Genetic and Cytological Studies In The Genus Castanea

RICHARD A. JAYNES

1961

Cordially presented to
Dr. Jones and
The Connecticut Agricultural
Experiment Station
Dick Jaynes

GENETIC AND CYTOLOGICAL STUDIES IN THE GENUS *CASTANEA*

A Dissertation
Presented to the Faculty of the Graduate School
of Yale University in Candidacy for the
Degree of Doctor of Philosophy

By
Richard A. Jaynes

May 1, 1961

SUMMARY

are reported for the first time for six

Chromosome counts ~~of six new~~ species and several hybrids in the genus *Castanea* are reported. All had n = 12 or 2n = 24 except for two hybrids, one of which was a triploid with 3n = 36 ± 1. The results confirm the reports of previous workers who had concluded that the somatic number for the genus is 24. No evidence was found to support those reports where the somatic number had been given as 22.

The inheritance of catkin length in two different but related interspecific crosses is presented. In the cross of *C. crenata* × *C. dentata* over one-half the progeny had catkins longer than either parent, whereas in a cross of *C. mollissima* with a long-catkined *C. crenata* × *C. dentata* hybrid a majority of the progeny had catkins shorter than either parent. The genetic basis of this inheritance pattern is discussed.

It is suggested that everbearing or continuous flowering, a character of *C. seguinii*, is recessive in crosses with certain *C. mollissima* trees and is controlled by two unlinked genes.

Chestnuts are predominantly wind pollinated.

The time of maximum stigma receptivity is best judged with respect to the time the stigmas become fully expanded.

Experimental determination of the degree of self-compatibility has been confused by the possible occurrence of apomixis and outcrossing. Self-compatibility is not common, but does occur infrequently.

Male-sterility is relatively common in chestnut species and hybrids. In most of the male-sterile trees studied pollen abortion occurred before microsporogenesis. There is evidence to support the hypothesis of cytoplasmically transmitted male-sterility among a few of these trees.

Techniques are described by which high percentages of pollen germination can be obtained *in vitro*, and by which pollen can be stored in a functional condition for a month and possibly a year.

A new type of xenia is reported in which the male parent influences the period of dormancy of the nut in cold storage.

A review and discussion of all the first generation interspecific crosses within the genus *Castanea* is presented. Several interspecific crosses are reported for the first time. A majority of the possible F_1 interspecific combinations have been attempted, and crosses have been completed successfully between species of the three subgenera in the genus. Partial incompatibilities, as evidenced by poor fruit set, male-sterility, cracked-bark, and other abnormalities, do exist between certain species. The barriers are incomplete and, with the exception of fruit set, do not conform readily to any pattern. Crosses between species of different subgenera appear to produce fewer nuts than crosses within subgenera.

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ACKNOWLEDGEMENTS

My graduate work has been supported for four years by a fellowship sponsored by The Connecticut Agricultural Experiment Station. I wish to express my sincere appreciation to the Connecticut Station and to the following individuals who have helped to make this dissertation possible: to Donald F. Jones who initially made it possible for me to undertake the study and under whose guidance the program has continued; to Arthur H. Graves whose interest and enthusiasm has been a constant inspiration; to Francois Mergen for his advice and sincere interest in the work and who, with Harry T. Stinson, Jr., spent considerable time editing the original manuscript; to Benjamin W. McFarland for his professional help and personal interest in processing the photographs; to Mrs. David C. Walden whose help on the manuscript went far beyond that required of a typist; to James Cole who gave freely of his time when consulted on statistical problems; and finally, to my wife Sally, who in her own way made it all so much easier.

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INTRODUCTION

Background

The genus *Castanea* contains several species of trees which are economically important in many countries of the world. The trees are presently most valued for their production of nuts, though they also serve as a source of wood and tannin. Large numbers of orchard trees are grown in China and Japan; however, the nuts which enter international commerce are supplied primarily by Italy. The United States is one of the largest purchasers in the international market and imports about 13 million pounds of chestnuts a year.

Of the thirteen species in the genus the American chestnut, *Castanea dentata* Borkh., was at one time the most versatile. It was the predominant and most valued species in the hardwood forests of eastern United States and, in addition to its aesthetic value, was important for three reasons: 1) it was the primary source of domestic tannin for the tanning industry; 2) the wood is extremely resistant to rot fungi and was extensively used for lumber, railroad ties, telephone poles, and fence posts; and 3) the nuts were a staple in the diets of squirrels, turkeys, deer, and other game, as well as some domestic animals.

Unfortunately, virtually all the American chestnut trees have been destroyed by the chestnut blight fungus, *Endothia parasitica* (Murr.) And., which was introduced into this country about 1890. The only living evidence of these great trees ~~are~~ is sprouts, which continually push up from the old root systems. Young sprouts resist infection by the blight fungus, but the older ones are systematically killed back to the ground.

