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Chapter 5

Inbreeding and Crossbreeding
in Seed Development*

It is now generally recognized that the effects on growth of inbreeding and crossbreeding are intimately interwoven in the whole complex fabric of development and reproduction. Not only are the effects widespread and often of major consequence in the economy of the organism, but sometimes they are manifested in devious ways. Such is the case in the seed of flowering plants.

The success or failure of seed development turns primarily, not on the embryo which embodies the line of descent, but upon an accessory organ of reproduction, the endosperm. The novel origin and sensitivity of this latter tissue to changes in genetic composition render early seed development one of the critical stages in the life cycle of flowering plants. My colleague, D. C. Cooper, and I have been exploring these relations during the past decade. An attempt will be made here to review some of the evidence upon which our point of view rests, and to call attention to some of the broader implications of the main facts.

As a means of bringing the important aspects of the problem in flowering plants into focus, seed development in the angiosperms and gymnosperms will be compared. Essential features of the general hypothesis by which we have been guided will then be set forth. The central role of the endosperm in formation of the angiosperm seed and the responsiveness of this tissue to variations in genetic composition will be illustrated by a consideration of the immediate effects of self- and cross-fertilization in alfalfa. It will then be shown that the means by which the embryo in the common dandelion, an autonomous apomict, is nourished is of a type which would be expected according to the hypothesis proposed.

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An illustration will next be given of endosperm failure as an isolating mechanism. Finally, the significance of the present results for the problem of artificially rearing embryos whose development in the seed is blocked by endosperm disfunction will be pointed out.

Complete literature citations are not given. These may be found in the summary paper (Brink and Cooper, 1947) in which much additional evidence bearing on the present thesis also is presented.

The endosperm is a special structure intercalated between the female parent and the embryo, serving to mediate the relations between the two. The tissue originates from the central cell of the female gametophyte, following a fertilization distinct from that giving rise to the embryo. The secondary fertilization is unusual in that two identical haploid nuclei of maternal origin are united with one contributed by the pollen. The endosperm thus becomes $3x$ in chromosome number in contrast with the $2x$ condition of the embryo and the mother plant, respectively. Endosperm and embryo carry the same kinds of genes, but the genic balance may be unlike in the two tissues by virtue of the double contribution to the endosperm from the maternal parent. A further element of genetic heterogeneity in the seed arises from the fact that nucellus and integuments, which are maternal structures, may differ in genotype from the endosperm and embryo which they enclose, since they belong to the previous generation.

These facts, of course, have long been known. Certain of their implications, however, are only now becoming apparent. Particularly is this true of the secondary fertilization on which our attention will be focussed.

A word should be said at this point concerning the manner in which the endosperm should be visualized. Many are familiar with the tissue only in the mature seeds of species in which the endosperm persists as a storage organ. This condition, well known in the cereals, for example, is exceptional among flowering plants, and represents a secondary adaptation of significance mainly for the future seedling. In most species the endosperm either does not persist in the fully developed seed or occurs therein as a residue only. On the other hand, the endosperm is regularly a prominent organ in the juvenile seed. It is especially active directly following fertilization, during what may be termed the lag phase of embryo growth. This period is seldom longer than a few days, and varies according to the species. In spite of its typically ephemeral character, the endosperm plays a critical role in (1) transforming the mature ovule into a young seed and (2) nourishing the embryo during its initial period of growth. We are here concerned with the endosperm in these two relationships only.

THE SEED IN GYMNOSPERMS AND ANGIOSPERMS

It is helpful in understanding the significance of the secondary fertilization to compare the circumstances of seed development in the angiosperms with

those in the other great class of seed forming plants, the gymnosperms. A secondary fertilization does not occur in the gymnosperms. The endosperm is a haploid tissue derived from the megaspore by continuous cell division. The tissue is a part of the gametophyte rather than an integral structure distinct from both gametophyte and sporophyte, as in the angiosperms.

On the other hand, the endosperms in the two classes of seed plants have an important common function, namely, nourishment of their respective associated embryos. The genetic equipment with which the two kinds of endosperms are furnished differs in a fundamental respect. That of the gymnosperm is a sample half of the mother plant's inheritance, whereas the angiosperm endosperm, being of biparental derivation, has two chances instead of only one of receiving a physiologically effective genic complement. Insofar as the two tissues are autonomous in their functional properties, the angiosperm endosperm, therefore, is equipped to meet much more exacting requirements than its counterpart in the gymnosperms. A summary review of the differences in the gymnosperm and angiosperm ovules and seeds at fertilization, and during the immediately subsequent period, shows the importance of (or necessity for) a secondary fertilization in the flowering plants in order to maintain continuity of the life cycle at this stage.

The differences between the mature ovules of gymnosperms and angiosperms which appear to have a direct bearing on the present problem may be summarized as follows:

1. The seed coat in the gymnosperms approaches its mature size at the fertilization stage. The angiosperm seed coat undergoes extensive growth subsequent to fertilization. These facts are of interest in relation to the total food requirements of the two respective classes of growing seeds and the post-fertilization distribution of nutrients between the seed coat and the enclosed tissues.

