Genetic and Cytological Studies In The Genus Castanea

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GENETIC AND CYTOLOGICAL STUDIES
IN THE GENUS *CASTANEA*

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By
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SUMMARY

Chromosome counts are reported for the first time for six ~~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 $3n = 36 \pm 1$. 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.

TABLE OF CONTENTS

PART I

PART II

CHAPTER I

Page

CHAPTER II

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LIST OF TABLES

INDEX TO FIGURES

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 polyploid~~e~~s 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 through the efforts of Arthur H. Graves. Eleven of the 13 species designated by A. Camus (1929) in her monograph of the genus are represented at the Chestnut Plantation, as well as many interspecific hybrids. Table 1 lists the species recognized by Camus, and the common names and abbreviations used in this report.

Several attempts were made by the author to obtain division figures in leaf primordia by staining with Feulgen or acetocarmine. The methods attempted, however, were unsatisfactory; the primary difficulty being that the cells could not be flattened enough to spread the small chromosomes. Sax's method (1959) of using the parenchymatous tissue which forms in a bark wound was also tried but no satisfactory figures were obtained.

The mitotic figures reported herein were prepared from the meristematic region of root tips, and unless specified otherwise, the root tips were obtained from germinating nuts. After the hypocotyl or radical had elongated approximately two inches the tip was removed, placed in fixative, and later stained with Feulgen (see Appendix, Table 17 for schedule used). One drawback to the method used was that a new embryo was being dealt with and only one half of the chromosomes of the specific female parent in question were being observed, while the other half were of an unknown parent that had been contributed through the pollen. To avoid this problem and to determine the chromosome number of juvenile trees, and trees which appeared to be female-sterile, it became necessary to obtain cytological material from other than germinating nuts.

Since root tips had proved to be satisfactory, it appeared

Table 1. SPECIES IN THE GENUS CASTANEA

Chestnut Species (A. Camus, 1929)	Symbol	Common Name
I Eucastanon* (3 nuts per involucre)		True chestnuts
C. sativa Mill.	E	European
C. dentata Borkh.	A	American
C. mollissima Bl.	C	Chinese
C. crenata Sieb. and Zucc.	J	Japanese
C. seguinii Dode	S	Seguin
C. davidii Dode		David
II Balanocastan~~on~~ea (1 nut per involucre)		Chinkapin
C. pumila Mill.	P	Allegany chinkapin
C. ashei Sud.	As	Ashe
C. floridana Ashe	F	
Var. ~~typica~~ floridane Ashe		
margaretta Ashe		
arcuata Ashe		
C. ozarkensis Ashe	O	Ozark chinkapin
Var. arkansana Ashe		
C. alnifolia Nutt.	Aln	Alder-leaf chinkapin
C. paucispina Ashe		
III Hypocastan~~on~~ea (1 nut per involucre, extension of involucre over apex of nut virtually nil)		
C. henryi Rehd. and Wils.	H	Henry or Willow-leafed chestnut

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* According to the International Code of Botanical Nomenclature, 1956, Article 22, the name Eucastanon is invalid and should be replaced by the name Castanea. I quote from Art. 22, "The subgenus or section including the type species of the correct name of the genus to which it is assigned repeats that name unaltered as its epithet ...."

necessary only to root cuttings or make air layerings, but chestnut is notorious for being difficult to root (Hartmann and Kester, 1959). Mist propagation or the use of a plastic tent, combined with special hormone mixtures, shows promise of being a technique by which chestnut can be reliably rooted, but it is not yet satisfactory (Waxman, personal communication). In Europe a modified grafting and inarching technique has been used to root apple cuttings (Kemmer, 1958). I have used this method to root chestnut material and refer to it as the "buried-inarch technique." The procedure is described in detail in the Appendix.

Preparations of meiotic figures were obtained from divisions of the male gametophyte. Young flowers were collected at daily intervals, starting approximately three weeks before normal anthesis, and were continued on any one tree until tetrads were observed. The anthers were dissected out, stained, and mounted in acetocarmine.

Photographs and camera lucida drawings were utilized to record the observations. A microscope with phase attachment was used for all the photographs. The phase contrast was particularly effective for increasing the contrast between the cytoplasm and the chromosomes. The use of phase accounts, in part, for the intensity of the granular material in the cytoplasm as seen in some of the photographs. An attempt was made to reduce the camera lucida drawings to the size of the photographs; hence, the magnification of every figure should be the same. The distortion that appears between the drawings and photographs is primarily due to the mechanics of the camera lucida device. However, it should be noted that when a particle, or in this case a chromosome, is out of focus in the microscopic field its apparent position in relation to the infocus material is often distorted in a plane perpendicular to the line of sight.

Results and Discussion

Chromosome counts of ten species in the genus Castanea are reported, as well as several counts on various interspecific hybrids. Six "new" species were studied and confirmation of the chromosome number in four previously reported species is given. The six new species include representatives from all three sections of the genus. The species and hybrids, including seven male-sterile trees, from which counts were obtained, are presented in Table 2. Figures of the species and some of the hybrids studied, along with specific comments, are given on pages 8 to 10.

No attempt was made to evaluate the observations on the interphase nucleus reported by Gosselin (1947), Delay (1948), and Poucques (1950). Nucleoli were not obvious in most of the preparations, but an example of one which showed up particularly well is illustrated in Figure 5.

Apparent satellites were observed in some cells but they were seldom consistent or conspicuous enough within one plant to warrant definite conclusions. C. sativa may have a pair of satellited chromosomes; however, Almeida (1947) did not report nor indicate the presence of any satellites in his drawings of this species. Figures 34, 35, 36, and 37 are hybrids of C. sativa which show chromosomes that may have satellites. The possibility that these are just large

Figure 1[*] *Castanea henryi*, 2n = 24.

2 Camera lucida drawing of Figure 1.

3 *C. henryi*, n = 12.

4 *C. pumila*, n = 12.

5 *C. pumila*, pachytene stage in a microsporocyte showing the nucleolus.

6 *C. ozarkensis*, 2n = 24.

7 Camera lucida drawing of Figure 6.

8 *C. ozarkensis*, n = 12.

9 *C. ashei*, n = 12.

10 Camera lucida drawing of Figure 9.

11 *C. ashei*, 2n = 24.

12 Camera lucida drawing of Figure 11.

13 *C. alnifolia*, 2n = 24.

14 Camera lucida drawing of Figure 13.

15 *C. alnifolia*, n = 12.

16 Camera lucida drawing of Figure 15.

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[*] Note for Figures 1-43: When a species name is followed by "2n" the material for the figure was a root tip and thus somatic in origin, whereas a species name followed by "n" indicates microsporocyte tissue was used and the figure is of the reduction division. Magnification of all figures is approximately the same, × 2,000.


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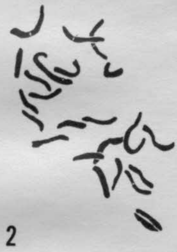
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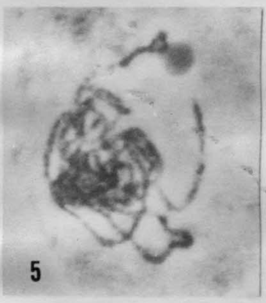
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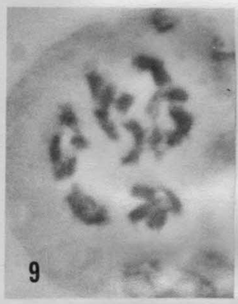
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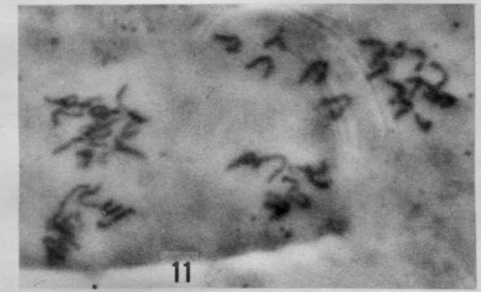
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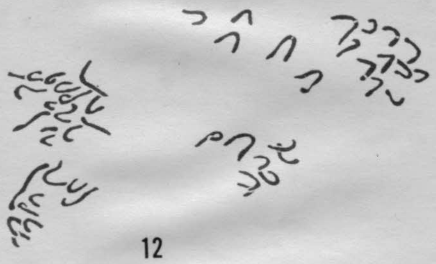
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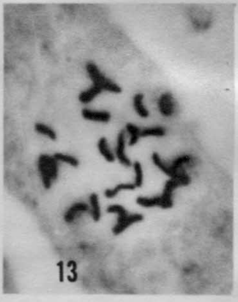
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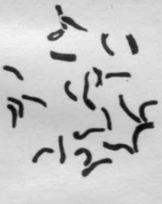
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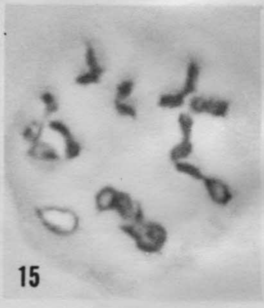
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Figure 17 *C. crenata*, 2n = 24.

18 Camera lucida drawing of Figure 17.

19 *C. dentata*, 2n = 24.

20 Camera lucida drawing of Figure 19.

21 *C. mollissima*, 2n = 24.

22 Camera lucida drawing of Figure 21.

23 *C. sativa*, 2n = 24.

24 Camera lucida drawing of Figure 23.

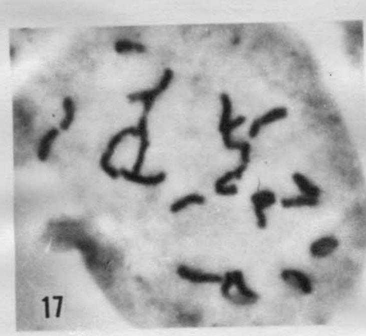
25 *C. seguinii*, 2n = 24.

26 Camera lucida drawing of Figure 25.

27 A male-sterile, complex hybrid of the following pedigree: [(*C. dentata*)×(*C. crenata* × Chinkapin?)] × [*C. mollissima*]. The somatic chromosome number is 25.

28 Camera lucida drawing of Figure 27.

29 The same hybrid as described for Figures 27 and 28. The cell is in prophase of a mitotic division. Notice the small chromosome which has precociously condensed.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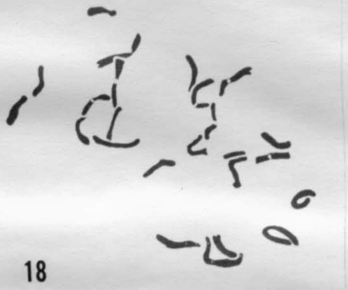
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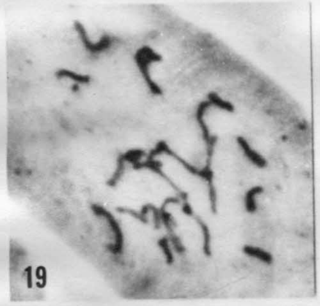
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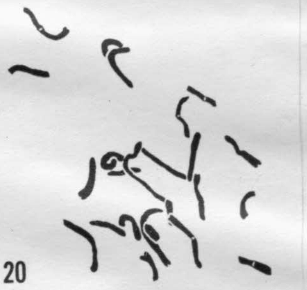
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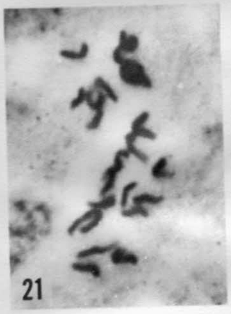
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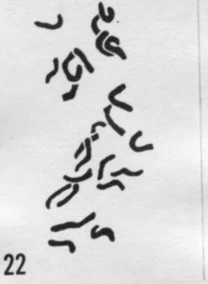
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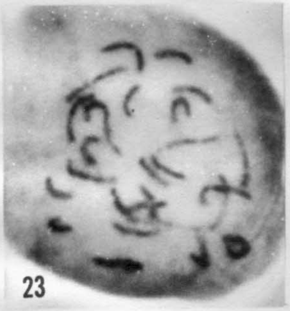
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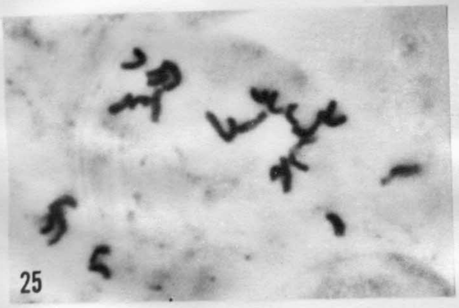
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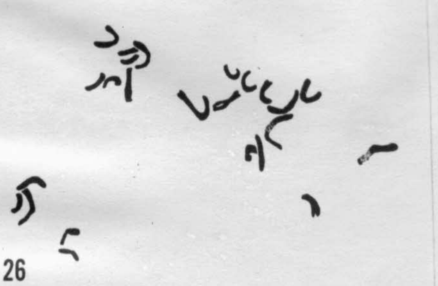
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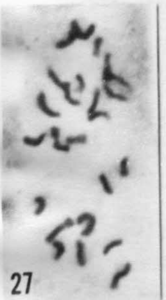
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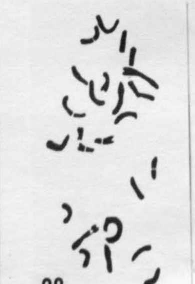
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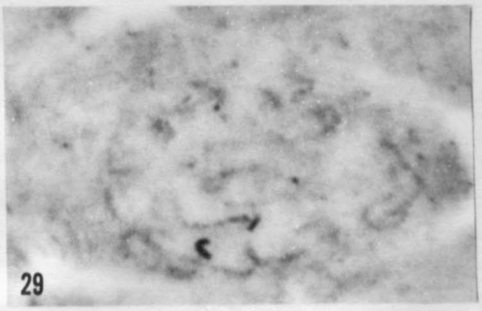
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Figure 30 A *C. mollissima* × *C. dentata* hybrid with a somatic chromosome number of 36±1.

31 Camera lucida drawing of Figure 30.

32 A male-sterile, *C. crenata* × *C. dentata* hybrid, 2n = 24.

33 Camera lucida drawing of Figure 32.

34 A *C. sativa* × *C. crenata* hybrid, 2n = 24. Notice what appear to be satellites on two chromosomes.

35 Camera lucida drawing of Figure 34.

36 A *C. sativa* × *C. seguinii* hybrid, 2n = 24. Notice what appears to be a satellite on one chromosome.

37 Camera lucida drawing of Figure 36.

38 A male-sterile, *C. dentata* × *C. mollissima* hybrid, 2n = 24.

39 Camera lucida drawing of Figure 38.

40 A male-sterile, *C. mollissima* × *C. dentata* hybrid, 2n = 24.

41 Camera lucida drawing of Figure 40.

42 A male-sterile, *C. sativa* × *C. mollissima* hybrid, 2n = 24.

43 Camera lucida drawing of Figure 42.

heterochromatic centromere regions which are near terminal in position was not eliminated.

