuoles (fig. 4, A and B). The cells are continuing to increase in size at 28 hours. Some of

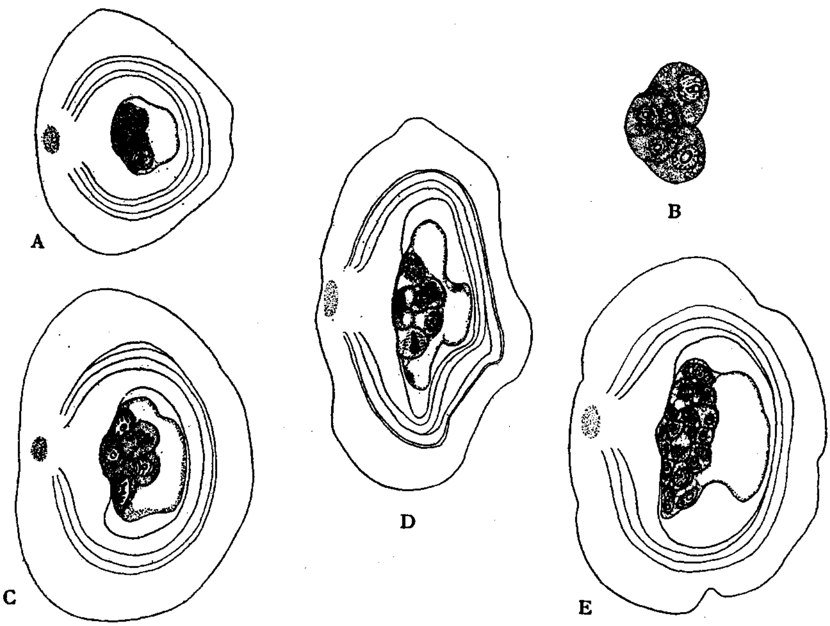

FIG. 4.—Transverse sections of developing grains of *Hordeum jubatum* through the mid-region of the antipodal tissue at like intervals following cross pollination, *Secale cereale* being used as the staminate parent.

A. 10 hours. A few of the antipodal cells increase somewhat in size while the majority remain small. All cells are densely cytoplasmic and there is little evidence of vacuolization. ×69.

B. 10 hours. A group of antipodal cells showing their small and uneven size and the dense nature of their cytoplasm. ×142.

C. 28 hours. The antipodal cells have increased in size and are, for the most part, densely cytoplasmic. Nuclei of two of the cells are in stages of division. ×69.

D. 48 hours. The antipodal tissue is increasing in size due to nuclear and cell division. ×69.

E. 72 hours. A large compact antipodal tissue is formed as a result of the increasingly meristematic condition of its cells. ×69.

the nuclei are in stages of mitosis at this time (fig. 4 C). Nuclear and cell divisions are frequent at 48 hours (fig. 4 D) and continue at 72 hours (fig. 4 E). By 96 hours, however, the hyperplastic antipodal tissue characteristic of the hybrid seed is breaking down.

The metamorphosis of the antipodals in the normal *H. jubatum* seed is further illustrated in figures 5 to 8. These are outline drawings based on 15-micron serial sections of typical seeds and are intended to reflect volume changes. A comparison of figures 5 and 6 shows the marked increase in antipodal size associated with normal fertilization. Figure 7 shows the *H. jubatum* antipodals

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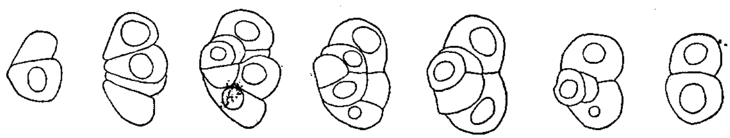

