THE INHERITANCE OF A PAIR OF SPOROPHYTIC CHARACTERS IN SPHAEROCARPOS

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Received August 4, 1924

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INTRODUCTION

It is well known that certain species of Sphaerocarpos are characterized by the persistent union of the four spores formed by the division of a spore mother cell, each tetrad being surrounded by a resistant wall. According to Miss HAYNES (1910), however, the spores of *Sphaerocarpos Donnellii* Aust. separate at maturity; HAYNES and HOWE (1923) say that the "spores in *S. Donnellii* become free at full maturity"; and AUSTIN (1877), in the original description of the species, seems to refer to the same characteristic: "Coccus deeply lobed, . . . sometimes quite fragile."

In my cultures of *S. Donnellii*, on the contrary, with exceptions noted later, the spores regularly remain united in tetrads. The wall surrounding each tetrad is comparatively thick, black at maturity, with a characteristic sculpturing distinct from that of the walls of the enclosed spores. The tetrad wall persists both at and after maturity; in the latter case, it is immaterial whether the spore-containing capsule is dried or is left in the culture until the capsule wall, the enclosing involucre, and the contiguous portions of the maternal gametophyte decay and the spore tetrads become scattered upon the soil. The spores are similarly united from the time of their formation until that of maturity; but until a comparatively late period the tetrad wall is colorless and transparent, its sculpturing apparently not having yet developed, and the outlines of the included spores and the structure of their walls are then clearly visible.

Whether this persistent union of the spores, differing from the specific character as described from out-of-door material, is consequent upon greenhouse conditions or is characteristic of particular, perhaps geographically localized, races, is still uncertain. Each of these possible explanations finds some support in the observations described below. My cultures originated in material from Sanford and Miami, Florida; the type locality for the species is Jacksonville, Florida.

The work here discussed has been aided by grants from the research fund of the UNIVERSITY OF WISCONSIN; and I have had the assistance, at various periods, of Messrs. H. W. RICKETT, A. M. SHOWALTER and J. A. LOUNSBURY. A portion of the results have already been briefly reported (ALLEN 1923).

THE SEPARATE-SPORE CHARACTER

In 1919, it was observed that the spores borne by the sporophytes resulting from a mating in which clone R27E (which began with an isolated tufted female plant from Sanford, Florida) was the female parent, were not united in the manner that had thus far appeared characteristic of the species. This fact was noted in an earlier paper (ALLEN 1919, p. 294, footnote), and the results, with respect to other characters, of this and other matings of clone R27E have since been reported in detail (ALLEN 1924, 1925). In all, five matings have been made of this clone, all with like results. The males concerned represented three distinct clones. Two of these three male clones (R19δ1aB and 19.27) have also been mated with numerous other female clones, such matings invariably resulting in sporophytes whose spores were united in tetrads. It is thus evident that the separateness of the spores borne by the sporophytic offspring of clone R27E is not a result solely of exceptional external influences; it represents, on the contrary, a tendency inherent in, or inherited through, this maternal clone. The union or non-union of the spores will for the present be treated as a sporophytic character, which it apparently is; although it is true that this character may conceivably be rather an expression of the genotypic constitution of the maternal gametophyte, influencing in some way the development of spores within the attached and dependent sporophyte.

For simplicity of statement, spores firmly enclosed within a tetrad wall will be spoken of as "united"; those not so enclosed, as "separate." In the latter case, when they are released by a breaking of the capsule wall, some, though never a considerable proportion, of the spores often retain the tetrahedral arrangement; but a touch separates them. Sometimes, when such adherent spores are separated, there appear what may be fragmentary

remnants of the original mother-cell wall; but nothing showing the thickness, color, or structure of the wall that surrounds each tetrad of "united" spores. If a capsule of a separate-spore strain is immature and the spores are still green or greenish, they sometimes manifest a greater tendency to adhere in tetrads than at a later stage; but in such a case, also, they are easily separable. On the other hand, in those instances in which the spores are described as "united," the tetrad wall can be ruptured only by a considerable pressure, which is about equally likely to break the walls of the individual spores. Thus, save for the very few exceptional cases mentioned below, the sporophytes resulting from all the matings that have been carried out fall into two sharply distinct categories: they have produced either "united" or "separate" spores. The difference seems to result from the development in the one case, and the non-development in the other, after the division of the protoplast of the spore mother cell, of a tetrad wall in addition to, or replacing, the mother-cell wall.

In no instance has any marked difference with respect to union or separateness of spores been observed between the various sporophytes resulting from a single mating. As a general rule, several to many sporophytes from each mating have been examined; a few matings produced only one or two sporophytes each.