Some difficulty was experienced in staining the chromosomes dark enough without overstaining the cytoplasm. The use of the phase contrast system was helpful in overcoming this problem. Fortunately the mitotic chromosomes were contracted enough at metaphase so that special treatments were not necessary. However, when the chromosomes are lined up at the metaphase plate they clump together, and to spread them for counting a considerable amount of pressure and tapping on the cover slip is needed. The preparation of satisfactory slides of the reduction division was unpredictable and often a very frustrating task. There was little difficulty in ascertaining when anthers were at approximately the proper stage of division, but metaphase and anaphase figures were at times most elusive, even when prophase and telophase figures were present in the same preparation.

The evidence, with the exception of one C. mollissima × C. dentata hybrid and one male-sterile hybrid of a complex pedigree, indicates that the normal somatic number of the species and interspecific hybrids in the genus Castanea is 24. The somatic figures were not good enough to make satisfactory karyograms or detailed observations on the morphology of the chromosomes. However, as can be seen in the photographs and drawings, there are considerable size differences in the chromosomes, the longest chromosomes being about twice the length of the shortest. The sizes at metaphase varied between about 2 and 4µ. Poucques' drawings (1950), using his scale of magnification, indicated that the length is something between 1 and 3µ.

Table 2. CHROMOSOME COUNTS OF CASTANEA SPECIES AND HYBRIDS

Subgenus	Species	Identification*	n	2n	
Hypo- ea castanon	C. henryi	R32T3		24	
	" "	R32T1	12		
Balano- ea castanon	C. alnifolia	RBeT3	12	24	
	C. ashei	NW Spr	12		
	" "	W Spr		24	
	C. ozarkensis	RGaT3	12		
	" "	RAlT2		24	
	" "	REpT3		24	
	C. pumila	RZeT2	12		
Castanea	C. seguinii	R3T8	12	24	
	C. crenata	R7T7		24	
	C. dentata	Rox 4W		24	
	C. mollissima	R1T12		24	
	" "	R15T9		24	
	C. sativa	R13AT3		24	MS
	" "	AW74 Fr		24	BI
- - - - -					
Hybrids	C. sativa × C. crenata	#14-59		24	CP
	C. sativa × C. seguinii	#10-59		24	CP
	C. sativa × C. mollissima	R19T12		24	MS,BI
	C. crenata × C. dentata	R9T10		24	MS
	[C. dentata × (C.crenata × Chinkapin?)][C.mollissima]	R7T10		25	MS
	C. dentata × C. mollissima	R10T10		24	MS
	C. dentata × C. mollissima	R10T12		24	MS
	C. mollissima × C. dentata	R17T3		24	MS
	C. mollissima × C. dentata	R4T5	Abnormal reduction	36±1	BI

* = This column identifies the tree at the Chestnut Plantation from which the nuts or scions were taken for study.

BI = Root tips obtained from a rooted scion by the buried-inarch technique.

CP = Root tips obtained from hypocotyls of nuts resulting from a controlled pollination.

MS = Male-sterile.

One exceptional chromosomal count was obtained from root tips of a tree that resulted from a cross of C. mollissima with C. dentata. It is an apparent triploid having 36±1 chromosomes (Figures 30, 31). The tree has shown rapid growth but has been irregular in habit. The leaves are most striking and often range between 9 and 12 inches in length. Graves and Nienstaedt (1952) stated that this tree was both male- and female-sterile; however, between the years 1939 and 1952 the Connecticut Station records show this tree has been used in 14 different pollinations: ten times as the female parent and four times as a male parent. Eight of these pollinations resulted in no nuts; of the remaining pollinations a total of nine nuts were obtained when the hybrid was used as a female tree, and ten when it was used as a male tree. Two pollinations subsequent to 1952, one with the tree as the male and the other as female, yielded no nuts. The evidence demonstrates that the tree is not completely sterile. Up to one per cent of the pollen is capable of germination in vitro. Studies of the reduction division in the anthers revealed no cells in which the chromosomes could be readily counted. It was difficult to determine if failure to observe good reduction figures was due to the abnormal behavior of the material or to a flaw in technique. Early meiotic stages were observed and the chromosomes appeared to be clumped abnormally and grouped in irregular numbers.

This triploid, C. mollissima × C. dentata, is quite susceptible to the chestnut blight fungus, although the female parent was Chinese and resistant to this disease. The chromosomes cannot be distinguished in respect to their origin in this hybrid, but because the hybrid more closely resembles the female parent in morphological characters and because a gamete with an abnormal number of chromosomes would have a better chance of survival if it came through the egg instead of the pollen, we might expect that the female parent contributed the extra set to the zygote. If this was indeed the case, we would have an example where a single complement of chromosomes of *C. dentata* is able to impart susceptibility to a tree which also has a double set of chromosomes of the normally resistant species, *C. mollissima*.

The other exceptional chromosome number was observed in the root tips of germinating nuts of a male-sterile hybrid of the following pedigree: [*C. dentata* × (*C. crenata* × Chinkapin?)] × [*C. mollissima*]. The extra chromosome appears to be a very small one and precocious in condensation (Figures 27, 28, 29). There is little evidence to indicate the specific origin of this extra chromosome. Reduction figures would be helpful, but they could only be obtained from studies of the egg because abortion of the anthers occurs before microsporogenesis. Somatic material of this male-sterile tree, other than from open-pollinated nuts, will have to be observed before it can be known if the true somatic number is 25 or 26.

Summary

Chromosome counts of ten species and several interspecific hybrids in the genus *Castanea* are reported. Six ~~new~~ not previously reported on species were studied: *Castanea seguinii*, *C. henryi*, *C. pumila*, *C. ashei*, *C. ozarkensis*, and *C. alnifolia*, which include representatives from the three subgenera of the genus. Counts are also given for four previously reported species belonging to the subgenus Castanea. All the species have a haploid number of chromosomes equal to 12 or a diploid number equal to 24. The hybrids examined also have 2n equal to 24 with the exception of two trees: one is a hybrid of C. mollissima × C. dentata and the other is a male-sterile hybrid with a complex pedigree. The former was a triploid with a somatic chromosome number of 36±1, whereas open-pollinated nuts of the latter tree had a somatic number of 25. The results confirm the reports of previous workers who had concluded that the somatic number for the genus Castanea is 24. No evidence was found to support those reports where the somatic number had been given as 22.

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PART II

GENETIC STUDIES

Chapter I

FLOWERING AND RELATED PHENOMENA IN CHESTNUT

Introduction

The purpose of this chapter is to bring together certain information bearing on flowering and reproduction in chestnut. This information has relevance to future genetic studies with this group. It is hoped that some of the material will be of interest and use to those working with hardwoods other than chestnut. The literature review, discussion, and conclusions have been handled separately in the individual sections. A general summary will be found at the end of the chapter.

The Chestnut Flower

Chestnuts are monoecious and bear their flowers on catkins on the current year's growth. Two types of inflorescences are found: all-male catkins and bisexual catkins. The all-male catkins occur on the proximal portion of new shoots and consist entirely of staminate flower clusters that are arranged in spiral fashion around the axis of the catkin. The bisexual catkins are distil to the all-male catkins and have not only the staminate flower clusters but, in addition, from one to several clusters of pistillate flowers near the base of each catkin. Flowers on the unisexual, staminate catkins usually dehisce first, then the pistillate flowers become receptive, and finally, the staminate flowers of the bisexual catkins dehisce. This situation of male, female, then male maturation of flowers has been termed duodichogamy (Stout, 1928; Vilkomerson, 1937).

Catkins are normally borne erect and, depending somewhat on the species, are between 5 and 20 cm long; the bisexual catkins are shorter than the staminate catkins. Flowering usually begins from the end of May to the first of July, and is completed by the end of July in the New Haven, Connecticut area.

Exceptions to statements in the previous paragraph have been observed and are dealt with in the following two sections on "Catkin Length," and "Inheritance of the Seguin Everbearing Character."

Catkin Length

In following a trait such as catkin length in interspecific crosses one might expect the trait to be quantitatively controlled and to have an expression in hybrids intermediate to that of the parental species. But this is not always the case as shall be demonstrated for two interspecific crosses. The following three species, which normally have catkins of the indicated length, were involved in the crosses: C. *dentata*, 15-20 cm; C. *mollissima*, 8-20 cm; and C. *crenata*, 5-20 cm (Camus, 1929).

Long, pendent catkins had been observed in the Boone chestnut, a *C. crenata* × *C. dentata* hybrid produced by Endicott in 1895 (Blake and Edgerton, 1945), and long catkins had been noticed by Graves (1937) in several *C. crenata* × *C. dentata* hybrids. No quantitative studies have been reported.

In the summer of 1960 catkin length was measured on all the *C. crenata* × *C. dentata*, or J × A, hybrids available in the local plantings. Representative trees of the two parental species were also sampled along with some *C. mollissima* × (*C. crenata* × *C. dentata*), or C × JA, hybrids. Each tree was sampled after anthesis of the staminate flowers; all the catkins were measured on two flowering branchlets.

The number of catkins on each branchlet varied considerably, as did the length of individual catkins. However, the maximum catkin length on separate branchlets in any one tree was quite consistant. Thus, the representative catkin length for the various trees was derived by taking the average length of the *three longest catkins* from each of two branchlets of every sampled tree. The measurements for the two branchlets were then averaged. These data are listed in Tables 3, 4 and 5.

Unfortunately the original *C. dentata* parents, five in number, of the JA hybrids were not available to measure catkin lengths and only two of the five *C. crenata* parents were available. But photographs taken several years ago show that catkins on two of the other *C. crenata* parent trees were not exceptionally long. This leaves unaccounted for the catkin length of six parental trees.

Table 3. CATKIN LENGTHS (cm) OF *C. CRENATA* × *C. DENTATA* HYBRIDS AND THEIR RESPECTIVE PARENT SPECIES

C. crenata	*C. crenata* × *C. dentata*	*C. dentata*
24.3	34.7[P]	23.8
23.2[P]	34.5[P]	21.7
20.0	32.3	21.0
19.7	31.8[P]	19.2
	30.7[P]	19.0
	30.7	17.8
	29.5	16.2
	25.3	15.2
	25.0	14.4
	25.0	12.6
	24.8[P]	
	24.8	
	22.2	
	21.2	
	20.8	
	20.0	
	16.5	
	16.2	
	9.7[*]	
Ave. 21.8 (n=4)	Ave. 26.2 (n=18)	Ave. 18.1 (n=10)

[*] Not included in average because the tree was severely blighted

[P] Pendant catkins as opposed to the normal erect type

Long catkins have not been reported for either parental species and it would be illogical to believe that these six representatives had long catkins. Thus it is assumed that one parent of every long-catkined JA hybrid could not have had long catkins. To get an approximation of catkin length for the parental species, catkins of local representatives were measured, as well as catkins on herbarium sheets in the case of the American species.

Table 4. CATKIN LENGTHS OF PROGENY FROM FOUR CROSSES OF C. MOLLISSIMA × (C. CRENATA × C. DENTATA) AND THEIR RESPECTIVE PARENTS

Identification	Catkin length (cm)	Ave. of CJA sibs (cm)	Identification	Catkin length (cm)	Ave. of CJA sibs (cm)
C	20.8		C	18.5	
C×JA	15.0	(n=1) 15.0	C×JA	17.5 18.2 15.0	(n=3) 16.9
JA$^+$	31.8		JA$^+$	31.8	
C	14.0		C	21.2	
C×JA	20.2 20.7 17.8 13.5 12.0	(n=5) 16.8	C×JA	17.0 21.0	(n=2) 19.0
JA$^+$	31.8		JA$^+$	31.8	

+ Used as male parent in all cases
C = *C. mollissima*, J = *C. crenata*, A = *C. dentata*

As can be seen from Table 3, the range of catkin length is considerable for the *C. dentata* trees studied, 12.6 - 23.8 cm. Only four *C. crenata* trees were sampled but each represents a different selection or type within the species; the respective range of catkin length for these Japanese trees was 19.7 - 24.3 cm. Thus the average catkin measurement of all the JA hybrids, 26.2 cm, was more than the maximum measurement for each of the parental species. The hybrids had catkin lengths varying over a wide range, 9.7 - 34.7 cm; however, the tree with the extremely short catkins may distort the true range,

Table 5. SUMMARY OF CATKIN LENGTHS FOR TWO INTERSPECIFIC CROSSES

Identification	Number of trees	Catkin length (cm)	
		Observed	Expected if parents have equal effect in hybrid
C	4	18.6	
C×JA	19 (all have common JA ♂ parent)	16.6	25.2
JA	2 grafts of same hybrid	31.8	
J	4	21.8	
J×A	18	26.2	20.0
A	10	18.1	

C = *C. mollissima*, J = *C. crenata*, A = *C. dentata*

since this tree was severely attacked by the chestnut blight fungus. Presumably the catkins were stunted because of the general weakening of the tree from the blight.

Of considerable interest are the *C. crenata* × *C. dentata* hybrid trees with extremely long catkins (see Table 3). The long catkins are not characteristic of all the hybrids from the same cross, yet they are known to occur only in Japanese × American crosses.

The JA × JA hybrids are very susceptible to the blight and none ~~were~~ was available for catkin measurements. However, we did have a number of *C. mollissima* × (*C. crenata* × *C. dentata*), or C × JA, hybrids and 19 of these, all of which had a common, long-catkined JA parent, were studied. The data are summarized in Tables 4 and 5.

The striking result was that crosses between a JA hybrid with long catkins and Chinese trees which have relatively short catkins, produced trees with catkins no longer than the short-catkined parent. Certain progeny even had catkins considerably shorter than either parent.

The character of weeping or drooping catkins is closely correlated with length, but as seen in the data in Table 3 long catkins are not necessarily pendent.

The long catkins characteristic of some of the JA hybrids may result from overdominance or heterosis. In the C × JA hybrids the Chinese chestnut genes may completely dominate those of the JA male parent, but there is some indication that an interaction occurs such as to decrease catkin length below that of the Chinese parent. The parental Japanese, American, and Chinese trees used in these crosses could be heterozygous for genes effecting catkin length since cross-pollination is the rule for the respective species. Thus transgressive segregation and gene recombination may be occurring in the J × A crosses as well as in the C × JA crosses. Obviously, the long catkin length in the JA hybrids is not transmitted to the progeny of the C × JA crosses. A more extensive analysis of hybrid trees resulting from crosses between these three species is necessary before more definitive conclusions can be made about the inheritance of catkin length.

Inheritance of the Seguin Everbearing Character

Some of the *C. seguinii* trees are unique among most all other chestnut species by virtue of their "everbearing" habit.[1] Instead of ceasing to bloom after a single flush of flowers in June, the trees produce unisexual and bisexual catkins throughout the entire growing season.

Clapper (1954) reported, "Everbearing is a dominant character in first-generation hybrids from crossing non-everbearing with everbearing, and is inherited in a ratio of at least three to one in the second generation, with the character varying from the two-crop type to true everbearing." No statement was made in regard to the species used in the crosses with the everbearing Seguin in arriving at this conclusion, and no data were given as to the number of crosses analyzed or the number of offspring studied.

Table 6 summarizes the information from the flowering progeny of our crosses between the everbearing Seguin and non-everbearing species. Only one of seven first generation *C. mollissima* × *C. seguinii* hybrids was everbearing. Two siblings of the one everbearing F_1 were normal or non-everbearing. No further crosses have been completed with the everbearing F_1 or its two siblings. All the crosses involving F_1, *C. mollissima* × *C. seguinii* hybrids were made with one or more of four non-everbearing siblings of a cross completed in 1934.