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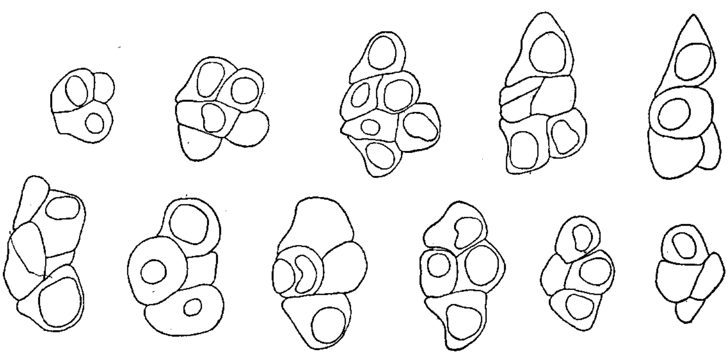

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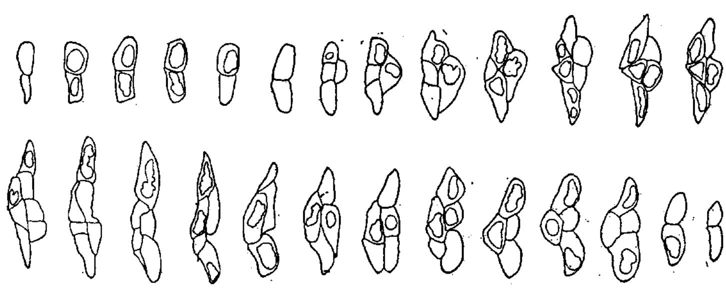

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FIG. 5.—Serial transverse sections of the antipodal tissue showing its size and extent in the mature megagametophyte of *Hordeum jubatum*. ×124.

FIG. 6.—Serial transverse sections of the antipodal tissue from a megagametophyte two hours after self pollination and immediately following fertilization showing the great increase in the size of both cells and nuclei. ×124.

FIG. 7.—A like series at 28 hours following self pollination showing the great increase in size of antipodal tissue. ×124.

FIG. 8.—A like series at 48 hours following self pollination showing the flattened condition of the antipodal cells. ×124.

at their maximum size, 28 hours after pollination. By 48 hours, as illustrated in figure 8, a marked shrinkage of the tissue has taken place.

Only one illustration of the *H. jubatum*×*S. cereale* hybrid is included in this series, namely, figure 9. This is based upon the antipodals in a seed collected at

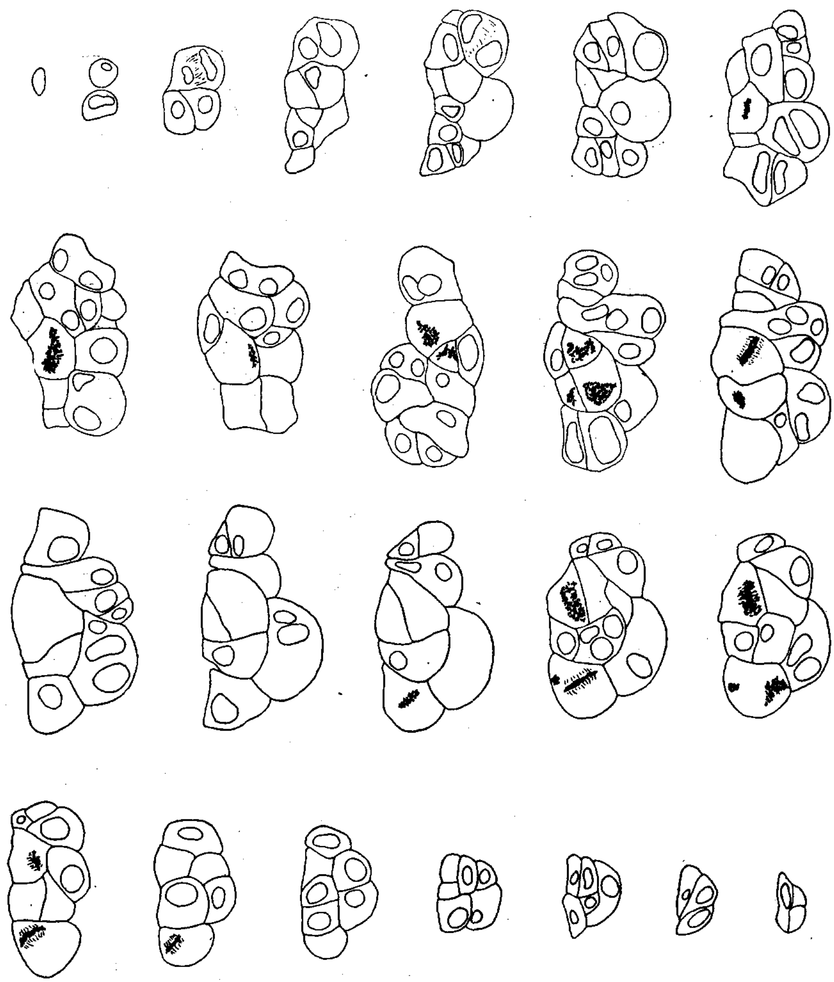
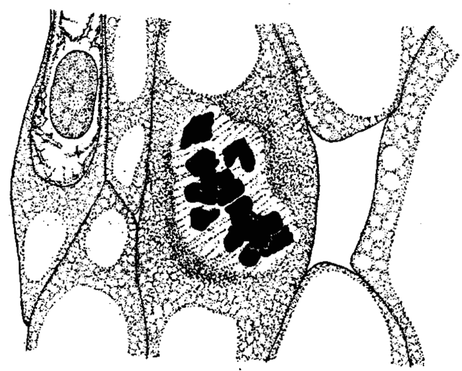