Purpose

Emphasis in this paper has been placed on summarizing and contributing to botanical knowledge of the genus Castanea. The genetic and cytological studies supply information upon which future chestnut genetic and breeding programs could be developed. It is hoped that some of the information will be of value specifically to those working with woody plants.

The study consists of two parts. Part one is a cytological study and was undertaken to determine if there were major chromosomal differences among the chestnut species. The second part is concerned largely with genetic studies. The first chapter of part two is on flowering and related phenomena, and includes discussion of catkin length, type of flowering, self-compatibility, male-sterility, and xenia. The second and final chapter, dealing with interspecific crosses, was undertaken in an effort to draw conclusions on the relationships of the chestnut species to one another. A general discussion is presented which relates some of the findings to practical applications, and in the Appendix a technique is described for rooting chestnut.

PART I

CYTOLOGY

Introduction and Literature Review

Members of the genus *Castanea* are generally recognized as having n=12 chromosomes, which is the same as in most of the other Fagaceae studied to date (Darlington and Wylie, 1955). However, the chromosome number of only four of the thirteen species in the genus *Castanea* have been reported, and as recently as 1948 Delay attributed *Castanea vulgaris* Lam. with 2n=22. This species, *C. vulgaris*, was not recognized by A. Camus in her monograph of the genus (1929) but refers to the genus as a whole or specifically to *C. sativa* Mill. Wetzel (1929), after studying reduction figures of *C. sativa* and *C. crenata* Sieb. and Zucc., also concluded the genus had 2n=22. Schad *et al.* (1952) found n=11 and 12, but more often the latter, in *C. crenata*, *C. sativa*, and *C. mollissima* Bl. They also observed a few natural polyploides among these three species and their hybrids, 2n=31-48.

In 1930, Jaretzky did an extensive cytological study of the Fagales and reported the haploid number of the genus as n=12. He made preparations of three species but reported counts for only *C. sativa* and *C. dentata* Borkh. He had difficulty obtaining good figures of the latter species and failed to get counts from preparations of *C. crenata*. Almeida (1947) reported n=12 for *C. crenata*, and his mitotic and meiotic counts on a putative hybrid of *C. sativa* × *C. crenata* showed n=12 and 2n=24. The reduction division in the anthers of the hybrid was noticeably abnormal when compared to that of *C. crenata*. He found nonequational divisions, lagging chromosomes, and univalents with bivalents in some figures. In 1950, Poucques reported somatic counts from root tip preparations of *C. mollissima* and *C. dentata* which both proved to have 2n=24.

The literature contains sparse information on the morphology of *Castanea* chromosomes. Jaretzky (1930) divided the twelve meiotic chromosomes of *C. sativa* into three distinct groups according to size: five small, three of average size, and four large ones. Poucques (1950) reported that the chromosomes of *C. dentata* are thinner and longer at the end of prophase and in metaphase than those of *C. mollissima*.

From studies of the resting or interphase nucleus, Gosselin (1947) claimed the nucleus was of the euchromocentric type as opposed to chromocentric or prochromosomic. He differentiated between the interphase and resting nucleus as follows: the interphase type of nucleus is found in the meristematic cells of a root tip, whereas the nuclei of the differentiated root cap cells are in the resting stage. In the former type the nuclear plasm is nonreticulate and the euchromocenters are united against the nuclear membrane. Gosselin further stated that in this interphase nucleus the number of euchromocenters should also equal the number of chromosomes. In the resting nucleus, however, the nuclear plasm is reticulate and the number of chromatic granules, which now lie on the reticulum, is more than the number of chromosomes. Delay (1948), on the other hand, stated that the resting nucleus contains only 10-15 euchromocenters, a number less than the 22 chromosomes he attributed to the genus.

Poucques (1950) reported that the interphase nucleus of the root tip meristem of C. mollissima is 5-7μ in diameter, and possesses a large nucleolus of 2.5μ in diamoter, and a visible satellite. The euchromocenters were more numerous, 20-25, in C. dentata than in C. mollissima. The nucleolus of C. dentata reportedly attains a diameter of 3μ and, like C. mollissima, there is a large satellite. Wetzel (1929) reported the resting nucleus diameter of C. sativa and C. crenata as being between 7 and 8μ, which was the smallest of all the Fagales he studied. He also observed that the reduction division occurs rapidly, and there are often in the same field of vision resting nuclei, as well as nuclei in the prophase, metaphase, tetrad, and pollen stages of development. He concluded that synapsis and the tetrad stage were relatively long.

The present cytological study was undertaken to determine if there were any major chromosomal differences among the species in the three subgenera of the genus Castanea. Counts had been reported previously for species only in the subgenus Castanea, and there was disagreement as to whether the diploid number for these species is 22 or 24. Furthermore, the observation of incompatibilities among certain interspecific crosses (see final chapter) suggests the possibility of chromosomal differentiation at the species level.

Materials and Methods

The majority of the trees used were growing in the Sleeping Giant Chestnut Plantation at Mount Carmel, Connecticut, on state-owned land. These trees were acquired from several sources and primarily