2. The female gametophyte in the gymnosperms is an extensively developed multicellular (multinucleate, in some higher forms) structure. Its counterpart in the angiosperms typically consists of only seven cells. The potential disadvantage of the extreme reduction of the female gametophyte in the flowering plants will be considered below.

3. Generally speaking, the gymnosperm ovule is rich in food reserves, whereas the angiosperm ovule is sparsely supplied. This means that in the latter, the large volume of nutrients required for growth of the endosperm, embryo, and seed coat must be moved in from other parts of the plant. In the gymnosperms an extensive supply is directly at hand.

4. So far as may be inferred from the published accounts, fertilization in the gymnosperms initiates a new cycle of growth in the embryo only. Other parts of the ovule do not appear to be stimulated. Double fertilization in the angiosperms, in contrast, not only marks the inception of endosperm and embryo formation, but also incites pronounced mitotic activity and en-

largement of the cells in the integuments. Thus, with the exception of the nucellus which is broken down and absorbed by the rapidly expanding endosperm, all the elements of the young seed which were previously quiescent, suddenly spring into active growth following syngamy.

Consideration of these differences between the seeds of gymnosperms and angiosperms led us some ten years ago to explore the hypothesis that the secondary fertilization in angiosperms is essentially a means of enhancing the competitive power of the endosperm relative to the maternal portions of the seed—by conferring upon the endosperm the advantages of hybridity. The nutritive requirements of the young seed suddenly are raised from a low to a high level since fertilization starts a new cycle of growth in the massive integuments. The nutrient supply, on the other hand, quickly falls to the plane which can be maintained by movement of foods into the seed from other parts of the plant as a result of exhaustion of the limited ovule reserves.

It seemed reasonable to assume that, within the seed, the incoming nutrients would tend to be partitioned between the different tissues according to the respective amounts of growth occurring in them. On this basis, the extensively developed integuments would consume the major portion. The diminutive endosperm and embryo would receive but a small fraction of the total. Under these conditions, failure of the young seed through starvation of the embryo could arise, unless the endosperm—as the nutritive agent of the embryo—were endowed with special properties which offset its initially small size. It seemed essential that the endosperm, by one means or another, be enabled to quickly acquire a position of physiological dominance in the juvenile seed in order to insure continued development.

Two genetic characteristics of the endosperm suggest themselves as being important in this connection. The first is the triploid condition of the nuclei. Little is known of the physiological effects of ploidy in general, and virtually nothing of its meaning in special situations of this kind. One suspects, however, that the endosperm gains some advantage from its extra chromosome garniture, as such, in mediating the relations between the diploid maternal parent and the young diploid embryo. It is also probably significant that, whereas the embryo inherits equally from the two parents, two-thirds of the endosperm's genic complement is derived from the plant upon which it is nutritionally dependent and one-third of the complement from the male parent.

Heterozygosis is the second characteristic of the endosperm which might enhance the inherent physiological efficiency of this tissue. The possibility of heterozygosity arises, of course, from the biparental origin of the endosperm mother nucleus. The condition is realized in matings between genetically different plants. Haploidy of the endosperm, as occurs in the gymnosperms, appears to be genetically insufficient for seed development in the flowering plants. Early post-fertilization circumstances, particularly the dependence

upon and competition for an outside nutrient supply in the latter, require that the tissue shall share in the advantages of sexuality. The advantage gained is not that of amphimixis in general, as in the embryo, but solely the extra vigor of growth associated with the union of unlike nuclei in the mother cell. Thus hybrid vigor in the endosperm has some claim to uniqueness. The sole object gained by entry of a sperm into the nuclear makeup of this sterile tissue is the added vigor of growth thus acquired. Some of the evidence by which the validity of this point of view may be tested will now be considered.

INBREEDING AND CROSSBREEDING EFFECT ON
SEED COLLAPSE IN MEDICAGO SATIVA

Two classes of matings on seven alfalfa plants were carried out under favorable growth conditions in a greenhouse. After removal of the anthers from the flowers used, a part of the flowers were pollinated with pollen from the same respective plants. This constitutes the self-fertilized series. Other flowers on the same plants were cross-pollinated, the pollen being derived in each case from an unrelated plant within the group. These matings comprise the cross-fertilized series.

Since alfalfa is regularly cross-fertilized, the second series of matings is designed to maintain the level of heterozygosity normal to the endosperm and embryo in this species. The enforced self-fertilization, on the other hand, would be expected to reduce heterozygosity in the endosperm mother nucleus and the zygote by 50 per cent. It is proposed to review the consequences for seed development of this sharp reduction in heterozygosis.

Following the above two series of matings, the pistils were collected at 30, 48, 72, 96, 120, and 144 hours and imbedded in paraffin. After sectioning and staining, data were taken on fertility of the ovules, frequency of fertile ovules collapsing, number of cells in the embryo, and number of nuclei in the endosperm. Detailed observations were made subsequently on growth of the integuments.