FIG. 9.—Serial transverse sections of the antipodal tissue of *Hordeum jubatum* at 72 hours following pollination using *Secale cereale* as the staminate parent. The tissue is actively meristematic and the cells vary greatly in size and number of nuclei per cell. ×142.

72 hours. The persistence at this comparatively late stage and the large size of the tissue are noteworthy. The cells are irregular in size and several mitotic figures are present. Figure 9 illustrates the maximum development of the antipodals in the hybrid seed.

Two types of division figures are to be found in the antipodals of the hybrid seed, both of which may occur in the same tissue. One type is characterized by giant chromosomes present in the normal number. This class is represented in figures 10 and 11, showing, respectively, 14 large chromosomes approaching the equatorial plate at 42 hours after pollination and 14 longitudinally split giant chromosomes, 10 hours later, in a cell in which the nuclear membrane has disappeared. The nuclei possessing giant chromosomes do not give rise to



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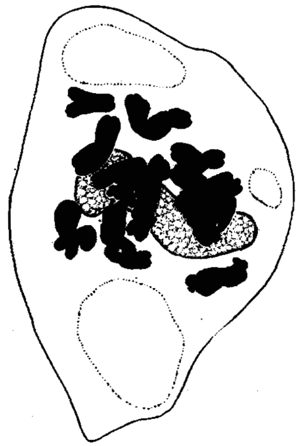

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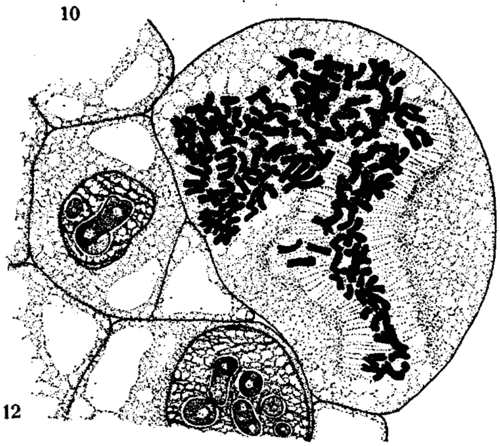

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FIGS. 10 and 11.—Mitoses in antipodal cells with 14 giant chromosomes. Figure 10, metaphase at 42 hours after cross pollination; figure 11, late prophase at 52 hours after cross pollination. ×686.