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[1] This character may be found in a few forms of *C. crenata* growing in southern Japan (Clapper, 1954).

Table 6. SUMMARY OF INFORMATION ON THE INHERITANCE OF THE SEGUIN EVERBEARING CHARACTER

Cross	Normal	Two-Crop	Everbearing	Total
C × S	6		1	7
"y" × S	3		0	3
CS × C	29	1	0	30
CS × "y"	6		0	6
CS × CS	29	1	3	33
	73	2	4	79

C = *C. mollissima*, S = *C. seguinii*
"y" = trees other than *C. mollissima* or *C. seguinii*

Backcrossing these F$_1$, Chinese × Seguin, non-everbearing trees with the Chinese resulted in no everbearing progeny (30 trees). Unfortunately, progeny of the backcross to the Seguin were not available. Three out of 33 flowering trees of the second generation cross of Chinese × Seguin were characterized as everbearing. It is suggested that the everbearing character is recessive, at least in certain crosses of *C. mollissima* × *C. seguinii*, and is controlled by two unlinked genes.

The one F$_1$, Chinese × Seguin, everbearing tree already referred to does not readily fit into the above hypothesis. This tree could be accounted for if recessive genes for everbearing are present in some trees of *C. mollissima*.

The "two-crop" type of flowering, listed in Table 6 and mentioned by Clapper, refers to a second burst of flowers later in the summer in contrast to continuous flowering or everbearing.

However, this trait may have no relation to the everbearing character. In 1960, a second crop of flowers was observed on two American, three Chinese, and several hybrid chestnut trees which do not have C. seguinii parentage. The expression varies from a few catkins on one or two branches to a large number of catkins on many branches. Because this characteristic expressed itself in only two of the 79 trees which had C. seguinii in their pedigree, one should not attach much significance to it. The second crop of flowers for the single (Chinese × Seguin) × Chinese tree with this trait consisted of only two catkins and most certainly could have been unrelated to the everbearing trait.

If a Chi Square Test is performed on the hypothesis that 1 out of 16 trees will show the everbearing character in the F_2 generation, and if the observed ratio of 3 out of 33 is tested, then $P=.5$. If the single two-crop tree is included as an everbearing type and the ratio of 4 out of 33 is tested, then $P=.16$. Thus even by including the single two-crop tree that resulted in the F_2, the hypothesis of two recessive unlinked genes is not rejected on the basis of a Chi Square Test.

The Seguin, in addition to the everbearing trait, is characterized by prolificness, that is heavy flowering and fruiting. It also flowers at a young age and is dwarf in stature. These characters show some expression in the F_1 and segregation in the F_2, but no conclusions were reached as to inheritance patterns of of traits other than everbearing.

Wind and Insect Pollination

There has been much debate as to whether chestnuts are wind or insect pollinated. Groom (1909) described the various morphological characters of the flowers in respect to whether they were typical of an insect or wind pollinated plant. The male catkins, he stated, are predominantly designed for insect pollination: the catkins are conspicuous by their yellow color and by their length and grouping; they are often scented, and nectar is present; they are erect, and not pendant like most wind-pollinated species; pollen grains cling in groups, and bees and other insects are often seen on the flowers. The female flowers, on the other hand, appear best suited for wind pollination: the flower is inconspicuous, devoid of odor, and the rod-like stigma-bearing styles agree in size with wind pollinated species. Groom thus concluded that the chestnut is in a transitional stage, degenerating from an insect to a wind pollinating mechanism. Porsch (1950), however, decided on the basis of flower morphology and observation of 103 insects visiting C. sativa that chestnuts are insect pollinated. Clapper (1954) concluded on the basis of tests in which pistillate flowers were screened from insects, but not from wind-borne pollen, that both factors are involved; however wind is the more important. Breviglieri (1955) was able to catch large numbers of pollen grains (70,000 - 500,000 on a 20 × 20 in. surface) carried by the wind at a distance of 200 feet from the chestnut blossoms by using special lanolin coated glass plates. He also pointed out that some of the best bearing C. sativa clones are those which are nearly male-sterile and which produce little or no nectar. Although few insects visited these male-sterile trees, fruit set was good, thus indicating that the trees were wind pollinated.

This author has not performed specific tests to distinguish between the natural occurrence of wind and insect pollination. However, the observation that the locally grown male-sterile trees, which have little or no nectar, are highly "female" fertile supports the claims of Clapper and Breviglieri that chestnuts are predominantly wind-pollinated.

Female Receptivity

Several workers have attempted to determine the period during which the female flowers are receptive. Vilkomerson (1937, 1940) concluded from single pollinations of a series of pistils at various stages of development that they are probably receptive from the time the style branches spread until the stigmatic tips darken. Clapper (1954), in a series of small tests conducted over a number of years, concluded that the most advantageous time to pollinate is between 10 and 17 days after staminate flower anthesis of the mother tree. He found that with *C. crenata* and *C. pumila* high percentages of nut set were obtained when pollinations were made as late as 23 days after anthesis. Nienstaedt (1956) conducted similar studies using *C. crenata*, *C. mollissima*, and two *C. crenata* hybrids. He concluded that pollinations should give best results if performed between the 10th and 13th day after the beginning of the anthesis of the unisexual catkins. Schad, Grente, and Solignat (1955) and

Solignat (1958) found that stigmatic receptivity was greatest from one to two weeks after complete expansion of the stigmas had occurred.

The relative timing of anthesis of the staminate flowers to stigmatic expansion varies considerably between trees and within a tree during different years. However, expansion of the stigmas coincides more closely with the period of maximum receptivity of the stigmas than does anthesis of the staminate flowers. Stigmatic expansion, therefore, is probably the better criterion to use in timing pollinations. Satisfactory nut set was obtained in the pollination seasons of 1959 and 1960 by pollinating about one week after complete expansion of the stigmas.

Graves and Nienstaedt (1953; Nienstaedt, 1956) tested the effect of emasculation on nut yield. They found that, although bagging apparently has an injurious effect, emasculation did not injure the female flowers nor cause a reduction in the nut yield. In fact, they observed a slight beneficial effect from emasculation. They also noted that particular hybrids, such as C. crenata × C. dentata, are inherently low nut yielders in respect to the initial number of bagged female flowers and the subsequent expected nut yield.

Clapper (1954) observed that pollinations performed in the morning were more successful than afternoon pollinations. It was thought that the higher humidity conditions which exist in the morning might make the stigmatic surface more tacky and thus promote adherence of pollen to it. There is also the possibility that effective germination of pollen occurs only during the high afternoon temperatures and that pollinations are most successful when completed before this high temperature period. This supposition is based on the observation that pollen in vitro germinates at relatively high temperatures.

Self-Compatibility

There are many reports in the literature of isolated trees that have not borne fruit (Schneck, 1881; Taylor, 1935; Graves, 1931, 1937, 1944), and "selfing" experiments have indicated that chestnut trees are often self-sterile (Graves, 1934, 1937; McKay, 1942; Clapper, 1954). On the other hand, it has been demonstrated that self-fertilization apparently does occur in at least some chestnut trees (Breviglieri, 1951; Schad et al., 1952, 1955; Solignat, 1958).

Stout (1926, 1928, 1929) thought self-sterility might be due largely to dichogamy in the maturation of the male and female flowers. Though this mechanism may have some effect, other self-incompatibility mechanisms are also operative (Vilkomerson, 1937). The exact nature of the self-incompatibility mechanism is, however, unknown. Schad et al.(1952) suggested that it might be a polyallelic series such as occurs in Nicotiana. Clapper (1954) clipped the styles back on several trees before pollination to test whether or not pollen tubes would reach the ovules and fertilize them if the tubes traveled a shorter distance. Clipping of the styles did not enhance self-fertilization nor did it prevent cross-fertilization.

The tests of Breviglieri (1951), Schad et al. (1952, 1955), and Solignat (1958) under controlled conditions indicated that self-compatibility occurs regularly but generally at a low level, at least in C. sativa and C. sativa × C. crenata hybrids.

At the Connecticut Station there have been several attempts to self-pollinate chestnut trees. The author has adopted the practice in the past two years of placing at least four separate "selfing bags" on every tree which was being used for cross-pollinating. These selfing bags, enclosing the pistillate and staminate flowers of a branch, were placed on the trees at the same time the pistillate flowers were emasculated and bagged. They were also removed at the same time as the crossing bags, i.e. when the pistillate flowers were no longer receptive. Thus the selfing bags served in part as controls for our pollination work. When the nut set was high in these bags, there was reason, as explained below, to doubt the hybridity of the nuts formed in crossing bags of the same tree.

Contamination by pollen from surrounding trees is assumed not to be a major problem, but possible contamination by pollen of the trees bearing the pistillate flowers is more serious for three reasons: 1) When the bag over the emasculated pistillate flowers is removed the nearest staminate catkins, and thus the most likely source of pollen in the air, are from the same tree; 2) Occasionally pistillate flowers produce one or two functional anthers which go undetected at the time of emasculation and pollination; and 3) Any insects which might enter the neck of a bag would, more often than not, have crawled from surrounding branches on the same tree and carry local pollen.

Table 7 is a summary of the selfing experiments completed at the Station. It can be seen that not many of the self-pollinated trees set fruit, and the total nut yield was not high. The yield of nuts per bag from interspecific crosses would, on the average, be at least ten times that obtained upon selfing.

Table 7. SELF-FERTILIZATION OF CHESTNUT TREES

No. different trees selfed	No. trees set fruit	Total no. of bags	Total no. nuts set
48	14	282	64*

* Twenty of these nuts were derived from five bags on one hybrid tree.

Apparent successful self-pollinations have occurred on trees of C. mollissima, C. crenata, and some hybrids of C. crenata. These are listed as presumptive self-pollinators because the nuts could also have resulted from apomixis. Several selfing attempts of the following species have been unsuccessful: C. dentata, C. pumila, C. seguinii, and C. ozarkensis.

Thus, the degree of self-compatibility in chestnut is not entirely clear. Self-compatibility is not common but it apparently does take place occasionally in some trees and is, perhaps, more common in certain species.

Apomixis

Apomixis was reported by Morris as early as 1914 and later by McKay (1942) and Breviglieri (1951). McKay observed pollen tubes and male gametes near the egg apparatus in sections of flowers taken from a self-pollinated C. mollissima tree. Fertilization was not observed in these sections, but three per cent of the self-pollinated pistiles on this and certain other seedlings of the same species set fruit with embryos. The nuts were assumed to have resulted from apomictic development. McKay also obtained nuts from approximately five per cent of the unpollinated pistillate flowers on three out of four C. mollissima trees; one tree set no fruit.

I have made limited tests on seven trees including five different species which involved a total of about 200 flowers. In no case did the isolated, unpollinated pistillate flowers form nuts. The data in Table 7 show that 34 out of 48 self-pollinated trees failed to produce nuts. It is apparent that these 34 trees are not only highly self-incompatible but non-apomictic as well. Apomixis, therefore, has not been observed in our material unless the 14 trees, which were thought to show some selfing, produced fruit apomicticly.

It is concluded that if apomixis occurs in chestnut, it is probably less common than self-fertilization. However, if the process of self-pollination triggers apomixis then the experimental distinction between the two events would be difficult.

Sterility

Sterility as reported here refers to malfunction of the gametes or flower parts and not to the self-incompatibility mechanism.

A tendency towards either male-sterility or female-sterility in certain specimens of *C. sativa* was observed by Borzi in 1920, who viewed it as a tendency towards dioeciousness. The same phenomenon and interpretation was reported by Breviglieri (1951).

Borzi categorized the pollen producing ability of a tree according to the length of the stamens: longistaminée, mesostaminée, and brachistaminée, the latter tending toward sterility. Most European writers have followed this pattern of classification with the addition of a fourth group, the astaminate or completely male-sterile tree (Petri, 1924; Breviglieri, 1951; Solignat, 1958; Baldini, 1959). Baldini classified eight cultivars of *C. sativa* as astaminate. Of the two reports by American workers on male-sterility, neither categorized the degree of fertility by stamen length (McKay, 1940; Graves and Nienstaedt, 1952).

McKay (1940) observed three completely male-sterile trees. One, a *C. crenata* tree, produced flowers which opened and had normal quantities of nectar, but microscopic examination showed that stamen primorida were not formed; the catkins appeared normal in that they attained full size and did not prematurely drop from the tree. The second tree was a presumed hybrid of *C. sativa* and *C. dentata*, and sterility was similar to that in the first except the flowers did not open. The third, a *C. sativa* tree, lost its staminate catkins before the normal maturation date. No reasons or causes were proposed for the sterility in these three trees.

Graves and Nienstaedt (1952) reported that male-sterility had been encountered in several instances in *C. dentata* × *C. mollissima* and C. crenata × C. dentata hybrids. The male-sterile trees produced a normal or even excessive number of female flowers and were prolific nut producers. (The trees listed in Table 8 include some of those studied by Graves and Nienstaedt).

This writer's observations on male-sterility are in general agreement with those reported by Graves and Nienstaedt (1952). Pollen sterility is not sporadic in a given individual, but it is uniform throughout the flowering branches. The arrangement of the individual flowers is normal; however, when the flowers open, one can see with the aid of a hand lens 3-6 tiny, membranous perianth-segments for each small flower. The segments are whitish in color and more or less connected at their bases. Within the perianth there are no signs of anthers, but only fine hairs. Staminate catkins take on a brownish color and eventually drop off, whereas in androgynous catkins the staminate portion withers but may remain attached. Figure 45 shows fertile and sterile male flowers on catkins at comparable stages of development.

Several reasons may be suggested for sterility in interspecific hybrids: the chromosomes may fail to pair normally at meiosis, thereby giving rise to unbalanced gametes; it may be genically determined, the result of bringing together diverse genomes; or there may be a gene-cytoplasm interaction.

If staminate primordia are not formed, or if the primordia fail to form anthers, it is apparent that the breakdown in fertility occurs before meiosis, and sterility cannot be due to meiotic irregularities. The staminate flowers of all the male-sterile trees listed in Tables 8 and 9 abort before meiosis with the exception of two. Thus male-sterility resulting from meiotic phenomena is ruled out in most of these trees. Observations of female-fertility on these trees are incomplete but as yet there is no evidence of reduced fertility. In fact, as reported by Graves and Nienstaedt (1952), there is reason to believe that the male-sterile trees may be exceptionally good nut producers.

This being the case it is suspected that male-sterility of the pure species and cultivars may be genic, whereas the predominant type in the hybrids is either chromosomic or the result of a gene-cytoplasm interaction. The various types should be distinguishable by progeny testing or, if there are major chromosomal abberations, by cytological studies.

Cytological studies of seven male-sterile trees revealed normal chromosome counts in all but one (See Part I).

Table 9 lists all the available flowering trees derived from male-sterile parents. There is some indication that the inheritance of male-sterility may be cytoplasmically controlled in a few of these trees. Note particularly the progeny from trees R10T10 and R10T12 which have a common C. dentata parent. There is one fertile tree which is bothersome but not necessarily unexpected on the hypothesis of cytoplasmic male-sterility, e.g. it might be explained on the basis of restorer genes. Unfortunately, it is not known if the original American parent was male-sterile. Cytological studies of R10T10 and R10T12, as reported in Part I, revealed no gross chromosomal abnormalities.