Alfalfa was known previously to be partially self-incompatible. It was not unexpected, therefore, to find that only 15 per cent of the ovules became fertile after selfing in contrast to 66 per cent after cross-pollination. The new fact which emerged was the much higher incidence of collapse of ovules subsequent to fertilization in the selfed than in the crossed group. The data are summarized in Table 5.1. Fertilization occurred within about 30 hours after pollination under the prevailing conditions. It was somewhat delayed after selfing. Little evidence of breakdown of the seeds was found at 48 hours. In the 72 hour and subsequent collections, however, the phenomenon was common. The results presented in the table cover the period from 72 hours to 144 hours, inclusive, and are based upon 433 seeds and 1682 seeds in the selfed and crossed series, respectively.

Growth of the young seed at this stage appears to be quite independent

of that of its neighbors in the same ovary. Furthermore, the quickly succeed-
ing secondary effects of fertilization, such as enlargement of the surrounding
fruit, are at a minimum. Studies on the reproductive physiology of the flower-
ing plants are rendered difficult by the multiplicity of changes which are
eventually set in motion in the tissues of the seed, the fruit, and the maternal
plant following fertilization. The sequence and interrelations of the events
immediately subsequent to syngamy are simpler to analyze than those which
occur later, in view of the fact that each very young seed may be considered
to behave independently of the others.

The data in Table 5.1 show that, for each of the seven plants tested, the

TABLE 5.1

FREQUENCY OF FERTILE OVULES COLLAPSING IN SEVEN ALFALFA
PLANTS FOLLOWING SELF- AND CROSS-FERTILIZATION. DATA BASED
ON COLLECTIONS AT 72, 96, 120, AND 144 HOURS AFTER POLLINATION
(AFTER COOPER AND BRINK, 1940)

	SELF-FERTILIZATION			CROSS-FERTILIZATION		
Plant Selfed	No. of Fertile Ovules		Percentage Collapsing	Plants Crossed	No. of Fertile Ovules		Percentage Collapsing
	Total	Collapsing			Total	Collapsing	
A........	37	9·	24.3	A×B.....	187	13	7.0
B........	37	19	51.4	B×C.....	110	5	4.5
C........	20	7	35.0	C×D.....	171	13	7.6
D........	17	7	41.2	D×E.....	171	16	9.4
E........	39	8	20.5	E×A.....	146	9	6.2
F........	109	39	35.8	F×G.....	228	14	6.1
G........	55	19	34.5	G×F.....	198	16	8.1
Total..	314	108	34.4	Total..	1211	86	7.1

frequency of seeds collapsing is much higher in the selfed than in the crossed
series. The proportions vary in different individuals from about 3 to 1 to
over 11 to 1. On the average, approximately five times as many seeds con-
taining inbred endosperms and embryos collapse within the first six days
after pollination as in the crossbred group. Since other factors were not
varied, the decrease in survival in the selfed series must be attributed to
the inbreeding.

The evidence, both general and particular, points to the endosperm
rather than the embryo as the seat of the inbreeding depression effect. The
endosperm in alfalfa is free nucleate up to about 144 hours after pollination,
although it develops as a cellular tissue thereafter. Successive waves of
mitotic divisions traverse the tissue, the number of nuclei being doubled in
each cycle. Thus growth during this period proceeds at an exponential rate.

The concurrent development of the embryo, on the other hand, is relatively slow. The zygote divides to form a two-celled proembryo. Successive divisions of the apical cell give rise first to a six-celled proembryo and then to the initials of the definitive embryo.

The pronounced difference in rate of development of the two tissues is illustrated by the fact that at 144 hours the modal number of cells in the embryo is only 16, whereas the typical number of nuclei in the endosperm at this time is 128. Rapid and precocious development of the endosperm as seen in alfalfa is characteristic of the angiosperms in general. The much higher level of activity of the endosperm is presumptive evidence that this tissue, rather than the embryo, is especially subject to developmental upsets in the young seed. Data available in the present instance provide direct confirmation of this interpretation.

The comparative rates of growth of endosperm and embryo in the selfed and crossed alfalfa series up to 144 hours after pollination are illustrated in Figure 5.1. Not only are the values for the embryo low, but also there is little difference between those for the inbred and crossbred series. The conclusion appears warranted that the direct effect of inbreeding on the embryo at this stage, if indeed there is a demonstrable effect, is too small to account for the high frequency of seed collapse. In contrast, there is a very sharp decline in rate of nuclear division in the endosperm, following enforced self-fertilization of this naturally cross-fertilized plant. The lower rate is shown from the first division onward. There are about twice as many nuclei present at 144 hours in the crossbred as in the inbred endosperms.

Due to the partial self-incompatibility in alfalfa, fertilization on the average, is slightly delayed following selfing. A comparison of the rate of growth of the two classes of endosperms independent of time as shown in Figure 5.2, however, establishes the reality of the difference in rate of growth between the inbred and crossbred endosperms. When the seeds are arrayed in terms of cell numbers of the enclosed embryo, it is found that for all nine classes occurring in the material the endosperms are more advanced in the crossbred than in the inbred series. That is to say, the embryos at a given stage of development have associated with them more vigorously growing endosperms following cross-fertilization than after selfing. Moreover, the decrease in size resulting from the inbreeding is so large that one is led immediately to suspect that herein lies the primary cause of the frequent seed collapse following selfing.