FIG. 12.—Section of a large antipodal cell showing the large number of chromosomes of normal size which may be present in such cells. The metaphase plate was coiled within the cell and extended through six sections, one of which is shown, so that many times the gametophytic number (14) of chromosomes was distributed on it. ×686.

daughter nuclei although division appears to proceed to various stages short of this before being arrested. Considerable hypertrophy of the antipodal nuclei and increase in size of chromosomes occurs normally in *H. jubatum* selfed. This type of antipodal nucleus in the hybrid seed is similar. The most apparent difference, however, is that the nuclei in the normal seed do not show division stages beyond prophase. The second type of antipodal division figure is found in very large cells in which high numbers of chromsomes of normal size are present. Such an antipodal cell in a hybrid seed taken at 56 hours is shown in figure 12. This cell extended through seven sections cut at 15 microns thickness. The equatorial plate was coiled within the cell and many times the gametophytic number (14) of chromosomes was distributed on it. Figure 12 is based on but one of the six sections in which portions of the equatorial plate were found. This is an extreme case. Numerous other antipodal cells were observed, however, whose nuclei contained greatly increased even if lesser numbers of chromosomes.

The fact is established, therefore, that irregularity in chromosome number in the nuclei of *H. jubatum*×*S. cereale* seeds is not limited to the endosperm, which is of hybrid origin, but may arise also in the antipodal tissue whose nuclear complement is derived entirely from the maternal parent. This circumstance makes it probable that hybridity of the nucleus in itself is not the direct cause of the aberrant mitotic behavior of the endosperm.

DISCUSSION

It has been pointed out in the previous paper that the immediate cause of seed failure following fertilization of *Hordeum jubatum* by *Secale cereale* is starvation arising from impaired development and, eventually, a complete breakdown of the endosperm. The latter tissue appears to be a necessary intermediary between the parent sporophyte and the developing embryo in the nutrition of the latter. Dissolution of the endosperm, however, is not autonomic but takes its origin in profound nuclear disturbances which arise in association with irregular behavior of the antipodals during fertilization and immediately thereafter. Analysis of the problem, therefore, leads back to the relation of the antipodals to development during the crucial period when the mature ovule is undergoing transformation into the juvenile seed. Some of the morphological investigations of an earlier period are pertinent in this connection and since no attempt hitherto has been made to relate them to the general problem of seed failure following species hybridization a brief review will be given.

WESTERMAIER (1890) first showed that the antipodals may play an important role in the nutrition of the embryo. HOFMEISTER (1849), as LLOYD (1902) has noted, had suggested in his classical study on the origin of the embryo in angiosperms that the function of the antipodals was nutritive. It remained for WESTERMAIER, however, to gather the evidence which made such a conclusion apparent. WESTERMAIER directed attention to (1) the position of the antipodals relative to the nutrient stream (2) the location of the cuticularized membranes in the ovule and (3) the distribution of starch in the ovule. Several later investigators have concurred in this general point of view as observations on antipodal behavior have been extended to additional species.

LLOYD (1902) found that development of the antipodals was highly variable, frequently even within families. A detailed study of the Rubiaceae showed that within the Galieae, for example, most of the species possess three antipodals, one of which is much elongated and seems to perform a haustorial function.

The antipodals in the genus Crucianella, on the other hand, are of very short duration and show no special morphological features. Their function, according to LLOYD, is at best but passive and is carried out by the mere giving up of their substance in death to the embryo sac. The antipodals in *Diodia virginiana* vary from four to ten cells which are arranged in a long series physiologically equivalent to the single elongated antipodal in the Galieae. LLOYD observes that in Richardsonia and Haustoria the antipodals take on the appearance of glandular cells and, for a brief period, are active in some kind of secretion.