Table 8. LIST OF MALE-STERILE TREES
In which the Female Parent was Male-Fertile
or could not be Determined

Pedigree (Siblings Grouped)	Abort before Meiosis	Pedigree (Siblings Grouped)	Abort before Meiosis
C	+	A×C•JA fp,fs1	+
E	+	A×C•JA fp	+
E	+	E×C fp	+
E	+	E×C fp,fs2	+
? C×A	+	J×A fp,fs4	+
C×A	-	JA×C fp	+
C×A	+	JA•J×JA•J	+
C×A	+	JA•J×JA•J	+
C×A	+	A•S8×C	+
C×A	+	A•S8×C	+
C×A	+	A•S8×OP	+
C×JA	+	A•S8×OP	+
C×JA fs1	-		
A×C	+		
A×C	+		
A×C	+		

fp = the pistillate parent is known to have been male-fertile
fs = indicates that siblings were fertile, the number specifies how many
C = C. mollissima
E = C. sativa
A = C. dentata
J = C. crenata
S8 = C. crenata hybrid
OP = open or uncontrolled pollination
\+ = No anthers formed
\- = Anthers formed; pollen inviable

Additional progeny of the male-sterile trees are on record at the Connecticut Station but at the time of this study they were either not available or they were from recent crosses and not old enough to flower. Observations of one more year should add considerably to the validity of the hypothesis that cytoplasmically inherited male-sterility occurs in Castanea. But final proof will depend on completing enough successive backcrosses to accomplish genome substitution in male-steriles by their male-fertile parent.

Table 9. ANALYSIS OF POLLEN FERTILITY OF PROGENY FROM MALE-STERILE TREES

Pollination Number	Pedigree	Location of Parent ♀	Location of Parent ♂	No. of Progeny	Data for 1960
13-54	JA×C	R10T9	R1T15	29	4 Flowered: 4 ♂ Fertile
48-50	AC×C	R10T12	R1T7	8	1 Flowered: 1 ♂ Sterile
45-51	AC×C	R10T10	R1T7	11	2 Flowered: 1 ♂ Fertile 1 ♂ Sterile
29-48	AC×C	R10T10	R1T15	10	5 Flowered: 5 ♂ Sterile
OP-49	AC×OP	R10T10	?	1	1 Flowered: 1 ♂ Sterile

J = C. crenata

A = C. dentata

C = C. mollissima

OP = Open or uncontrolled pollination

No cases of complete female-sterility have been detected among the flowering trees under observation. It is a more difficult phenomenon to observe than male-sterility, particularly in young trees. A few of the F_2 progeny of the *C. mollissima* × *C. seguinii* cross are suspected of being female-sterile. They have borne female flowers but no nuts; however, they are still too young for definite conclusions to be drawn. The triploid chestnut discussed in Part I of this paper is partially female- as well as male-sterile, as might be expected.

Pollen: Germination and Storage

Because flowering of the different chestnut species is not synchronous many interspecific crosses can be obtained only if pollen of the male parent can be stored in a functional condition. Several authors have attempted to germinate and store pollen, but with the methods used pollen germination always has been 50% or less. A germinating technique by which higher percentages of germination have been obtained and a successful method to store pollen are here described.

In germination tests, Vilkomerson (1937) consistantly obtained negative results using an agar or distilled water medium with varying concentrations of sucrose and yeast, and even decoctions of crushed style tips.

Urquijo (1944) stored pollen under varying temperature and humidity conditions and in partial vacuums. Initial pollen germination was 45% and after 90 days approximately 30%, decreasing rapidly thereafter. There were no significant differences between treatments, accounted in part, perhaps, by the rather low initial germination.

Schad et al. (1952) germinated pollen in petri dishes on a two per cent agar gel. The surface of the gel was moistened with a 5-20% sugar solution immediately before sowing the pollen. They found that 10 and 15% sucrose gave the best results with germination varying between 6.5 and 54% in counts made after 48 hours. Evidence that all the viable pollen grains were not germinating was obtained by staining with potassium iodide.

Nienstaedt (1956) examined the effects of temperature, sugar content, and boric-acid concentration on pollen germination. Pollen was extracted by dropping the catkins on a glass plate and scraping the pollen off the glass with a razor blade into a vial. For germination tests pollen was suspended in a drop of medium from a glass slide in a moist chamber. The basic medium was distilled water. Best results were obtained at temperatures of 28 and 37 C. The effect of the addition of 10 and 20% sucrose was not clear; sucrose appeared beneficial in some tests while in others there was no effect. C. henryi definitely grew better in 10 and 20% sucrose. Boric acid was added at the rate of 0.1, 0.5, and 1%. The two higher concentrations had a retarding effect, whereas the 0.1% solution was beneficial with pollen from some trees but not with others. Pollen of C. crenata, C. henryi, C. mollissima, and a C. crenata hybrid was germinated. Per cent germination was not reported.

I have made germination tests using the following technique for collection and storage of pollen: catkins are dropped onto a piece of wax paper, and the pollen is scraped off onto a small stick inserted in a cork, which is then fitted into a glass vial. Room is left in the bottom of the vial below the stick for a small amount of desiccating material such as calcium sulfate.

Pollen of *C. henryi* and *C. dentata* was used to test the optimum sucrose concentration and the optimum temperature for germination. Pollen was placed in a drop of medium on a cover slip which was sealed in place by petroleum jelly over the depression of a Van Tiegham slide. Germination in distilled water, .01, .1, .5, 1, 2, 5, 10, 20, 40, and 50% sucrose was tested after 7-12 hours of incubation at 30 C. Germination occurred in distilled water and in the sucrose solutions up to a concentration of 40%. Germination was good and showed no sharp maximum between sucrose concentrations of .1 and 10%. In a second study the optimal temperature range for germination was determined by incubation at 18, 26, 29, 30, 33, and 39 C. The optimal temperature range for both species is 28-34 C. But *C. henryi* appeared to germinate better at slightly higher temperatures than *C. dentata*. One might expect the converse to be true since *C. henryi* flowers a month earlier than *C. dentata* and normally before the onset of high summer temperatures.

Thus, for determinations of pollen viability it was decided to use a .5% aqueous sucrose solution and to incubate at 29-31 C. Under these conditions much of the viable pollen germinated in one hour. A pollen grain was considered germinated when the pollen tube length was equal to, or more than, the diameter of the pollen grain. Estimates of germination were made by counting 100 grains on each of two slides after an incubation period of at least 12 hours.

Fresh pollen from trees of the following species has been germinated: C. dentata, C. crenata, C. seguinii, C. mollissima, C. ozarkensis, C. pumila, C. alnifolia, C. ashei, and C. henryi. Germination was routinely between 50-80%.

It was necessary to store pollen to cross-pollinate certain species such as C. henryi, which flowers late in May, with C. ashei or C. alnifolia, which do not flower until early July. It was found that pollen in vials with a desiccant added remained viable for several weeks at room temperature; in fact, low percentages of germination occurred in vitro after four months. When pollen was frozen at -15 C and stored with a desiccant, germination in vitro was still above 10% after one year.

Nuts were obtained in 1958 and 1960 from several pollinations in which pollen of C. henryi, C. ozarkensis, C. pumila, and C. dentata was stored at room temperature or at -15 C for a period of a few days to four weeks prior to its use. Two pollinations were attempted with pollen of a C. dentata and a C. crenata tree which had been stored at -15 C for one year. Germination in vitro was above 10% at pollination time, but no nuts resulted from pollinating 94 female flowers on a C. henryi tree.

These results show that chestnut pollen can be kept viable for at least a year, and though not yet demonstrated, it is likely that under optimal conditions, i.e. low temperature and dry atmosphere storage, the pollen could be kept functional for an equal period of time. Pollen is readily and easily germinated in a .1-10% aqueous sucrose solution at the relatively high temperature of 28-34 C.

Xenia

Effect of Male Parent on Dormancy

Introduction - Xenia: Xenia has been found to occur in many plants and no attempt will be made here to present a complete review of the subject. As applied in this paper xenia refers to the direct effect of a foreign pollen parent on nut tissue which is not entirely maternal in origin; specifically then this means the effect of the pollen parent on the embryo and endosperm. Focke used the term xenia in 1881 to describe the effect of the pollen parent on the endosperm in *Zea mays* L., and Mendel in his classical genetic studies described one of the best known cases of xenia, that is, the effect of the pollen parent in producing smooth or wrinkled peas. Recently a case of xenia and metaxenia was reported in a genus of trees somewhat related to chestnut. Schreiner and Duffield (1942) observed that pollen of *Quercus robur* crossed onto *Q. alba* produced larger nuts than found in the maternal species (xenia), and it also prolonged the period of maturation of the nut (metaxenia).

Blaringh~~a~~em (1919) in crosses of *Castanea sativa* and *C. dentata* observed that nut size and time of ripening were effected by the pollen parent; however, his observations were based on limited data. Several authors, utilizing the species *C. sativa*, *C. mollissima*, and *C. crenata*, presented data confirming the occurrence of xenia in chestnut (McKay and Crane, 1938, 1939; McKay, 1956; Gossard, 1956). They observed the effect of different pollen parents in determining the size of nuts and also in controlling the relative amounts of carotene and other carotenoids in chestnut kernels. McKay (1960) emphasized the possible significance of utilizing these seed characters in speeding up chestnut breeding. By using seed characters the success of an intended cross can be verified, and there is also the possibility of immediately analyzing the inheritance of various seed characters which may or may not have a direct bearing on the tree that will subsequently develop. To the geneticist there is the obvious advantage of obtaining results in the same year of pollination instead of waiting several years as is necessary with most genetic studies on trees.

I have obtained information which supports the findings of McKay and others on the effect of the pollen parent on nut size in chestnut. The effect is almost invariably correlated with the "normal" nut size of the tree used as the male parent. The findings reported here, however, are concerned with the effect of the male parent on the length of dormancy required by chestnut seed, a phenomenon which has not been previously described.

Effect of Male Parent on Dormancy: It was observed that nuts from most of the Chinkapin species (subgenus *Balanocastanon*) do not have a long dormancy requirement, and they germinate readily in the stratifying medium. The records at the Experiment Station show that large numbers of interspecific crosses have been completed between the Chinkapin species and species in the other two subgenera; however, few of the seedlings or trees had been raised. There was some evidence that these hybrid nuts were germinating prematurely while stratified, and then rotting before normal planting time in the spring. The question was raised as to which nuts were early germinating,and did the male parent effect the dormancy requirement. To answer this question a study was made of the nuts obtained from several crosses between species of the three subgenera.

In general, studies on germination and dormancy requirements involve placing a large sample of seed at the desired cold temperature; small subsamples are periodically removed to some higher temperature to test for germination. The limited number of hybrid chestnut seeds available precluded testing in this manner.

In this experiment the chestnut seed was stratified in a sandy loam at a constant temperature of 4 C, and the nuts were examined while still in the cold room to determine the degree of germination. Different crosses on the same female tree were all handled in the same manner so as to minimize differences in treatment effect. Observations on some of the crosses are presented in Table 10.

The data were treated statistically to determine if the various male parents differed significantly in their effects on dormancy when crossed to a common female parent. In Table 10 crosses grouped by any one bracket were not significantly different ($P=.05$) from each other. Because of different harvest dates direct comparisons cannot be made between crosses involving different female parents. For the statistical treatment the data were set up in $2 \times k$ contingency tables, in which the observations were dichotomized as germinated or not germinated, and tested against the null hypothesis with Chi Square.

It is obvious from the results that the male parent can affect nut dormancy. Furthermore, certain conclusions can be drawn about the general effect of a species when used in different crosses.

Table 10. EFFECT OF MALE PARENT ON NUT DORMANCY AT 4 C

Date of Observations	Pollination number	Cross	Significant difference *	Degree of hypocotyl extension** 0	1	2	3	4	% Germination	Total no. nuts
1/17/61	a) 25	O × J	┐	22	7	1			26.7	30
	23	O × S	│	6	7	2			60.0	15
	22	O × O	┘	9	12	2			60.9	23
	15	O × P	]				8	5	100.0	13
1/17/61	b) 37	O × E	┐	5	2				28.6	7
	35	O × A	│	7	3				30.0	10
	5	O × H	│	5	4	2			54.5	11
	50	O × C	┘	20	39	14			72.6	73
	21	O × Aln	┐		1	1	2	4	100.0	8
	32	O × As	┘					5	100.0	5
1/17/61	c) 1	H × H	]	6	13	5	2		76.9	26
	4	H × O	]	4	6	24	7		90.2	41
	11	H × J⊙				2			100.0	2
	9	H × P⊙						1	100.0	1
1/17/61	d) 36	J × J	┐	21					0	21
	28	J × H	┘ ┐	3					0	3
	26	J × O	┘	7	6				46.2	13
	17	J × P	]	1			1	4	83.3	6
1/17/61	e) 88	A × C	┐	14					0	14
	47	A × S	┘	31	1				3.1	32
	68	A × As	]	8	1		1	2	33.3	12
2/13/61	f) 44	J × J	]	22	1				4.3	23
	8	J × H	┐	24	9	2			31.4	35
	29	J × J	┘	30	17				36.2	47
	27	J × O	┐	11	10	1			50.0	22
	42	J × P	┘	2	2	3			71.4	7
2/13/61	g) 92	C × A	┐	13	8				38.1	21
	70	C × C	┘	3	8				72.7	11

Data are grouped according to female parent: a,b,c, etc. A key to species abbreviations is given in Table 1.

* Crosses within brackets were not significantly different (P=.05)

** 0 = no external evidence of embryo growth } No germination

1 = perceptible cracking of pericarp and hypocotyl extension up to 2/16 inch
2 = hypocotyl extension between 2/16 and 5/16 inch
3 = hypocotyl extension between 5/16 and 10/16 inch
4 = hypocotyl extension 10/16 inch and over
} Germination

⊙ Insufficient data for valid conclusions

C. ashei, *C. pumila*, and *C. alnifolia* tend to decrease the dormancy period when crossed with other species, and *C. ozarkensis* has a similar, but less pronounced effect when crossed with *C. henryi* and *C. crenata*. Conversely there is a tendency for *C. dentata*, when crossed onto *C. mollissima*, to increase the period of dormancy.

It is apparent that there is considerable variation in the dormancy requirement of different trees within a species, as seen for example by comparison of crosses f)29 and f)44 in Table 10. This is not unexpected since all the species, except a few in the Chinkapin group, have wide geographic ranges and consequently have existed under conditions allowing, or favoring, the development of variation. It is known that for the example cited the two pollen parents were from different geographic regions.

The results support the conclusion that the length of dormancy of a stratified nut in cold storage is a function of the two parents which contribute to the formation of the embryo. Dormancy in chestnuts, at least as determined in this experiment, must be controlled largely by the embryo rather than by the seed coat. The Chinkapin species (subgenus *Balanocastanon*) are, with one exception, endemic to southeastern United States and apparently have shorter dormancy requirements than the True chestnut species (subgenus *Castanea*) which are endemic to more northernly latitudes.