Why should impairment in rate of endosperm growth lead to arrested seed development? The answer in the present case is clear. As was pointed out earlier, double fertilization initiates not only endosperm and embryo development, but also a new cycle of growth in the integuments. The latter compete directly with the endosperm for the nutrients moving into the young seed. If the endosperm is developing subnormally, a disproportionate amount of

the incoming nutrients is diverted to the integuments. As a result this tissue frequently becomes hyperplastic. The overgrowth in the case of alfalfa characterizes the inner integument. As Dr. Cooper observed, it begins at a point opposite the distal end of the vascular bundle where the concentration of nutrients may be assumed to be the greatest. The inner integument, which is normally two cell layers in thickness, becomes multilayered and somewhat callus-like in the region of the greatest mitotic activity. This pronounced overgrowth of the inner integument quickly reacts upon the endosperm, further impairing its development. In the seeds which fail, a complete
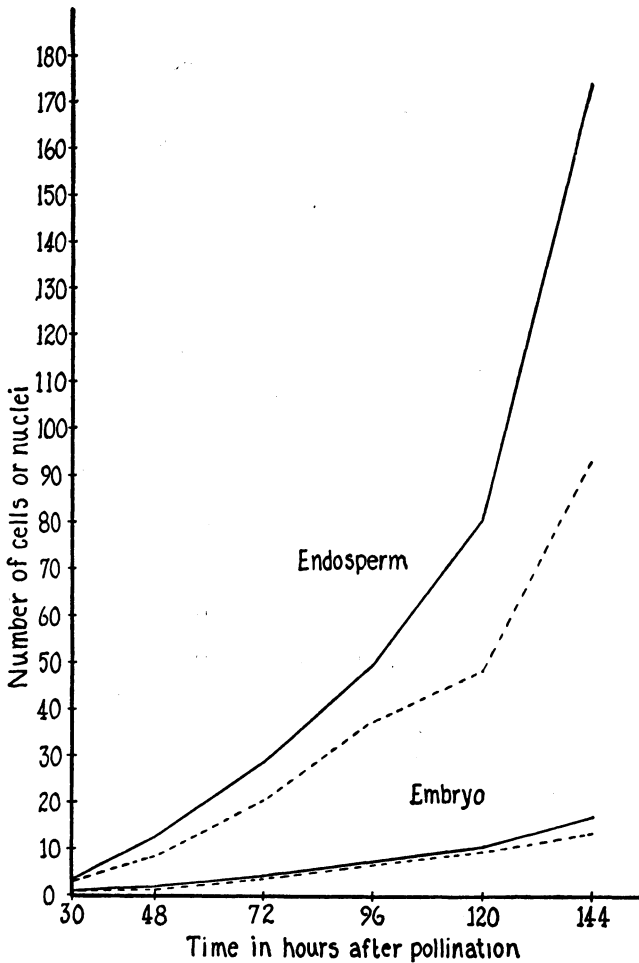

FIG. 5.1—Increase in number of cells in embryo and in number of nuclei in endosperm following self- (*broken line*) and cross-fertilization (*continuous line*). After Brink and Cooper, 1940.

collapse of the endosperm then ensues. Significantly, breakdown of the endosperm tissue begins in the region opposite the end of the vascular bundle where the inner integument is especially hyperactive. Following collapse of the endosperm, the young seed dies.

SEED DEVELOPMENT WITHOUT FERTILIZATION

There are a few species of flowering plants in which both endosperm and embryo develop without fertilization. These so-called autonomous apomicts
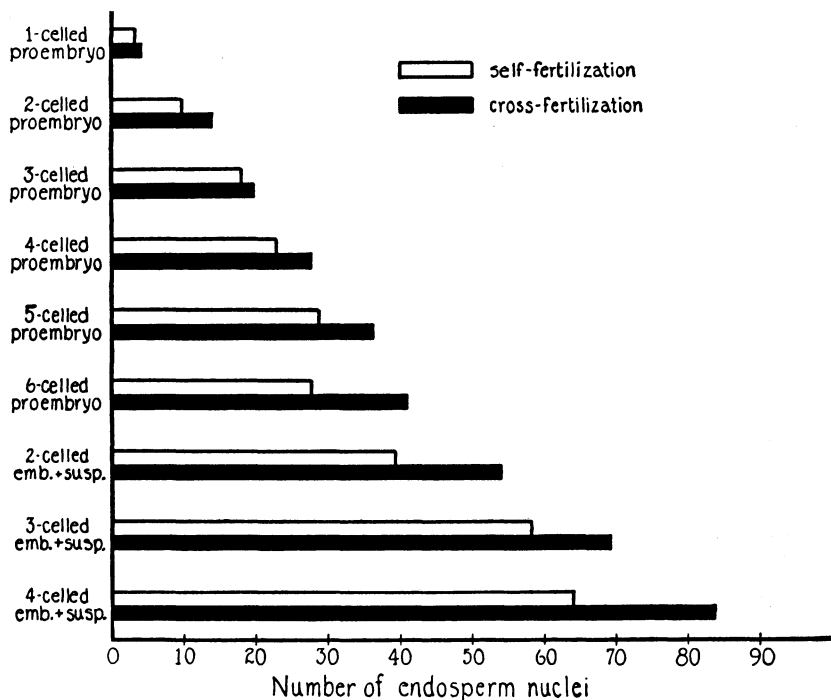

FIG. 5.2—Number of endosperm nuclei associated with proembryos and embryos at various stages of development following self- and cross-fertilization. After Brink and Cooper, 1940.

should provide an independent test of the hypothesis that aggressive development of the endosperm is requisite to seed development, and that the secondary fertilization is a device by which aggressiveness of the tissue is enhanced. On the basis of the reasoning applied to sexual species, one would expect to find in autonomous apomicts that the embryo is not basically dependent on an active endosperm for its nourishment. So far as I am aware, the evidence bearing directly on this question is limited to a single study which Cooper and I carried out on the common dandelion, *Taraxacum officinale* (Cooper and Brink, 1949).