Sporophytes have been obtained and examined that resulted from 113 matings in which neither parent was clone R27E or a descendant of that clone. Typical (♀ and ♂), tufted (♀ and ♂), and polycladous (♂) plants were involved. Of the 113 matings, 3 had occurred in mixed cultures before they were received; 12 were brought about within mixed cultures or between portions of two such cultures after their receipt from Sanford or Miami; 5 were between female clones (each developed from an isolated plant, or from a spore from an earlier mating) and male plants from the original cultures; and the remaining 93 were between clones of various origin. The sporophytes borne by 110 of the 113 matings in question bore uniformly united spores.

Of the three matings whose results were more or less divergent, two were of a single female clone (21.215) with different males. The sporophytes resulting from these two matings bore spores usually separate; but the occasional tetrads could sometimes be seen to be surrounded by a thin wall, evidently fragile but with the markings characteristic of a tetrad wall and distinct from those of the walls of the enclosed spores. In respect of the enclosing wall, then, these tetrads of adherent spores seemed to differ from those sometimes obtained from sporophytes descended from clone R27E. Matings of clone 21.215 with two other male clones resulted

uniformly in sporophytes bearing united spores. The third mating giving divergent results involved female clone 20.339; most of the spores were united, but a few were separate. In a mating of the same female with another male clone, only united spores were observed to be produced. Apparently, this and the two previous cases are to be explained by an incomplete development of the tetrad wall, perhaps in consequence of external conditions. They may suggest, too, that conditions in the natural habitat of the species are such as to check or inhibit the development of the tetrad wall, thus accounting for the observations already cited from the taxonomic literature. It is clear, at any rate, that the inherent tendency to the production of uniformly separate spores distinguishes clone R27E from all others thus far studied in culture.

THE F_2 GENERATION

If clone R27E and the males with which it was mated are considered the starting-point (the p_1 generation, following the symbolism used in previous papers), the sporophytes resulting from those matings belonged to the F_1 generation. These sporophytes, as has been seen, bore separate spores. From the germination of their spores arose the gametophytes of the f_1 generation. The mating of f_1 gametophytes, in turn, gave rise to F_2 sporophytes.

One hundred sixty-six matings that produced sporophytes have been made, in which one or both partners were f_1 progeny of R27E, and therefore of F_1 sporophytes bearing separate spores. Except in a few cases in which the material was too scanty, each f_1 female clone descended from R27E was mated with males of the same and of different origin; and each f_1 male clone so descended, with a few exceptions, was mated with females of the same and of different origin. Among the females used in these matings were typical and tufted clones; and among the males were typical, tufted and polycladous clones.

Of these 166 matings, 88 were between females and males, both progeny of R27E; these involved 47 female clones, of which 44 were progeny of mating 8 previously reported (ALLEN 1924), 1 of mating 10 (ALLEN 1924), and 2 of mating 15 (ALLEN 1925); and 27 male clones, of which 18 were progeny of mating 8, 2 of mating 10, and 7 of mating 15. In every case, the resultant F_2 sporophytes bore separate spores.

Sixty matings were between f_1 female progeny of R27E and males of other origins; 45 female clones were concerned, of which 42 were progeny of mating 8, one had resulted from mating 10, and 2 were progeny of mating 15. All the F_2 sporophytes produced by these matings likewise

bore separate spores. Thus all the f_1 female descendants of clone R27E, whether typical or tufted and however mated, gave rise, like the ancestral female clone, to sporophytes with separate spores.

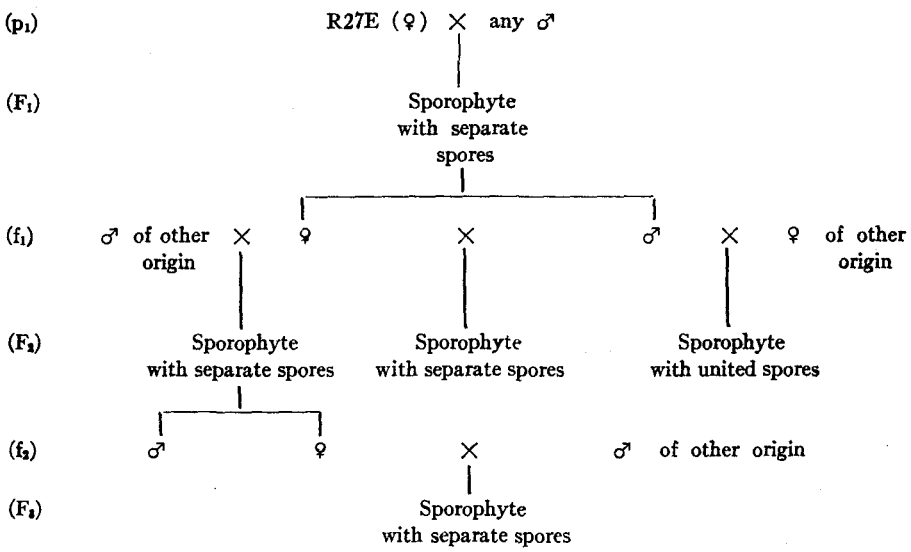
Eighteen matings were between f_1 male progeny of clone R27E and females of other origins. Seventeen male clones were involved; 15 were progeny of mating 8, 2 of mating 15. The F_2 sporophytes resulting from 16 of these matings uniformly bore united spores. In one of the two exceptional cases, three separate spores were seen, all the others observed being united; this instance may perhaps be classed with the exceptional cases noted in an earlier category, as due to a partial or entire failure of the tetrad wall to develop even when the possibility of such development is inherent. The other instance of divergent behavior can not be thus explained. The F_2 sporophytes resulting from this mating (22.86×20.88) bore uniformly separate spores, like the F_1 sporophyte from which the male clone here concerned was descended, although in the ancestry of the female clone only united spores had appeared.