Dormancy should not be ignored in commercial nut production, because the value of nuts depends on good keeping quality, including resistance to early germination. Orchard growers in the southeastern United States should take care that their orchard trees, which are presently C. mollissima, are not cross-pollinated by any of the native Chinkapins. For the tree breeder and geneticist knowledge of specific xenia effects, such as the one reported, is helpful in rapidly determining if the intended cross was successful, since selfing, apomixis, and certain outcrosses can be eliminated. In the following section evidence of male-parent effect on dormancy is used to determine the success of certain interspecific crosses.

Summary

The inheritance of catkin length in two different, but related, interspecific crosses is presented. Over one-half of the F_1 progeny of the cross C. crenata × C. dentata had catkins longer than either parent, and in the cross C. mollissima × (C. crenata × C. dentata) a majority of the progeny had catkins shorter than either parent. The genetic basis of this inheritance pattern is discussed.

Data are presented to support the hypothesis that everbearing or continuous flowering, a characteristic of C. seguinii, is recessive and is controlled by two unlinked genes in crosses with certain C. mollissima trees.

The arguments in support of the natural occurrence of insect as well as wind pollination are reviewed and further observations are added. It is concluded that chestnuts are predominantly wind pollinated.

Information on pistillate receptivity in chestnut is reviewed, and it is concluded that the time of maximum receptivity is best judged with respect to the time the stigmas become fully expanded.

The experimental determination of the extent of self-compatibility has been confused by the possible occurrence of apomixis and outcrossing. Self-compatibility is not common but it does take place occasionally in some trees and is, perhaps, more common in certain species.

If apomixis occurs in chestnut, it is probably less common than self-fertilization. However, if the process of self-pollination triggers apomixis then the experimental distinction between the two events would be difficult.

Several male-sterile trees, many of them hybrids, have been studied. Anther abortion in the staminate flowers in most of these male-sterile trees occurs before meiosis. The same trees show no depression in female-fertility. It is suggested on the basis of limited data that male-sterility may be cytoplasmically inherited in some of the observed cases.

Chestnut pollen can be readily germinated in a .1-10% aqueous sucrose solution and at a temperature of 28-34 C. Nuts have been obtained from crosses with pollen stored at room temperature or at -15 C for a period of a few days to four weeks. Pollen stored at -15 C in the presence of a desiccating agent for one year showed 10% germination in vitro.

Evidence is presented for a new type of xenia in which the male parent influences the dormancy period of the nut in cold storage.

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INTERSPECIFIC CROSSES

Introduction

The number of interspecific crosses attempted in the genus Castanea is impressive. There is, however, little descriptive information on the progeny from these crosses, and no review dealing with interspecific hybridization in chestnut has been undertaken. Moreover, the possible occurrence of self-fertilization, apomixis, and outcrossing cast doubt on the success of certain interspecific crosses. In reporting crosses there unfortunately has been a tendency to state that a cross was successful simply on the basis of obtaining nuts, or perhaps seedlings, but without any analysis to confirm their hybrid origin.

This report is intended to summarize and supplement the information available in the literature on interspecific crosses and to serve as a basis for drawing conclusions about the crossability and relations of the several species in the genus. The question of species-relationships in the genus Castanea is not a new one, but in the past it has generally been assumed that the species are freely intercompatible (Graves, 1937b, 1949b; McKay, 1945; Clapper, 1954). This is interesting, if true, since representatives of the genus occur on three different continents and several of the species have been geographically isolated from each other for several million years.

This paper presents evidence which supports the hypothesis that at least weak genetic barriers have developed between certain species.

Progenies of several interspecific crosses were studied and the inheritance of specific characters is given to verify their hybrid origin. The evidence presented allows an estimate to be made of the amount of out-crossing, self-fertilization, and apomixis that occurred.

Crane (1937) made the following statement on the possibility of outcrossing and in reference to the commonly used pollinating technique: "This method is both tedious and impossible to control so that contamination from other pollen will be avoided." As stated earlier, though, by this author the most serious problem is contamination by pollen from the tree that is being pollinated. In any case, the possible occurrence of outcrossing, self-fertilization, and apomixis make it essential to verify the hybridity of progeny from interspecific crosses.

The interspecific hybrids dealt with in this section are primarily from first generation crosses. Second, third, and even fourth generation crosses have been reported in the literature or are listed on the records at the Connecticut Experiment Station, but the majority of these were within the subgenus Castanea and it was felt that little would be added by including them in the present study.

A separate literature review is included; however, complete and specific references to F_1 crosses will be found in the section on "Results."

Literature Review

Chestnut breeding began in the United States before the chestnut blight fungus, Endothia parasitica (Murr.) And., was discovered. In 1888 George W. Endicott began a search for an American chestnut tree, C. dentata, blooming early enough to supply pollen for use on the pistillate flowers of the "Japan Giant" variety of C. crenata. It was from such a cross in 1895 that Endicott produced what became known as the Boone variety (Crane, Reed, and Wood, 1937).

Detlefsen and Ruth (1922) gave a fairly detailed report on the F_1 and F_2 JA hybrids produced by Endicott. Only three first generation trees were grown but these reportedly showed hybrid vigor, i.e. rapid growth rate, flowering at an early age, and prolificness. Over 175 trees raised by Endicott from nuts obtained from the Boone tree showed a typical F_2 segregation: growth and size of the trees was uneven, the time of nut ripening showed wide variation, and the range in nut size was considerable with some of the segregates closely resembling the American and Japanese parents.

In 1894 Walter Van Fleet pollinated the pistillate flowers of the European chestnut, variety Paragon, with pollen of the native American chestnut (Van Fleet 1914, 1920). The seedlings were intermediate between the parents in vegetative and fruit characters. However, at about 14 years of age the trees were attacked, and shortly thereafter killed by the chestnut blight fungus. Between 1894 and 1921 Van Fleet made additional F_1 crosses utilizing several chestnut species. He stated that practically all the seedlings grown, over 200, showed unmistakable evidence of hybridity. The hybrid crosses with C. crenata were precocious, often blooming in their second year, whereas crosses involving C. dentata and C. sativa were generally susceptible to the blight. A detailed list of successful crosses and evidence of hybridity in the progeny was not presented. Nuts from the F_1 trees showed a rather low germination percentage and the seedlings were variable in their foliage and branching habit.

Blaringhem (1919), working in France, made reciprocal crosses between C. sativa and C. dentata. Though he does not mention seedlings, we can conclude from his observations of xenia, i.e. the effect of the male parent on nut size, that the cross of C. dentata ♀ × C. sativa was successful.

In 1954 Clapper summarized, for the period between 1925 and 1949, the results of the chestnut breeding program that had been conducted under the auspices of the United States Department of Agriculture in Beltsville, Maryland. He reported that 173 different interspecific combinations were completed during the 24 year period. Nuts from the numerous crosses were germinated in a greenhouse after stratification and then transplanted to a testing garden. The success of a cross and the number of hybrid plants was determined by the number of seedlings transplanted from the greenhouse to the test garden. These seedlings were presumably hybrids, but little or no evidence of hybridity of specific crosses was given. Over the 24-year period, 18,511 nuts were obtained from the crosses and germination was reported to be less than 60%. Unfortunately, Clapper gave no indication of the number of unsuccessful crosses, i.e. those which failed to produce nuts and those which produced nuts but did not germinate. Information on the direction of the cross, or whether reciprocal crosses were attempted and completed, was not given in most cases.

McKay and Crane (1938), also with the USDA in Beltsville, demonstrated in a study on the occurrence of xenia in chestnut the success of crosses between the species *C. mollissima*, *C. crenata*, and *C. sativa*.

The chestnut program at The Connecticut Agricultural Experiment Station stems directly from a project initiated by Arthur H. Graves in 1929 while at the Brooklyn Botanic Garden. Graves made his first crosses in 1930 between *C. crenata* and *C. dentata*. In the 30 years since the program was initiated, approximately 300 different interspecific combinations have been attempted and about 18,000 presumed hybrid nuts have been produced. Only a few of these hybrids have been described sufficiently in the literature to verify their hybrid origin. Among these are crosses of *C. mollissima* × *C. seguinii*, *C. crenata* × *C. dentata*, and *C. mollissima* × *C. dentata*.

Urquijo (1944), in Spain, has successfully completed crosses between *C. crenata* and *C. sativa* as evidenced by his morphological descriptions of the parents and progeny.

Pavari (1949) discussed briefly his efforts to establish a breeding program at Florence, Italy. Results of crosses completed at Florence were reported by Breviglieri (1951), but no evidence as to the hybrid nature of the resulting progeny was presented.

Schad *et al.* (1952, 1955) and Solignat (1958) have reported results of crosses attempted at Brive, France. In the four years from 1948 through 1951 approximately 1,130 presumptive hybrids were produced.

Most of these were crosses of C. crenata × C. sativa. Data on blight resistance and hardiness of the progeny were given, but distinctive evidence regarding their hybrid nature was not presented.

Other references to chestnut hybridization do not give information on the crosses attempted and hybrids produced (Krstic, 1955; and Hershey, 1937).

Materials and Methods

Controlled Pollinations

The method I used to make pollinations was very similar to the technique described by Graves (1937b) and Clapper (1954). Female flowers were prepared for bagging shortly before the stigmas of the pistillate flowers started to spread and before anthesis of the staminate flowers. All the staminate catkins and the male flowers of the bisexual catkins were removed. Then the branch shoots with the pistillate flowers were enclosed with Kraft "Pollen-Tector #1250" paper bags. The mouth of the bag was secured about the branch with a piece of copper wire, which was given only a single twist so that it might be easily removed and replaced. When the pistillate flowers were receptive the bags were removed one at a time, and for as short a period as possible, and a male catkin was brushed lightly over the stigmatic surface. Catkins were taken from previously bagged trees to lessen the possibility of contamination and to obtain anthers from which the pollen had not been shaken or eaten by insects. Because fresh chestnut pollen cakes readily, it was not practical to extract it as is commonly done with other trees. Stored pollen was employed occasionally when the flowering of the two species did not overlap sufficiently to use fresh pollen. The storage technique is described in the preceding chapter.

The pollinating process formerly was repeated two or three times at two day intervals (Graves, 1937b). But the few additional resulting nuts neither warranted the extra time required to repeat the pollinations nor the risk of contamination which increases with each successive pollination. Hence the practice of repeat pollinations was, for the most part, not practiced during the 1959 and 1960 pollinating seasons.

Identification tags were placed on the branches at pollination time and approximately three weeks later, after the pistillate flowers were no longer receptive, the paper bags were removed. Before the burs ripened coarse mesh cloth bags were placed over the branches so that the nuts would not be misidentified or drop to the ground and be lost to squirrels. When the burs started to dehisce the nuts were harvested and placed in cold storage.

Nuts with long dormancy requirements were generally stratified at temperatures of about 35 F until late in March when they could be planted outside in seed beds. Nuts which were found to germinate at cold temperatures and after a short period of stratification were started in the greenhouse.

To verify hybrid origins and check for male parent characters the trees were examined in the field and leaf samples or twigs were removed from each plant for examination in the laboratory.

Results
Including a Review of Interspecific Crosses

A summary of first generation interspecific crosses within the genus *Castanea* is reported. With the exception of a single cross between *C. paucispina* and *C. mollissima*, the species *C. davidii* of the subgenus *Castanea* and *C. paucispina* of the subgenus *Balanocastanon* are omitted, because to date these two species have not been used in chestnut breeding programs. The crosses are divided into five groups according to whether they are within a subgenus or between subgenera: 1) Interspecific crosses within the *Castanea*, or True-chestnut subgenus, 2) Interspecific crosses within the *Balanocastanon*, or Chinkapin subgenus, 3) Crosses between the species within the *Castanea* and *Balanocastanon* subgenera, 4) Crosses between the species of the subgenus *Castanea* and the single species in the *Hypocastanon* section, and 5) Crosses between the *Hypocastanon* and *Balanocastanon* species. With the exception of several crosses within the subgenus *Castanea* the crosses are listed in tabular form.

For each interspecific cross a summary is presented of what is known about the cross as determined from reports in the literature. Following this is a resumé of information obtained from observations and experiments by this author, as well as from unpublished records of his predecessors at the Connecticut Station. Where possible, presumptive hybrids were examined for the presence of characters from the male parent. For the most part morphological characters were used: pubescence of the stem and leaf; the type of hairs and glands and their distribution; the angle or shape of the leaf apex and lamina base; twig color; bud shape; ratio of length to width of leaf;

and serration and venation of the leaf.

A capital "DU" indicates that the direction of the cross is not known. Figure 50 is a summary chart of the F_1 crosses attempted or completed in the genus Castanea.

INTERSPECIFIC CROSSES WITHIN THE SUBGENUS CASTANEA

C. mollissima × C. dentata: Graves, 1935, 1949c; Clapper, 1943, 1952, 1954.

From the description of the progeny given in the literature for these crosses there is little doubt of their hybrid nature. Some of the hybrids from a cross made by Clapper were fast growing, straight bolled trees which reportedly had hybrid vigor; however, they did canker from attacks of the blight fungus. Other similar crosses yielded progeny of inferior form and poor growth rate.

The Chinese × American hybrids are intermediate in resistance between the two parents and attain a larger size than C. dentata when exposed to the chestnut blight, but, like the American parent, they eventually succumb to the disease. Male-sterility has been observed among these hybrids but apparently occurs only in certain families (see Table 8; Figure 45 illustrates fertile and sterile flowers). Clapper (1954) reported an anomaly in seedlings of one cross which he described as "cracked bark" and attributed it to a mutation. This trait is similar to one I have observed in seedlings of C. sativa × C. mollissima and interpreted as a hybrid weakness.

At the Connecticut Station 59 crosses have been attempted;

39 produced one or more nuts and yielded a total of 405 seeds. Thirteen seedlings derived from four crosses in 1959 and examined at the end of the first year's growth, all resembled the female parent in the morphological characters studied. Whether these were outcrosses or true hybrids that would show intermediate characters when mature could not be determined. Older hybrids derived from similar crosses are clearly intermediate between the two parents in leaf shape, dentation, and pubescence.

C. dentata × C. mollissima: Graves, 1935.

Twenty-one crosses have been attempted with 16 of them yielding one or more nuts for a total yield of 210 nuts. Only four F_1 trees were available for observation but they appeared similar to the hybrid trees of the reciprocal cross described above; however, three of the four trees are male-sterile.

C. mollissima × C. crenata: Van Fleet, 1914, 1920, DU; McKay and Crane, 1938; Graves, 1937a; Clapper, 1943, 1954, DU.

Clapper noted that progeny from this cross did not show the hybrid vigor reported characteristic of hybrids of C. dentata × C. mollissima and C. dentata × C. crenata. And these same Chinese × Japanese hybrids were no more resistant or susceptible to the blight fungus than either parent, which may be interpreted to mean that the control of resistance is not genetically different and independent in the two species.

At the Connecticut Station nine pollinations of this cross were attempted, seven of which yielded nuts for a total of 40 presumed hybrid seeds. Thirteen one-year-old seedlings were available for study and six of these had small, mottled leaves indicative of abnormal chlorophyll production. Of the seven seedlings still alive the second year all appeared to be hybrids. They were intermediate to the parents for the following characteristics: number of hairs on the underside of the leaf, length of hairs on the petiole, stem color, number of secondary veins in the leaves, and leaf dentation. The expression of lepidote glands, which are present on the underside of leaves in the male parent species, is suppressed in the hybrids. Glands are present, but they are waxy, amorphous dots much like those that occasionally occur on the leaves of the Chinese parent. (See Figure 44).