The common dandelion is triploid ($3x = 24$). The regularity and abundance of seed production in the plant is well known. A full complement of seed

forms in the absence of pollination, as may be demonstrated easily by re-moving the corollas and anthers—by cutting off the distal portion of the head in the bud stage. Ordinarily the anthers do not open in the intact mature flower.

The female gametophyte is formed without reduction in chromosome number of the nuclei. Otherwise it is a typical eight-nucleate, seven-celled structure lying in direct contact in the mature ovule with the innermost layer of cells of the single thick integument. The polar nuclei fuse to give a hexaploid primary endosperm nucleus. The single layer of cells comprising the nucellus disintegrates during formation of the embryo sac.

Sexual forms of the common dandelion are not known to occur. Accord-ingly another species, *T. kok-saghyz*, the Russian dandelion, was examined as a control. *T. kok-saghyz* is diploid $(2x = 16)$ and, since it is self-incom-patible, requires cross-pollination for seed formation. A comparative study of *T. officinale* and *T. kok-saghyz* was made with a view to discovering, if possible, the means by which the former is enabled to dispense with the secondary fertilization, which is essential to seed formation in the latter. Heads were collected at four stages: late bud, just prior to anthesis, open flower, and with seeds ranging up to six days of age. After sectioning and staining, the number of cells in the endosperm and embryo was determined, and observations were made on the amount and distribution of food ma-terials.

Seed formation in *T. kok-saghyz* follows the course typical of the angio-sperms. Endosperm and embryo development are initiated by double fertilization. Subsequently, the two tissues grow very rapidly, and in tune with each other. Cell number in the endosperm increases exponentially. The endosperm, however, is somewhat less precocious than in most flowering plants. The seed is mature 9–12 days after fertilization.

A markedly different set of relations present themselves in the seed of the apomictic *T. officinale.* The seed in this species begins development when the flowers are in the late bud stage. By the time the flowers open, there may be 100 cells or more in the endosperm, the embryo, or in both tissues in some seeds. A further significant fact is the extraordinary amount of variability in the size ratios of endosperm and embryo from seed to seed of even age. There is a positive relation between cell number in endosperm and embryo over the period studied—as would be expected in view of the fact that in most seeds both tissues are growing. As measured by the correlation co-efficient, this value is low $(r = .57)$ compared with that for *T. kok-saghyz* $(r = .76)$.

Average cell number in the embryo in relation to endosperm size is de-picted for the two species in Figure 5.3. Cell number in the endosperm in-creases geometrically, so that size of the tissue may be expressed appropriate-ly in terms of division cycles. Embryo cell number, in contrast, increases

arithmetically. It will be noted from Figure 5.3 that the mean embryo cell number in *T. officinale*, before the endosperm mother cell divides (0 cycle), is about 16. The corresponding value *T. kok-saghyz* is 1. This is a reflection of the fact that the embryo in the apomictic species usually starts growth in advance of the endosperm. Although they start from different levels, the two curves are not greatly dissimilar. The embryo in the common dandelion, on the average, is consistently larger in the young seed than that of *T. kok-saghyz*, relative to given stages in endosperm development.
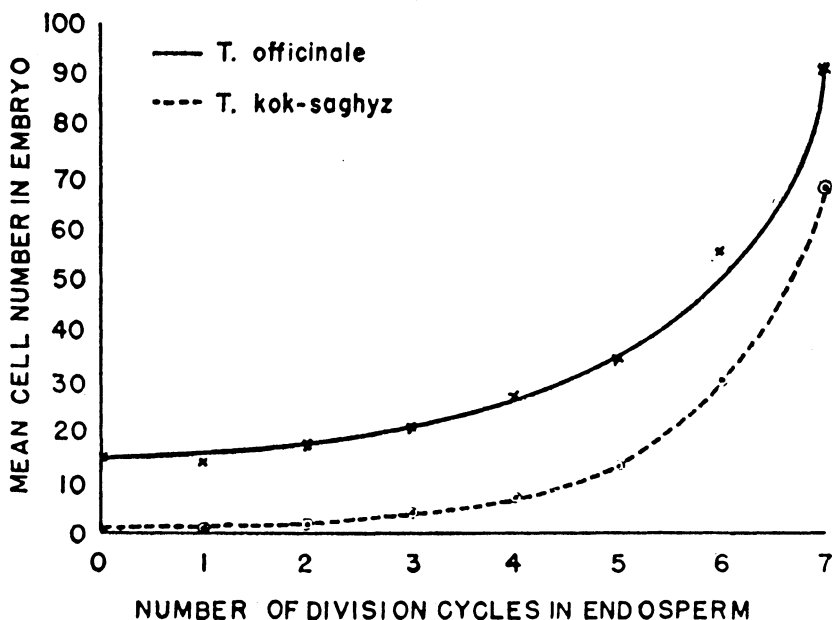

FIG. 5.3—Early growth of embryo of *T. kok-saghyz* and *T. officinale* in relation to endo-sperm size. After Cooper and Brink, 1949.