THE F_3 GENERATION

Seven matings have been made between 5 f_2 female descendants of clone R27E (progeny of mating 11) and males of other origins. The maternal f_1 ancestor (20.124) of these f_2 females had produced sporophytes with separate spores in matings with two males of similar, and three males of different, origin (all included among the matings above reported). Each of the 7 matings of the f_2 females resulted in F_3 sporophytes with separate spores. Thus the separate-spore character had been transmitted from the p_1 female (R27E) through an F_1 sporophyte, an f_1 *female* gametophyte, an F_2 sporophyte, and an f_2 *female* gametophyte, to the F_3 sporophytes.

DISCUSSION

Apart from one distinctly exceptional case, the inheritance of the alternative separate-spore and united-spore characters among the descendants of clone R27E, so far as it has been observed, has followed the course shown in the following scheme:



The tendency of a sporophyte of whatever generation to produce separate or united spores is inherited through the maternal, and not (with one apparent exception) through the paternal, gametophyte. The transmission of either of these alternative tendencies, therefore, parallels the transmission of the X chromosome from female gametophyte to sporophyte, and thence through the latter's gametophytic offspring to the sporophyte of the succeeding generation. Whether a character expressed in the asexual generation of a bryophyte should be termed "sex-linked" is questionable; but the pair of alternative characters here considered evidently bear essentially the same relation to the X chromosome that the sex-linked characters of an animal bear to its X (or Z) chromosomes. Apparently, in view of recent descriptions of sex chromosomes in Lychnis, the comparison is equally close with the sex-linked character in that plant described by BAUR (1912) and more fully studied by SHULL (1914).

If the union or separation of the spores is to be considered as imposed upon the parasitic sporophyte by the genotypic constitution of the maternal gametophyte,—and thus, in effect, a gametophytic rather than a sporophytic character,—its relation in inheritance to the X chromosome is equally obvious. From this point of view, being a character of the sexual generation, it would be "sex-linked" in the strictest sense.

The close linkage of this pair of characters with sex is contrasted with the entire absence of linkage between either of the pair on the one hand and tuftedness or polyclady on the other. This result agrees with previ-

ously reported observations, which indicate that tuftedness and polyclady are inherited independently of sex, as well as independently of each other.

The occurrence of separate spores in the sporophytes resulting from one mating (22.86×20.88), whereas all other results lead to the expectation of united spores in these sporophytes, might be explained by a mutation in the female clone (22.86). However, discussion of this problem may well await further results which will eliminate all possibility of an error in the records or of one due to contamination of the cultures. The general rule obtaining in the inheritance of the characters here discussed is clearly demonstrated.

SUMMARY

1. The sporophytes resulting from the mating of one female clone (R27E) of *Sphaerocarpos Donnellii* with any male bear separate spores; the spores of sporophytes produced by other matings are regularly (under greenhouse conditions) united in tetrads, each tetrad being surrounded by a resistant wall.

2. The formation of a tetrad wall is rarely (in culture) partially or completely inhibited, probably in consequence of external conditions, in strains in which the possibility of producing such a wall is inherent.

3. The separate-spore tendency is regularly transmitted, among the descendants of clone R27E, through the female gametophytes. With one possible exception, the genotypic constitution of a male gametophyte has no influence upon the union or non-union of the spores of its sporophytic progeny.

4. The transmission of the tendency to produce separate or united spores follows the same course as does the transmission of the X chromosome. This pair of characters is thus comparable with the sex-linked characters of animals and of Lychnis.

5. The characters of the pair in question are independent in inheritance of the tufted *versus* non-tufted and of the polycladous *versus* non-polycladous pairs.

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