<u>C. crenata × C. mollissima</u>: Graves, 1938a; McKay and Crane, 1938; Breviglieri, 1951; Schad *et al.*, 1952, 1955.

Since 1938 sixteen crosses have been attempted at the Station of which 12 have yielded nuts for a total of just over 200 seeds. All but two of 48 trees examined appeared to be hybrids. Morphological characters of the trees were similar to those described for the reciprocal interspecific cross.

In a family of 20 seedlings four had mottled, abnormal leaves much like those described for the reciprocal cross. However, this trait did not appear in 24 seedlings of another cross made the same year involving different parental trees of the same species.

With true reciprocal crosses, barring cytoplasmic inheritance, one normally expects the progeny of both crosses to show the same traits. However, if the reciprocal refers only to a reversal in the *species* of trees used as parents and not to the *particular trees*, then differences in the progeny can be expected. The two crosses reported above illustrate the point: one was a reciprocal between the trees used in the *C. mollissima* × *C. crenata* cross which had progeny with abnormal leaves. Progeny of the true reciprocal also had abnormal leaves. However, another cross of *C. crenata* × *C. mollissima* between different trees resulted in normal seedlings. Differences of this sort will be noted in other crosses.

C. mollissima × C. sativa: Clapper, 1954, DU.

Seven crosses were made and 44 nuts obtained. Four trees were available from two crosses completed in 1951 and 1952. These showed no evidence of the male parent characters.

C. sativa × C. mollissima: McKay and Crane, 1938; Breviglieri, 1951; Schad *et al*., 1952, 1955; Graves, 1956.

At the Station 13 crosses were attempted; 11 produced one or more nuts, and yielded a total of 294 seeds. Seven ten-year-old trees were examined and all appeared to be hybrids. Pubescence of the twig, leaf, and bud scales all tended towards the type of the staminate parent, *C. mollissima*. The fall twig color is intermediate between the purple of the pistillate parent and yellow tendency of the male parent. At least two of these seven trees are male-sterile and two male-fertile; four of the trees have been attacked by the chestnut blight; and, in addition, three have thick, corky, mottled, checked bark which is not characteristic of the parents (see Figure 46).

Thirty-nine two-year-old seedlings examined appeared to be hybrids and were intermediate for the taxonomic characters mentioned above, except that they had leaf bases which were more similar to the staminate parent. Eleven of these 39 seedlings had small, crinkled, mottled leaves — characteristics which might be interpreted as being symptomatic of an incompatibility between the parental species. One of these 11 phenotypically abnormal seedlings was further characterized by very contracted internodes. (See Figure 47)

C. crenata × C. dentata: Graves, 1949c; Clapper, 1954, DU.

This cross was first completed in 1895 by Endicott. The description of the first and second generation trees raised by Endicott leaves little doubt that most were true hybrids (Detlefsen and Ruth, 1922). Graves (1931) made his first chestnut pollination in 1930 using these two species. Since that time 81 pollinations have been attempted at the Station; 52 have produced nuts with the total nut production being 831.

There has been no indication of total blight resistance among the hybrids. They are more resistant to the blight fungus than the C. dentata parent, as was noted for the C. mollissima × C. dentata crosses, but they eventually succumb to the disease, a characteristic which in itself is very strong evidence for the success of the intended cross. The hybrids often have catkins much longer than either parent (see the preceding chapter). Another common feature is that of the yellowing of the foliage, particularly as the summer progresses. This may be due to the presence of aphids, Callaphis castanea, on the leaves, a pest which as Graves noted (1949a) selectively attacks only one of the parental species, namely, C. dentata. However,

Figure 44 a) *C. crenata.* Lepidote gland on the underside of the leaf. Notice the structure and arrangement of the cells in forming the doughnut like structure. Approx. ×200.

b) Same material as "a" but approx. ×100.

c) *C. crenata* × *C. mollissima.* Underside of leaf. Note lack of distinct lepidote glands. Smaller glands are present which are much like amorphous waxy dots. Approx. ×100.

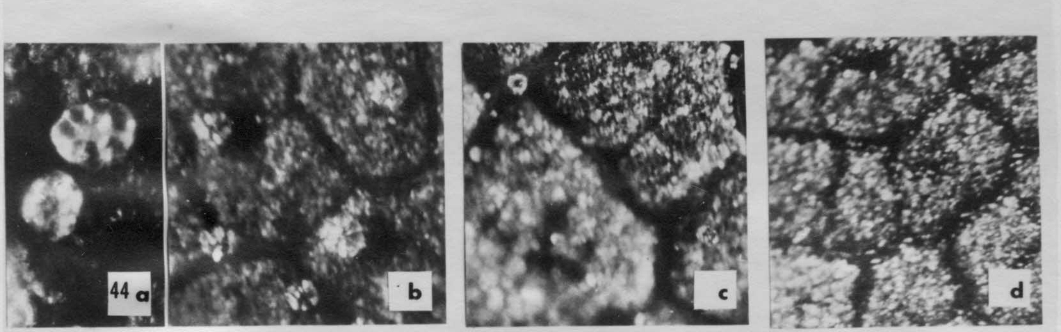
d) *C. mollissima.* Underside of leaf. No glands present. Approx. ×100.

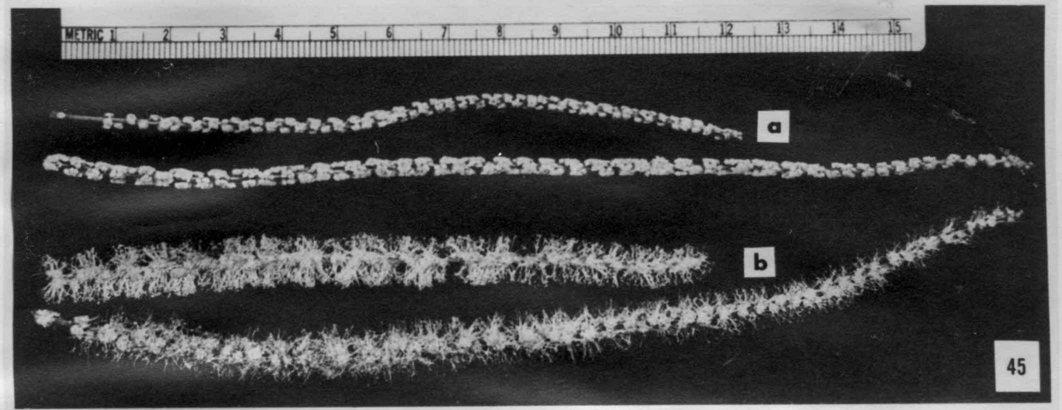
45 a) Two catkins bearing typical male-sterile flowers in which the anthers have aborted. The stage of development of catkins labeled "a" is comparable to catkins labeled "b".

b) Two catkins bearing typical male-fertile flowers. The flowers are open and the stamens are at the stage of shedding pollen.

46 a) A twig and small branch of *C. sativa* × *C. mollissima* which demonstrates the cracked, corky, and mottled bark that is common to some of these hybrids.

b) A twig and small branch of *C. sativa* × *C. mollissima* which demonstrates the normal appearance of chestnut bark.


44 a
b
c
d


METRIC 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
a
b
45

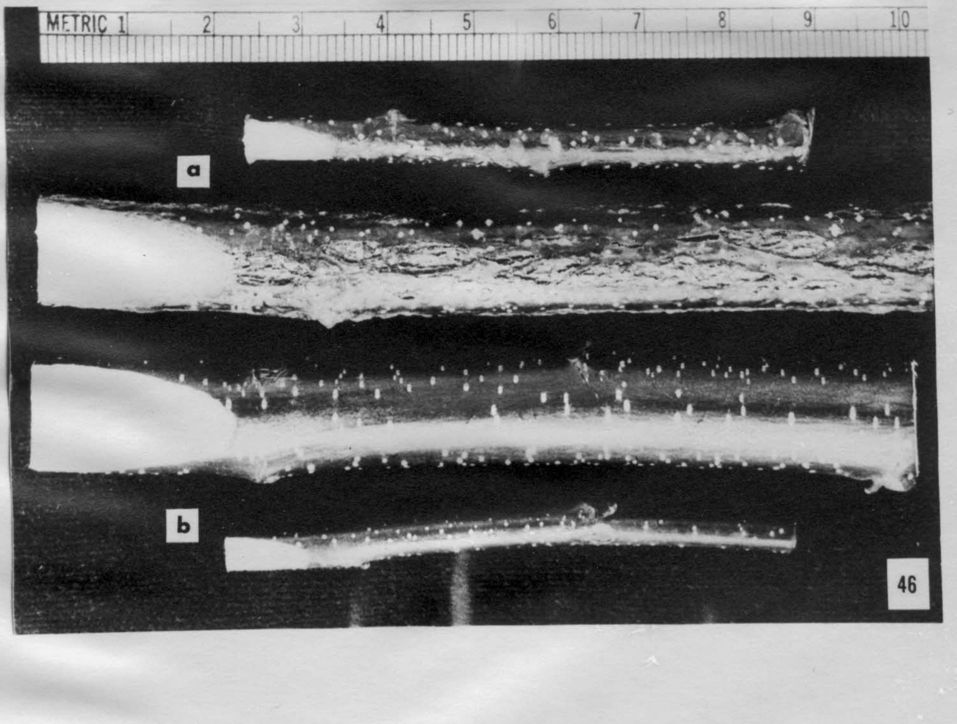

METRIC 1 2 3 4 5 6 7 8 9 10
a
b
46

Figure 47 a) Leaf from a normal two year old *C. sativa* × *C. mollissima* hybrid.

b) Abnormal leaf from a sibling of the tree described for "a", showing reduced size, mottled chlorophyll pigmentation, and undulating lamina with depressed lateral veins.

c) Twig from a sibling of "a" and "b" showing the abnormal characters mentioned above plus an extreme reduction in internode length.

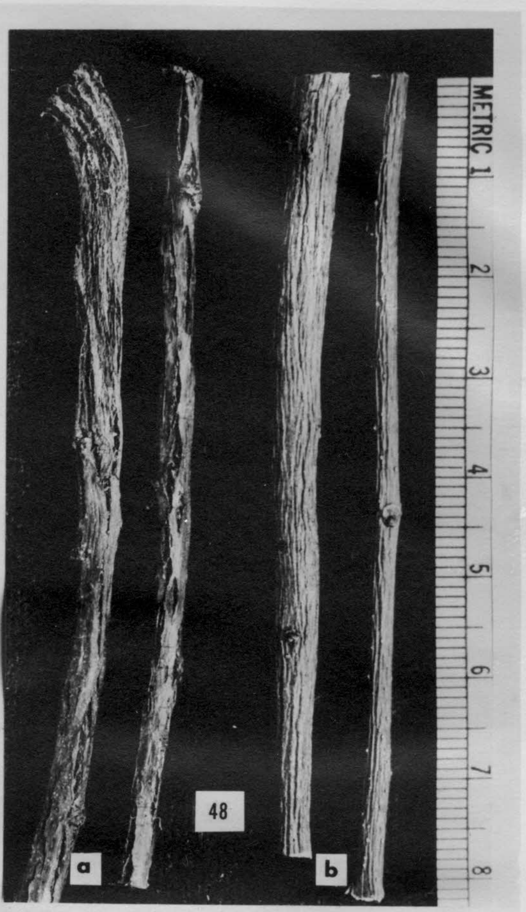
48 a) Two stems from one year old hybrid seedlings of *C. dentata* × *C. crenata* showing the deformity referred to in the text as cracked-bark.

b) Two stems from normal one year old seedlings. The wrinkles in the bark are due to desiccation previous to photographing.

49 Typical leaves of *C. ozarkensis*, "O"; *C. henryi*, "H"; and *C. ozarkansis* × *C. henryi* "O" × "H". Notice the difference in the leaf margins and the ratio of length to width.

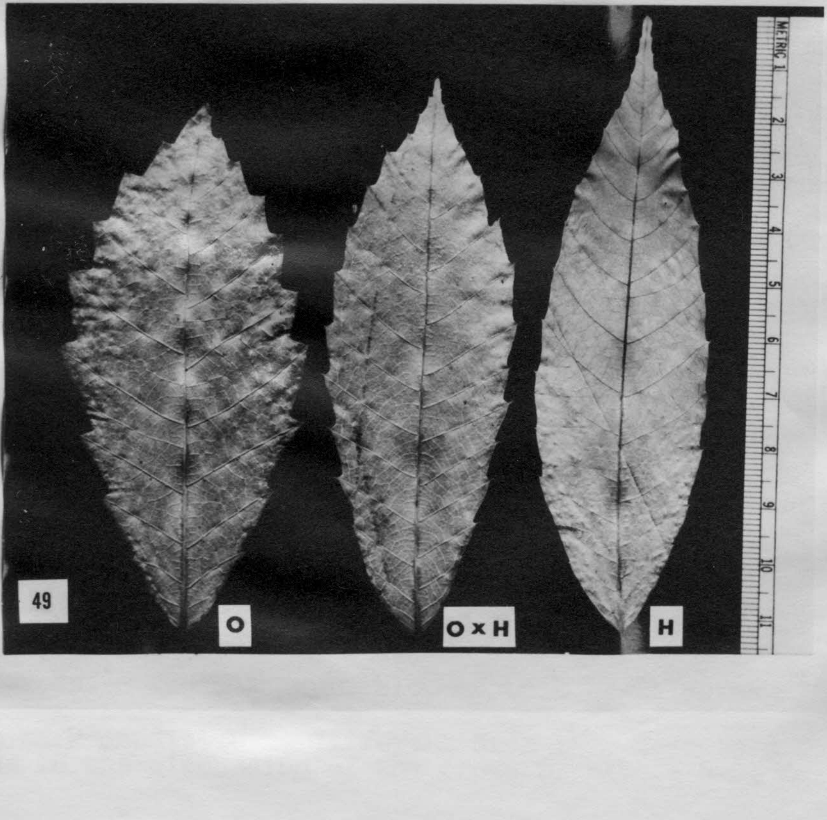
47

a
b
c
METRIC 1
2
3
4
5
6
7
8
9
10
11
12
13



48

a
b
METRIC 1
2
3
4
5
6
7
8



49

O
O×H
H
METRIC 1
2
3
4
5
6
7
8
9
10
11

the aphids generally do not cause as extensive yellowing on the American chestnut as on the hybrid. One male-sterile tree of this interspecific combination is known (Table 8). Clapper (1943) and Graves (1935, 1942) reported that the Japanese × American trees show hybrid vigor in respect to growth rate, but quantitative studies are lacking.

C. dentata × C. crenata: Graves, 1934.

Until 1958 four crosses had been attempted at the Station; two of the crosses set six nuts and two set no nuts.