More instructive than the mean values on which Figure 5.3 are based, is the variability in the frequency distributions concerned. The data are sum-marized in Table 5.2. A logarithmic scale was used in expressing embryo sizes merely as a convenient way of summarizing the widely dispersed values. As mentioned above, growth of the embryo during this period is approxi-mately linear.

Table 2 reveals that the variability is low in embryo cell number at suc-cessive stages of endosperm development in *T. kok-saghyz*. This means that embryo and endosperm are closely synchronized in their growth in the sexual species. The variability in embryo size in the apomict, on the other hand, is enormous. For example, in seeds in which the endosperm is still at the mother cell stage (0 cycle), the associated embryos are distributed over all size classes from 1 to 128. The standard deviation for embryo cell number is

15.6, a value equal to the mean. The range is even greater in the class of seeds having 128-cell endosperms, and the standard deviation rises to 51 cells.

The extreme variability in embryo size for given stages of endosperm development in *T. officinale* is a fact of cardinal importance in the present analysis. Inspection of Table 5.2 reveals certain details which emphasize the significance of the summary data on dispersion. Note, for instance, that

TABLE 5.2

DISTRIBUTION OF EMBRYOS BY CELL NUMBER RELA-
TIVE TO ENDOSPERM DIVISION CYCLE
(AFTER COOPER AND BRINK, 1949)

ENDO-SPERM DIVISION CYCLE	SPECIES	TOTAL SEEDS EX-AMINED	EMBRYO CELL NUMBER—LOGARITHMIC CLASS VALUES									STAND-ARD DEVIA-TION
			1	2	4	8	16	32	64	128	256	
0.......	T. officinale	227	9	16	33	57	66	38	7	1	....	15.6
	T. kok-saghyz	All	All	....	....	....	....	....	....	....	....	0
1.......	T. officinale	253	23	11	37	70	55	50	6	1	....	13.6
	T. kok-saghyz	77	31	46	....	....	....	....	....	....	....	0.5
2.......	T. officinale	145	18	11	7	23	43	33	9	1	....	17.0
	T. kok-saghyz	32	1	31	....	....	....	....	....	....	....	0.2
3.......	T. officinale	108	12	6	6	19	27	25	12	1	....	21.1
	T. kok-saghyz	25	....	....	22	3	....	....	....	....	....	0.7
4.......	T. officinale	111	4	1	2	9	39	40	14	2	....	19.2
	T. kok-saghyz	34	....	....	5	27	2	....	....	....	....	1.9
5.......	T. officinale	115	6	....	4	4	23	50	23	5	....	24.2
	T. kok-saghyz	68	....	....	....	24	40	4	....	....	....	4.1
6.......	T. officinale	99	1	1	....	....	7	31	46	13	....	29.9
	T. kok-saghyz	55	....	....	....	....	10	41	4	....	....	9.0
7.......	T. officinale	60	2	....	1	....	1	8	17	28	3	51.0
	T. kok-saghyz	19	....	....	....	....	....	3	16	....	....	16.7

among the seeds still in the endosperm mother cell stage (0 cycle) one contains an embryo in the 128-cell class and seven have embryos in the 64-cell class. Similar, although less extreme, cases occur in the 1-cycle and 2-cycle endosperm distributions. Study of the histological preparations shows that the seeds in which the embryos are found are growing vigorously and appear capable of completing development. This can mean only that either very small endosperms in *T. officinale* are extraordinarily efficient structures, or embryo growth in this species is not dependent on an endosperm.

At the opposite corner of the table, on the diagonal, two seeds are entered in the 7-cycle endosperm array in which the embryos are still in the one-cell stage. These seeds also appeared to be healthy and capable of continued

development. These extreme examples point unmistakably to the conclusion that in the apomictic dandelion the endosperm, as the master tissue in the young seed, has been disestablished. Embryo growth must be sustained by other means.

The substitute arrangement for nourishing the embryo in *T. officinale* was disclosed by a histological study of the ovules of this species and *T. kok-saghyz*. Basically the structure of the ovule is the same in both. As the female gametophyte expands, the nucellus disintegrates so that the gametophyte comes to lie in direct contact with the endothelium which comprises the innermost layer of cells of the massive integument. The endothelium persists and appears to function in the transfer of nutrients during the course of seed development. In *T. kok-saghyz* the inner layers of integumentary cells adjacent to the endothelium lose their contents during formation of the gametophyte, and contain shrunken and misshapen nuclei when the ovule is mature. The cells of the integument immediately surrounding this depleted region are densely cytoplasmic and possess well-defined nuclei. The outermost parenchymatous cells of the integument are highly vacuolate. The single vascular bundle makes an arc about the greatest circumference of the ovule in both species. Only limited amounts of stainable reserve food materials occur anywhere in the *T. kok-saghyz* ovule.