IKEDA (1902) in a study of the antipodals in *Tricyrtis hirta* observed the extraordinary behavior of the nuclei. As the antipodal cells attain their maximum length at, or just following, fertilization, the nuclei, which are at first small, enlarge greatly and show a very considerable increase in content of chromatin. The latter substance becomes aggregated into dense, highly stainable, usually angular, masses. IKEDA interprets the nuclear behavior as indicative of intensive metabolic activity in the antipodals and suggests that the tissue is the center for the absorption, elaboration and transportation of nutrient materials for the embryo sac. He states that several investigators have observed chromatin aggregation as an accompaniment of secretory activity in glandular cells in both animals and plants. The nutritive function of the antipodals appears to continue to the beginning of endosperm formation at which time the large chromatin masses gradually disappear and the whole tissue regresses. It is probably significant that in Tricyrtis a special group of columnar cells is differentiated in the chalaza connecting the vascular bundle with the embryo sac during the time the antipodals are active and in rather direct physical association with them. These cells doubtless serve for conduction of nutrients and water.

LÖTSCHER (1905) recognizes three general classes of antipodals. Type I shows a low grade of differentiation of the antipodal cells and these probably play only a very minor developmental role. The antipodals form a more prominent tissue in Type II which is effective in the manufacture and transmission of materials required by the embryo sac. This type is represented, among other families, by the Gramineae. The antipodal cells in Type III form an elongated structure, serving the embryo sac as a haustorium. LÖTSCHER considers that the antipodals are a part of the total mechanism by which the embryo is nourished, their share in this task varying considerably between species and standing in reciprocal relation to other structures serving the same end.

The antipodal apparatus in the Gramineae has been studied by SHADOWSKY (1926). The number of cells observed varied from three to 43, species having multiple antipodals preponderating. SHADOWSKY believes that the antipodals function in transmitting nutrients to other parts of the embryo sac and thinks it significant that the tissue persists in this family until the endosperm starts growth.

SCHNARF (1929), in a critical survey, emphasizes the fact that the antipodals are the most variable constituent of the embryo sac in number, form of cell and nucleus and in persistence. Usually three only are present, but additional ones may be derived from these. The increase in number is often associated with increased size, persistence or hypertrophy of the nuclei. There may be considerable variability in the antipodal apparatus between species within a family. SCHNARF points out that small antipodals usually disappear early whereas large ones may reach their maximum development after fertilization and remain active until the beginning of endosperm formation. He attributes a significant nutritive role to the antipodals in species in which the tissue is well developed. The general grounds for this view are summarized in the following statement from SCHNARF which is quoted in translation:

"(1) The position of the antipodals at the base of the embryo sac where in general the conducting tissue of the ovule terminates points to the assumption that the incoming material must pass through the antipodal region.

"(2) The form and particularly the finer structure of the antipodal cells point to a function similar to that of secretory cells.

"(3) The female gametophyte may be looked upon as an independent individual, so to speak, which is nourished by the plant less directly than a bud or flower; an agency is necessary to take up nutrients from the sporophyte and transform them into other substances which the growing gametophyte may absorb. This agency is frequently the antipodals."

SCHNARF calls attention to the fact that the "hypertrophied" nuclei characterizing highly developed antipodals are not peculiar to this tissue but are found also in anther tapetum, in endosperm, particularly in endosperm haustoria, in the suspensor cells of many embryos and in various other plant parts. This form of nucleus may be indicative of a function common to all these tissues which may well be secretory.

The prominent development of the antipodals athwart the nutrient stream in *Hordeum jubatum*, the rapid enlargement of these cells at fertilization associated with a corresponding increase in size of the embryo sac and the extraordinary changes through which the nuclei and chromosomes pass all point to an active participation of the antipodals in the transformation of the ovule into a young seed. The resemblance of the nuclei of antipodals of this class to those of glandular cells in general, noted by earlier investigators, definitely suggests a metabolic role. Substances necessary for the initial growth of the endosperm are probably built up in the antipodals from materials conveyed thence by the vascular bundle, and then secreted into the endosperm mother cell. The endosperm evidently becomes the dynamic center of the entire structure since growth stops upon its breakdown in the hybrid seed. The antipodals function for a brief period only, but since this period falls at the crucial stage of endosperm initiation their influence may be decisive for the whole subsequent course of seed development.