In 1958 this author utilized pollen from a C. crenata tree in Cheshire, Connecticut, to pollinate pistillate flowers on sprouts of four different C. dentata trees in Roxbury, Connecticut. One-hundred and sixty-five nuts were harvested and of 105 seedlings examined after one and two years growth all but one appeared to be true hybrids. The exceptional seedling possessed no traits of the tree used as the pollen parent in the cross. As with the reciprocal hybrids the trees were often affected by aphids. Multicellular, lepidote glands, which literally cover the underside of the leaves of the pistillate parent and are a key character of C. crenata, were not present in two year old hybrid seedlings. The only glands present were numerous small, amorphous, waxy dots which are common to young leaves of the American parent.

More pertinent to the question of species relationships was the observation of "cracked bark" among several of the seedlings of this cross.[1] Typical expression of this trait on an affected

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[1] This bark condition is different from that described for older trees in the discussion of the cross C. sativa × C. mollissima.

stem of a seedling can be seen in Figure 48. The cracking appeared not only on the stem but on the roots as well. Superficially the fissures resemble frost cracks, but it was noted in early spring after one year's growth that the cracks did not open down to the wood, and callus had formed around the edges of the cracked areas. When the hybrids were examined near the end of the second growing season several of the trees were observed to have cracked bark on the current year's growth. The abnormal condition occurred in 12 to 42% of the progeny of all four crosses. The observations on this trait are tabulated in Table 11.

Table 11. CRACKED BARK IN *C. DENTATA* × *C. CRENATA* HYBRIDS

	Observations					
	End of 1 year's growth			End of 2 year's growth		
Pollination No.	Total No. Plants	No. Cracked Bark	% Cracked Bark	Total No. Plants	No. Cracked Bark	% Cracked Bark
55-58	34	8	24	24	9	38
56-58	26	11	42	16	2	12
57-58				7	1	14
58-58	___	___	___	35	8	23
Total	60	19	(Ave.) 32	82	20	(Ave.) 24

No cracked bark individuals were observed in 73 open-pollinated seedlings of the American parent or in 15 open-pollinated seedlings of the staminate parent. There is good reason to believe that these seedlings grown from open-pollinated nuts were the result of <u>intra</u>-specific crosses, because each of the parent species is isolated from other chestnut trees, except those of the same species, by at least a quarter of a mile.

The cracked bark condition is semi-lethal and whether a tree with this trait could attain a large size is questionable. Most of the seedlings observed with this trait at the end of the first year either died over the winter or were killed back to ground level. However, the presence of this trait did not appear to affect the first year's growth.

<u>C. sativa × C. crenata</u>: Urquijo, 1944, DU; Breviglieri, 1951; Clapper, 1954, DU; Schad <u>et al</u>., 1952, 1955, DU.

Two crosses were attempted at the Station and 39 nuts were obtained. Nine seedlings from one pollination were studied. Six of these seedlings appeared to be hybrids in that they had scattered lepidote glands on the underside of the leaves, a trait which is characteristic of the staminate parent. It was questionable if three of the seedlings were hybrids for they did not possess the lepidote glands not any other distinct traits of the pollen parent.

<u>C. crenata × C. sativa</u>: Breviglieri, 1951

Almeida (1947) reported evidence for the natural occurrence of this F_1 hybrid, DU. He cites not only morphological characters but also cytological characters. He observed abnormal reduction figures in the presumptive hybrid. At the Station two crosses were attempted and only two nuts were obtained from these. No seedlings were available for analysis.

C. sativa × C. dentata: Blaringhem, 1919.

Walter Van Fleet (1914, 1920) successfully completed this cross in 1894 and reported the characters of the progeny to be intermediate between those of the parents. The trees came into bearing at about 12 years of age but were killed back to the ground by the chestnut blight two or three years later. The writer made two crosses between these species in 1960 and obtained 60 nuts.

C. dentata × C. sativa:

Blaringhem (1919) observed the effect of the *C. sativa* pollen in increasing nut size of *C. dentata*. He made no report on growing the progeny, but his observation of xenia is evidence that the cross was successful. The cross was tried unsuccessfully by this author in 1960.

The summary of first generation interspecific crosses is continued in Tables 12-16.

Female* parent	Male* parent	References	No. of crosses, CAES only	Nuts or seedlings obtained	Verified as hybrid	Comments
Seguinii	Mollissima	Graves, 1938a		+	-	The leaf and twig characters are not as distinctive traits between the parents as are the physiological responses of flowering time, growth habit, winter hardiness, and chestnut blight resistance. However, one leaf character that does distinguish the Seguin from the Chinese is the presence in the former of many lepidote glands on the underside of the leaf. The Seguin, in contrast to the Chinese, is typically dwarf, not winter hardy in Connecticut, blight susceptible, and everbearing. Eight presumptive hybrids were studied and to varying degrees all tended to have the dwarfing habit, susceptibility to the blight, and winter injury. With one exception all were normal flowering. The lepidote glands, as with the C. mollissima × C. crenata cross, were not evident in the F_1 hybrids. Nut size is intermediate in the F_1, and in the F_2 segregation is highly variable, but there is a bias towards the small sized nut of the original staminate parent. As indicated in the chapter on "Flowering and Related Phenomena" the continuous flowering trait of C. seguinii appears to be controlled by two independent genes which are recessive in crosses with C. mollissima.
		Clapper, 1954, DU		+	-	
		CAES	13	31 nuts	-	
	Reciprocal	Graves, 1935		+	-	
		" & Nienstaedt, 1953		+	+	
		CAES	14	8 seedlings	+	
Seguinii	Crenata	Clapper, 1954, DU		+	-	
		CAES	3	11 nuts	-	
	Reciprocal	Breviglieri, 1951		+	-	
		Graves, 1936		+	-	
		CAES	3	21 nuts	-	
Seguinii	Sativa	Graves, 1938b		+	-	
		CAES	3	3 nuts	-	
	Reciprocal	CAES	1	36 nuts	-	
Seguinii	Dentata	Graves, 1938b		+	-	
		Clapper, 1943,1954,DU		+	-	
		CAES	9	9 nuts	-	
	Reciprocal	CAES	1	33 nuts	-	

* Female and male parent respectively except where a reciprocal cross is indicated
DU Direction of cross not known
CAES Connecticut Agricultural Experiment Station; records go back to project started by A. H. Graves in 1930
\+ Yes
\- No

Table 13. INTERSPECIFIC CROSSES WITHIN THE SUBGENUS BALANOCASTANON

Female* parent	Male* parent	References	No. of crosses, CAES only	Nuts or seedlings obtained	Verified as hybrid	Comments
Ashei	Pumila	CAES	1	12 nuts	-	Of the 30 possible crosses between the six species in the subgenus Balanocastanon, which includes reciprocals, only one has been reported in the literature. I have attempted to complete several crosses within this group in the past two years; however, it is too soon to analyze the resulting progeny for their hybridity. Only interspecific combinations attempted are listed.
Ashei	Alnifolia	CAES	3	17 nuts	-	
Ashei	Ozarkensis	CAES	1	21 nuts	-	
	Reciprocal	CAES	1	5 nuts	-	
Ozarkensis	Pumila	Graves & Jaynes, 1959		+	-	
		CAES	2	17 nuts	-	
	Reciprocal	CAES	2	14 nuts	-	
Ozarkensis	Alnifolia	CAES	1	10 nuts	-	
	Reciprocal	CAES	1	14 nuts	-	
Alnifolia	Pumila	CAES	1	1 nut	-	
	Reciprocal	CAES	1	0		

*For key to symbols and abbreviations see Table 12.

Table 14. INTERSPECIFIC CROSSES BETWEEN THE SUBGENERA *CASTANEA* AND *BALANOCASTANON*

Female* parent	Male* parent	References	No. of crosses, CAES only	Nuts or seedlings obtained	Verified as hybrid	Comments
Seguinii	Pumila	Graves, 1938b		+	-	
		Clapper, 1954,DU		+	-	
		CAES	3	9 nuts	-	
	Reciprocal	CAES	1	0		
Seguinii	Ashei	CAES	1	0		
	Reciprocal	CAES	2	0		
Seguinii	Floridana Reciprocal	Clapper, 1954, DU		+	-	
Seguinii	Ozarkensis	Clapper, 1954, DU		+	-	
		CAES	2	0		
	Reciprocal	CAES	1	16 nuts	-	
Seguinii	Alnifolia	Clapper, 1954, DU		+	-	
		CAES	1	0		
	Reciprocal	CAES	1	0		
Crenata	Pumila	Graves, 1941		+	-	Two crosses attempted in 1960 yielded no fruit. Yet, two other crosses, in which the same two pistillate trees were pollinated on the same day with pollen of another *C. crenata* tree and a *C. mollissima* tree, made good sets of fruit. The *C. pumila* pollen was presumably good; *in vitro* germination at pollination time was above 20%. The effect of the male parent on dormancy (see Table 10, xenia) verifies that this interspecific cross can be completed, at least to the point of forming a hybrid embryo. It is suggested that a partial incompatibility with respect to pollination or fertilization exists between certain *C. crenata* and *C. pumila* trees.
		Breviglieri, 1951		+	-	
		Clapper, 1954, DU		+	-	
		CAES	6	26 nuts	-	
	Reciprocal	Van Fleet,1914,1920		+	-	
		CAES	5	2 nuts	-	
Crenata	Ashei Reciprocal	CAES	1	13 nuts	-	

See Table 12 for key to symbols and abbreviations.

Table 14 continued.

Female* parent	Male* parent	References	No. of crosses, CAES only	Nuts or seedlings obtained	Verified as hybrid	Comments
Crenata	Floridana	Graves, 1941		+	-	This one tree appeared to be a hybrid; like the pollen parent, it had many small glands and stellate hairs on the underside of the leaves and was susceptible to the blight. It lacked the lepidote glands which are typical of the pistillate parent.
		Clapper, 1954, DU		+	-	
		CAES	1	1 seedling	+	
	Reciprocal					
Crenata	Ozarkensis	Clapper, 1954, DU		+	-	There is evidence of reduced seed set. One pollination was on a tree which also had been crossed by *C. crenata*. The number of fruit set over the number of flowers pollinated was 56% for the cross between the two Japanese trees but only 17% for the Japanese × Ozark cross. The effect of the male parent on dormancy (see Table 10, xenia) verifies that this interspecific cross can be completed, at least to the point of forming a hybrid embryo.
		CAES	2	42 nuts	-	
	Reciprocal	CAES	1	31 nuts	-	
Crenata	Alnifolia	Van Fleet,1914,1920		+	-	Of six different pollinations made on the same *C. crenata* tree on the same date using pollen of *C. henryi*, *C. ozarkensis*, *C. crenata*, *C. pumila*, and *C. alnifolia*, only the cross with pollen of the latter species resulted in no fruit. The *C. alnifolia* pollen was apparently good because fruit was produced when it was used on a *C. ozarkensis* tree.
		CAES	3	0		
	Reciprocal	Clapper, 1954, DU		+	-	

For key to symbols and abbreviations see Table 12.

Table 14 continued.

Female* parent	Male* parent	References	No. of crosses, CAES only	Nuts or seedlings obtained	Verified as hybrid	Comments
Mollissima	Pumila	Graves, 1940b		+	-	There is evidence of reduced seed set. A cross of *C. mollissima* by *C. mollissima* resulted in 19 nuts. The pistillate parent of this cross was also pollinated on the same date by *C. pumila*. There were no nuts obtained from the latter cross even though a greater number of pistillate flowers were pollinated.
		Clapper, 1954, DU		+	-	
		CAES	3	1 seedling	-	
	Reciprocal	Van Fleet,1920,DU		+	-	
Mollissima	Ashei	Clapper, 1954, DU		+	-	It is indicated by the fact that no nuts were obtained from three crosses that there may be an incompatibility between certain trees of these two species.
		CAES	3	0		
	Reciprocal	CAES	1	0		
Mollissima	Floridana	Clapper, 1954, DU		+	-	There is evidence of reduced nut set. The pistillate tree which was crossed by *C. ozarkensis* was also pollinated on the same date by *C. mollissima*. The intraspecific cross between the two Chinese trees yielded 19 nuts whereas the cross between the Chinese and Ozark trees, which belong to different subgenera, produced only one nut. More pistillate flowers were utilized in the latter cross, and the Ozark pollen gave satisfactory nut set when used in another cross.
	Reciprocal					
Mollissima	Ozarkensis	Clapper, 1954, DU		+	-	
		CAES	1	1 nut	-	
	Reciprocal	CAES	2	88 nuts	-	
Mollissima	Alnifolia	Van Fleet,1920,DU		+	-	
		Clapper, 1954, DU		+	-	
		CAES	1	0		
	Reciprocal					
Mollissima	Paucispina	Clapper,1954, DU		+	-	This is the only report in the literature of someone using the species *C. paucispina*.
Dentata	Pumila	Clapper, 1954, DU		+	-	
		CAES	1	0		
	Reciprocal	Van Fleet, 1920		+	-	

For key to symbols and abbreviations see Table 12.

Table 14 continued.

Female* parent	Male* parent	References	No. of crosses, CAES only	Nuts or seedlings obtained	Verified as hybrid	Comments
Dentata	Ashei	CAES	2	12 nuts	-	
	Reciprocal	CAES	1	36 nuts	-	
Dentata	Floridana	Clapper, 1954 DU		+	-	
	Reciprocal					
Dentata	Ozarkensis	CAES	1	9 nuts	-	
	Reciprocal	CAES	1	10 nuts	-	
Dentata	Alnifolia	CAES	1	0		
	Reciprocal					
Sativa	Pumila	CAES	2	1 nut	-	There is evidence of reduced nut set. A cross of *C. sativa* × *C. dentata* yielded 25 nuts. The pistillate parent of this cross was also pollinated on the same date by *C. pumila*. Only one nut was obtained from the latter cross even though about the same number of pistillate flowers were pollinated in both crosses.
	Reciprocal	Van Fleet,1914,1920		+	-	
Sativa	Ashei	CAES	1	0		Four different pollinations were made on the same *C. sativa* tree with pollen of *C. sativa*, *C. crenata*, *C. dentata*, and *C. ashei*. Pollen of the latter species, the only species not in the subgenus *Castanea*, was the only one ineffective in producing fruit. More pistillate flowers were pollinated in the *C. ashei* cross, and the Ashe pollen was effective on the same date in a cross with *C. ozarkensis*. Thus, there is evidence for at least reduced nut set between *C. sativa* and *C. ashei*.
	Reciprocal					
Sativa	Floridana					
	Reciprocal					

For key to symbols and abbreviations see Table 12.

Table 14 continued.

Female* parent	Male* parent	References	No. of crosses, CAES only	Nuts or seedlings obtained	Verified as hybrid	Comments
Sativa	Ozarkensis	Graves & Jaynes,1959		+	-	The seedling was weak and died the first year. There is evidence of reduced nut set. One cross of *C. sativa* × *C. ozarkensis* set no nuts, whereas another cross on the same *C. sativa* tree made on the same date set 25 nuts. Pollen of the Ozark tree was apparently good because it was used successfully in other crosses.
		CAES	2	1 seedling	-	
	Reciprocal	CAES	1	8 nuts	-	
Sativa	Alnifolia	Graves & Jaynes, 1959		+	-	The seedling appeared to be a hybrid in many respects. It varied from the pistillate towards the staminate parent in the following ways: teeth on the edge of the leaves were not as acuminate nor as forward slanting; secondary veins were fewer in number; the leaf tip was not as acuminate nor was the leaf base as cordate; and the stem, leaf, and bud pubescence was greater. One cross of *C. sativa* and *C. alnifolia* produced no nuts whereas the same *C. sativa* pistillate parent when pollinated with pollen of *C. crenata* on the same date produced 25 nuts. There was no reason to believe that pollen of the *C. alnifolia* tree was nonfunctional since it was used in other crosses successfully. The evidence indicates a reduced nut set.
		CAES	2	1 seedling	+	
	Reciprocal					

For key to symbols and abbreviations see Table 12.