The *T. officinale* ovule differs conspicuously from that of *T. kok-saghyz* in possessing an abundance of reserve food. The cells of the integument just outside the endothelium enlarge as the ovule matures and become gorged with a homogeneous material which appears to be proteinaceous in composition. This substance also extends between the cells at the outer edge of the storage region proper.

This extensive prestorage of protein-rich food material in the integument provides an explanation of the fact that embryo development in the apomict may proceed normally in spite of very limited endosperm growth. The conditions render superfluous an aggressively functioning endosperm. The embryo draws directly on a food supply already at hand. From the physiological point of view, the nutritive mechanism in the apomict is analogous to that in the gymnosperms. In both these classes of plants certain of the processes essential to seed development, which follow double fertilization in sexual species of flowering plants, are pushed back into the ovule. The secondary fertilization, which through its effect on vigor of endosperm growth may be looked upon as a means of offsetting the tardy provision of nourishment for the embryo, thus can be dispensed with.

SEED DEVELOPMENT GRADE AND EMBRYO
GROWTH POTENTIALITIES

The conclusion that growth of the angiosperm seed is basically controlled by the endosperm has an interesting corollary. That is, that the grade of seed development attained after a given mating is not a definitive index of the

intrinsic vigor of the embryo. This statement is not intended to imply that the two phenomena are unrelated, but rather that they vary independently of each other to a significant degree. Many interspecific matings, for example, yield poorly developed seeds. Often the embryos in these seeds give rise to relatively weak plants. Sometimes, however, the embryos within such seeds are capable of forming plants of great vegetative vigor. In other words, the fact that development of the seed is impaired, even to a degree that calls for special methods of propagation, does not necessarily mean that the embryo is intrinsically weak. The hybrid during the seed stage may merely be the victim of a faulty endosperm. Only when released from this stricture can the inherent potentialities of the new individual be expressed.

Two examples of such intrinsically vigorous hybrids in which the conditions of seed development have been explored will be briefly mentioned. They differ in the grade of seed development attained. Small but nevertheless germinable seeds are formed in the one case, whereas in the other the embryo egularly dies unless special precautions are taken to save it.

Cooper and I found that when the diploid ($2n = 24$) Red Currant tomato, *Lycopersicon pimpinellifolium*, is pollinated with a particular strain of *L. peruvianium*, likewise a diploid, fertilization occurs with high frequency but all the seeds collapse before the fruit is ripe. Seed development follows a familiar pattern. The endosperm grows less vigorously than in normal *L. pimpinellifolium* seeds, and the endothelium enclosing it tends to become hyperplastic. Endosperm cells become highly vacuolate and starved in appearance. Densely staining granules of unknown composition accumulate in the chalazal region just outside the endosperm, suggesting that the latter tissue is incapable of absorbing the available supply of nutrients. All the seeds in the ripe fruit are shrivelled and incapable of germination.

Following the application of pollen from the same diploid strain of *L. peruvianium* to a tetraploid ($2n = 48$) race of *L. pimpinellifolium*, about one-half the fertile ovules develop into small but germinable seeds containing triploid embryos. The other seeds collapse at various stages of growth. Histological examination of the $4n$ *L. pimpinellifolium* $\times$ $2n$ *L. peruvianium* seeds shows retarded embryo development and a less rapid endosperm growth than occurs in the normally pollinated tetraploid parent. The endosperm in sixteen-day-old hybrid seeds lacks the rather densely packed starch reserves characteristic of tomato seeds at this stage. The peripheral layers of endosperm cells adjacent to the endothelium break down. An unusually large cavity is formed in the interior of the tissue as a result of digestion of the cells by the slowly differentiating embryo. Endosperm function is markedly impaired in this cross, but in many seeds remains somewhat above the threshold at which complete failure occurs.

The triploid plants resulting from germinable $4n$ *L. pimpinellifolium* $\times$ $2n$ *L. peruvianium* seeds are extraordinarily vigorous. Although partially

sterile, they considerably exceed both the parents in capacity for vegetative growth. The inference is clear that the genic combination resulting from this cross yields markedly different results in the endosperm and the sister sporophyte. The difference in part may be a consequence of the 2:1 balance of *L. pimpinellifolium* and *L. peruvianium* genes in the embryo as compared with the 4:1 ratio in the endosperm. The important point, however, is that the mechanism of seed formation in the flowering plants is such that the two products of a given double fertilization may be quite differently endowed in terms of the genes necessary to perform their respective functions.

The second example to be discussed in this connection will enable us to visualize the limits which may be reached in endosperm disfunction with retention of embryo viability.

Fertilization freely occurs when squirrel-tail barley, *Hordeum jubatum* is pollinated by cultivated rye, *Secale cereale*. The resulting seeds all die, however, within less than two weeks. Space does not permit me to recount here the steps leading to the breakdown. They have been described in detail elsewhere (Cooper & Brink, 1944; Brink & Cooper, 1944). The endosperm early becomes completely disorganized. Some of the embryos formed, however, reach a stage previous to collapse at which time they may be dissected from the seed and successfully reared on an artificial nutrient medium. A single plant was grown to maturity from an embryo treated in this way. The plant was thrifty, although sterile. Representatives of the parent species grown under comparable conditions were not available, so that a valid comparison of relative vigor could not be made. The hybrid, however, appeared to be intermediate in stature and number of tillers.