The sequence and possible character of the events leading to collapse of the *H. jubatum*×*S. cereale* seed may now be considered.

The antipodals are aroused to activity by the advent of normal sperm to the *H. jubatum* embryo sac. The promptness with which the antipodals respond is remarkable. Evidently the fertilization process does not proceed very far before an effect on the antipodals is produced. Probably the action proceeds from the polar nuclei and their associated sperm. Just prior to fertilization the two polar nuclei are closely adjacent to the egg; but immediately thereafter the three associated nuclei move to a position between the egg and the antipodals, approaching the latter. It is unlikely that the pollen tube, while still within the stylar tissue, is the source of the stimulus because in all embryo sacs which have been found showing enlarged antipodals sperms were visible in conjunction with egg and polar nuclei or fertilization had been completed. However this may be, the fact is that, following entrance of the sperm into the sac, the antipodals normally expand to several times their gametophytic size and the primary endosperm becomes greatly enlarged *before* the fusion nucleus begins to divide. This means that an opportunity is provided for even the first nuclear division in the endosperm to be conditioned by substances proceeding from the activated antipodals.

Now Secale sperm, when substituted at fertilization for Hordeum sperm in the *H. jubatum* ovule, fail to arouse the antipodals to their normal secretory activity. Evidently the stimulating substance which the sperm releases is specific, in some measure, so that the *H. jubatum* antipodals do not react fully to the *S. cereale* product. Hybrid fertilization, however, only slightly delays division of the Hordeum×Secale primary endosperm nucleus. Early development of the hybrid endosperm occurs, therefore, in the presence of partially activated antipodals whose position in the embryo sac is such that they can regulate the nutrient supply. If now it is assumed that the weakly functioning antipodals fail to secrete in sufficient amount some material essential for the growth and division of the endosperm nuclei, an explanation is at hand for the abnormal mitosis which arises in the hybrid tissue at this time. The hybrid seed may live beyond the antipodal stage but recovery of the endosperm is impossible because irregular assortment of the chromosomes is an irreversible change.

The hyperplasia of the antipodals often occurring in the *H. jubatum*×*S. cereale* seeds at 48 to 72 hours after pollination seems best interpreted as a side reaction arising from the irregular distribution of nutrients. Post-fertilization multiplication of antipodal cells appears not to occur when common barley is crossed with rye. Otherwise the course of events leading to seed collapse after the *H. vulgare*×*S. cereale* mating is parallel to that which has been described in detail for *H. jubatum*×*S. cereale.*

SUMMARY

The abnormal chromosome distribution characteristic of the hybrid endosperm in *Hordeum jubatum*×*Secale cereale* seeds is shown to be associated with a radically altered behavior of the antipodals.

The antipodals are prominently developed in the female gametophyte in a position athwart the nutrient stream. Their cells and nuclei are normally stimulated to a marked hypertrophy at fertilization. The antipodals appear to function in the secretion of substances necessary for endosperm growth up

to about 28 hours, after which they rapidly decline. Measurements of the volume show that *Secale cereale* sperm, on entering the *H. jubatum* embryo sac, exert only a feeble stimulus on the antipodals. The cytoplasm remains dense and only a limited and irregular enlargement of the nuclei occurs. The triple fusion hybrid nucleus nevertheless enters upon division promptly and early endosperm development proceeds in association with the weakly functioning antipodals.

It is suggested that the hybrid endosperm nuclei under these conditions fail to receive through the antipodals all the materials necessary for growth and reproduction. The starvation is reflected in gross mitotic disturbances which eventually so impair the endosperm that its development is arrested and the entire seed breaks down.

The fact is established that the time relations between stimulation of the antipodals and division of the primary nucleus (which is frequently abnormal) are such that the latter event could be conditioned by subnormal secretory activity of the antipodal tissue.

Confirmation of this explanation of collapse in *H. jubatum*×*S. cereale* seeds is seen in a similar sequence of changes leading to early seed failure following fertilization of common barley (*H. vulgare*) by rye.

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