The extreme character of the endosperm disturbances in the *H. jubatum* × *S. cereale* seed indicates that this hybrid could not arise under field conditions. Although the embryo is demonstrably capable of continued development its growth is terminated in the seed due to failure of the associated endosperm. Death of the embryo, as an indirect result of endosperm disfunction following wide crosses, appears to be commoner than was thought before the physiological implications of the secondary fertilization in flowering plants were recognized. Realization of this fact has stimulated additional interest in circumventing the phenomenon by excising such embryos from the seed and rearing them artificially.

Artificial methods of cultivating embryos removed from abortive seeds often have been used to extend the area within which gene transfers may be effected. Numerous interspecific hybrids have thus been grown which otherwise are not realizable. The nature of the general problem involved may now be seen in somewhat broader perspective. Two points of particular interest may be noted.

The first, briefly adverted to above, is that the frequency with which embryos are formed following matings between distantly related plants is much

higher than earlier believed. Various investigators have expressed the opinion that the mere presence of growing pollen tubes in the style causes enlargement of the ovules. This view now appears to be incorrect.

On the other hand, there is a steadily increasing amount of evidence to show that the incipient growth of the ovules, following many interspecific matings which do not yield functional seeds, is a response to fertilization. That is to say, the block in the reproductive cycle which was assumed to intervene prior to fertilization actually occurs following syngamy. Embryos are formed in these cases, but they perish when the young seed fails to develop. Some rather extreme examples of this phenomenon which have been observed in our laboratory include *Nicotiana glutinosa* $\times$ *Petunia violacea*, *N. glutinosa* $\times$ *Lycopersicon esculentum*, and *Medicago sativa* $\times$ *M. scutellata*.

It is not to be inferred that all hybrid embryos of this general class are capable of growing into mature plants. The fact that the seeds containing them collapse is not proof, however, of intrinsic inviability. An unknown but probably significant proportion of these novel zygotic combinations are potentially propagable. The problem is to discover the means by which they may be reared. This brings us to the second point—the nature of the problem to be faced in growing very small excised embryos.

With few exceptions, the embryos which have been successfully cultivated artificially have been removed from the seed at rather advanced stages of development. Unless they are multicellular and differentiation has at least begun, the embryos usually do not grow on the media which thus far have been devised. There are reasons for thinking that the nutritional requirements of these older embryos are simpler than those in a juvenile condition. Histological evidence shows that at the early stages of seed development the embryo is enclosed, or nearly enclosed, in the highly active, young endosperm. The endosperm cells adjacent to the proembryo and the very young embryo remain intact. A little later, as the embryo enlarges, these cells begin to break down and their contents disappear. Eventually all the endosperm tissue is consumed in most species.

One may infer from these facts that the embryo is dependent upon the endosperm for certain metabolites which initially the embryo is quite incapable of synthesizing. The endosperm may be pictured as secreting the needed materials at the early post-fertilization stage, and yielding them later in a more passive fashion as the tissue becomes lysed. Meanwhile the embryo becomes progressively less dependent upon the endosperm by acquiring for itself the synthetic capabilities previously limited to the nurse tissue. On this view the very young embryo is an obligate parasite on the endosperm. Once past the state of obligate parasitism, growth of the embryo may be effectively supported by comparatively simple nutrients such as may be provided in artificial culture media.

Visualized in those terms, the problem of cultivating very young, excised

embryos resolves itself into the discovery of means of duplicating the un-known but presumably special nutritive functions of the normal endosperm. Two possibilities suggest themselves in this connection. One is to determine natural sources of the special metabolites produced by the endosperm and then add these materials to the nutrient medium. Van Overbeek (1942) ob-tained significant improvement in the growth of small *Datura stramonium* embryos by supplying them with unautoclaved coconut milk. Blakeslee and Satina (1944) later reported that the coconut milk could be replaced by un-autoclaved malt extract. The other possibility is to cultivate the embryos artificially in association with actively functioning endosperm tissue. Cur-rent findings offer some encouragement that the latter procedure may prove efficacious.

Dr. Nancy Ziebur, working in our laboratory, recently has shown that the growth of very young embryos of common barley (0.3–1.1 mm. long) may be greatly improved by surrounding them on a nutrient agar medium with aseptically excised endosperms. The basic medium employed permits a satisfactory growth of older barley embryos but does not yield transplantable seedlings from embryos shorter than about 0.6 mm. except in conjunction with endosperms. Coconut milk and malt extract are ineffective with barley embryos. Water extracts of fresh barley endosperms gave positive, although smaller effects than the intact tissue. Further exploration of the living endo-sperm as a source of nutrients for very young, excised embryos should prove rewarding. The interrelationships of these two tissues in the juvenile seed give strong credence to this approach. The success which has so often at-tended efforts to grow older embryos artificially on rather simple media may have blinded us to the fact that the young embryo, divorced from the endo-sperm, may have quite different requirements.