Table 15. INTERSPECIFIC CROSSES BETWEEN THE SUBGENERA *HYPOCASTANON* AND *CASTANEA*

Female* parent	Male* parent	References	No. of crosses, CAES only	Nuts or seedlings obtained	Verified as hybrid	Comments
Henryi	Mollissima	Graves, 1950		+	-	Graves (1956), on the basis of unsuccessful pollinations in two successive years, concluded there was an incompatibility between these two species.
		CAES	5	15 nuts	-	
	Reciprocal	Clapper, 1954, DU		+	-	
		CAES	3	2 seedlings	+	Only one seedling appeared to be a hybrid. This seedling varied from the pistillate, Chinese parent towards that of the staminate, Henry parent by the following characters: dentation of the leaf edge was much less pronounced, the angle of the leaf apex was more acuminate as was the leaf base, the leaves had less pubescence, and the ratio of the length to width of the leaves was greater.
Henryi	Sativa	CAES	1	7 nuts	-	
	Reciprocal	Graves & Jaynes, 1959		+	-	
		CAES	2	1 seedling	+	The one seedling deviated from the European, pistillate parent towards the staminate parent by the following characters: pubescence was less on the petiole, veins, and buds; fewer glandular dots were on the underside of the leaf; and the leaf base was more acuminate. Thus it appears the cross was successful. However, the nut set was very poor in comparison to another cross of the same pistillate parent with pollen of *C. dentata* carried out on the same date. The pollen appeared functional, for satisfactory nut set was obtained when pollen of the *C. dentata* tree was used on another species.

For key to symbols and abbreviations see Table 12.

Table 15 continued.

Female* parent	Male* parent	References	No. of crosses, CAES only	Nuts or seedlings obtained	Verified as hybrid	Comments
Henryi	Crenata	Clapper, 1954, DU		+	-	The six seedlings studied all appeared to be hybrids. They varied from the Japanese parent by several characters: leaf apicies and leaf bases were more acuminate, very few lepidote glands were present, leaf petioles were longer, and the ratio of length to width of the leaf was greater. Two crosses were made on the same date using a single *C. crenata* tree as pistillate parent, and as staminate parents a *C. henryi* tree and a *C. crenata* tree. Fruit set with respect to the number of flowers pollinated was 56% for the cross between the two Japanese trees but only 10% for the Japanese × Henry cross. Pollen of both parents was fresh and presumably good.
		CAES	4	10 nuts	-	
	Reciprocal	Graves & Jaynes,1959		+	-	
		CAES	4	6 seedlings	+	
Henryi	Dentata	Clapper, 1954, DU		+	-	The lack of nut set from six different crosses is evidence of an incompatibility or at least a highly reduced nut set between the two species.
		CAES	6	0		
	Reciprocal	CAES	1	2	-	
Henryi	Seguinii	Clapper, 1954, DU		+	-	
		CAES	1	0		
	Reciprocal	CAES	1	0		

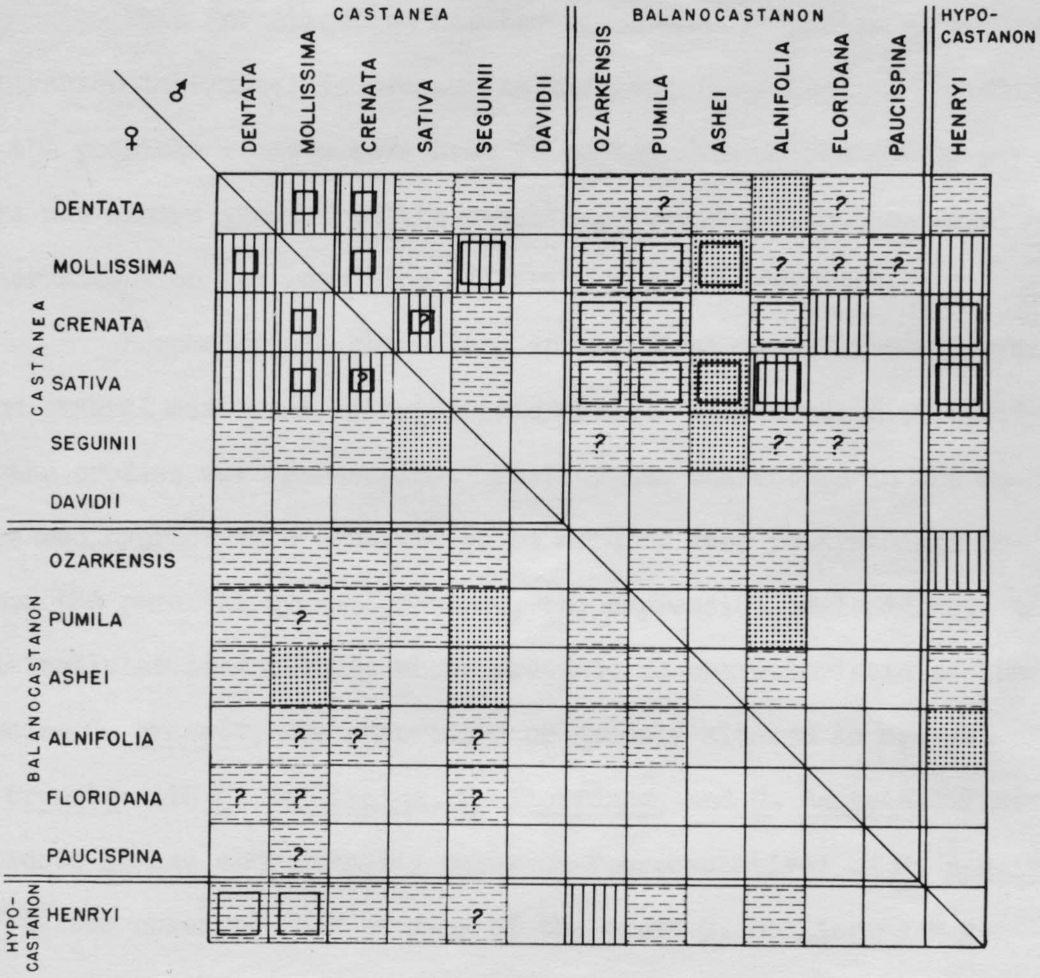
For key to symbols and abbreviations see Table 12.

Table 16. INTERSPECIFIC CROSSES BETWEEN THE SUBGENERA *HYPOCASTANON* AND *BALANOCASTANON*

Female* parent	Male* parent	References	No. of crosses, CAES only	Nuts or seedlings obtained	Verified as hybrid	Comments
Henryi	Pumila	Clapper, 1954,DU		+	-	Graves (1956) reported that one seedling grown from a nut of this cross was an albino. There appears to be a very reduced nut set;however, a positive statement to this effect cannot be made without appropriate control pollinations because the process of bagging is often detrimental to the pistillate flowers of *C. pumila* and causes them to drop off of the tree.
		CAES	6	9 nuts	-	
	Reciprocal	CAES	9	7 nuts	-	
Henryi	Ozarkensis	Clapper, 1954, DU		+	-	Six seedlings were studied and all were intermediate between the parental species for the following leaf characters: pubescence on the petiole and lower surface of the lamina, ratio of length to width, and dentation. See Figure 49 for illustration of typical leaves of the parental species and the respective hybrid.
		Graves & Jaynes, 1959		+	-	
		CAES	2	6 seedlings	+	
	Reciprocal	Graves & Jaynes, 1959		+	-	Eleven seedlings were studied and they were intermediate between the parents for the same traits as these described for the reciprocal cross above. (See Figure 49)
		CAES	2	11 seedlings	+	
Henryi	Ashei					
	Reciprocal	CAES	1	11 nuts	-	
Henryi	Floridana					
	Reciprocal					
Henryi	Alnifolia	CAES	1	1 nut	-	
	Reciprocal	CAES	1	0		

For key to symbols and abbreviations see Table 12.

FIGURE 50. INTERSPECIFIC CROSSES IN THE GENUS CASTANEA



	♂	CASTANEA						BALANOCASTANON						HYPO-CASTANON
♀		DENTATA	MOLLISSIMA	CRENATA	SATIVA	SEGUINII	DAVIDII	OZARKENSIS	PUMILA	ASHEI	ALNIFOLIA	FLORIDANA	PAUCISPINA	HENRYI
CASTANEA	DENTATA		▥ □	▥ □	▤	▤		▤	▤ ?	▤	░	▤ ?		▤
	MOLLISSIMA	▥ □		▥ □	▤	▥ ▢		▤ ▢	▤ ▢	░ ▢	▤ ?	▤ ?	▤ ?	▥
	CRENATA	▥	▥ □		▥ □ ?	▤		▤ ▢	▤ ▢		▤ ▢	▥		▥ ▢
	SATIVA	▥	▥ □	▥ □ ?		▤		▤ ▢	▤ ▢	░ ▢	▥ ▢			▥ ▢
	SEGUINII	▤	▤	▤	░			▤ ?	▤	░	▤ ?	▤ ?		▤
	DAVIDII													
BALANOCASTANON	OZARKENSIS	▤	▤	▤	▤	▤			▤	▤	▤			▥
	PUMILA	▤	▤ ?	▤	▤	░		▤			░			▤
	ASHEI	▤	░	▤		░		▤	▤		▤			▤
	ALNIFOLIA		▤ ?	▤ ?		▤ ?		▤	▤					░
	FLORIDANA	▤ ?	▤ ?			▤ ?								
	PAUCISPINA		▤ ?											
HYPO-CASTANON	HENRYI	▤ ▢	▤ ▢	▤	▤	▤ ?		▥	▤		▤			

⬜ CROSS NOT YET ATTEMPTED

░ CROSS ATTEMPTED BUT UNSUCESSFUL (NOT NECESSARILY EVIDENCE OF REDUCED NUT SET)

▤ NUTS AND/OR SEEDLINGS OBTAINED, NOT VERIFIED AS HYBRID

▥ PROGENY OBTAINED AND VERIFIED AS HYBRID

▢ EVIDENCE OF REDUCED NUT SET

□ EVIDENCE OF INCOMPATIBILITY, i.e. MALE-STERILITY, CRACKED BARK, ETC.

? DIRECTION OF CROSS UNKNOWN

SEE TEXT FOR REFERENCES AND MORE DETAILED EXPLANATION OF CROSSES

Discussion and Conclusions

This investigation has been concerned primarily with first generation interspecific crosses in the genus *Castanea*. A majority of the possible crosses have been attempted, but unfortunately progeny were not always grown from the resulting nuts or, when they were, observations on the resulting offspring were not recorded.

Morphological characters in the first generation offspring from several different interspecific crosses were studied to verify if the crosses were successful. Most of the characters in the F_1 presumed hybrids were observed to be more or less intermediate between the parental types. However, the expression of lepidote, multicellular leaf glands, whose presence is characteristic of the species *C. crenata*, was suppressed or grossly altered in crosses of *C. crenata* with *C. mollissima*, *C. floridana*, and *C. dentata*. These lepidote glands also normally occur on representatives of *C. seguinii*, and it was observed that progeny of the cross *C. mollissima* × *C. seguinii* also lacked these glands. The cross of *C. sativa* × *C. crenata* was the only cross in which the glands were present in large numbers in the F_1 where they were not characteristic of both parents.

Examination of progeny from several interspecific crosses confirmed the conclusions reached in the previous chapter about apomixis, self-fertilization, and outcrossing; that is, these events do not appear to be of frequent occurrence. However, it is still felt that progeny should be examined for traits of the male parent since, as was evidenced, the intended hybridization is not always successful.

Only very broad generalizations are possible in making conclusions about the relationships of the chestnut species to each other. Partial genetic incompatibilities occur between species, but they are not always specific for any two species nor do they as yet appear characteristic of particular subgeneric combinations. It was observed that progeny from two interspecific crosses involving different trees of the same respective species did not always show the same abnormalities. For example, one cross produced all fertile trees whereas another, using different trees of the same species, produced predominantly male-sterile progeny. This is to be expected when the species dealt with are heterozygous as are the chestnut species. Chestnuts are normally not only cross-pollinating but most of the species have wide geographic ranges which tend to maintain or increase further the variability within each species.

Of the traits studied reduction in nut set possibly portrays best the evolutionary relationships of the several chestnut species. It is unfortunate that more crosses were not made specifically to measure reduction in nut set. From the evidence presented it appears that crosses between the subgenera *Castanea* and *Balanocastanon* result in a lower nut set than crosses between species within the respective subgenera or from crosses within a species. Some evidence is also presented for reduction of nut set in crosses between species of the subgenera *Castanea* and *Hypocastanon*.

Other than nut set, evidence for the presence of partial incompatibilities is drawn from the results of crosses within the subgenus *Castanea* (see Figure 50). This does not deny the presence of partial incompatibilities between the species within the genus Balanocastanon or between subgenera, nor does it imply that the subgenus Castanea is not a natural group. It probably reflects the economic importance of the species in the subgenus Castanea and the fact that more crosses have been studied in this group.

It is concluded that genetic barriers between the species in the genus Castanea are being developed but they are as yet incomplete between species and variable within the respective species.

Summary

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. Partial genetic 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. The evidence on nut set indicates that crosses between species of different subgenera produced fewer nuts than crosses within subgenera. Interspecific crosses have been successfully completed between species of the three subgenera, and it is likely that all the remaining possible interspecific crosses could be completed.

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DISCUSSION

The following discussion emphasizes certain salient features of the genus Castanea which might be important in future breeding work with this group. Among the chestnut species there is tremendous variation and, fortunately, the genes controlling this variation can be recombined, with patience, in almost any way that might be desired. All the species have the same chromosome number and no complete incompatibility barriers between species have been demonstrated. A part of the variation inherent in the genus stems from its wide geographic range throughout much of the temperate zone of the world. Thus it should be possible to produce hybrids for a wide array of ecological situations. Development of trees fully competitive with the natural vegetation might not be easily accomplished, but synthesis of trees that would do well with a minimum of help from man should not be a major problem.

Specific ways of developing these tailor-made-trees will not be presented here, but general areas of practical importance and utilization of certain traits will be discussed. The four potentially or actually important uses of chestnut trees are conveniently categorized as follows: 1) As a forest tree, primarily for lumber and tannin; 2) As a wildlife and game food for squirrels, deer, pheasants, and other animals; 3) As an orchard tree for commercial nut production; and 4) As an ornamental and landscape plant. These categories are somewhat arbitrary, because the desire would often be for multiple use including two or more of the above four divisions. However, for purposes of discussion we will maintain the separation. It is recognized that resistance to common diseases and insects, and general adaptibility to the local environment, are important, regardless of the primary use of the tree.

In breeding for a forest tree it would be logical to work towards a type similar to the old American chestnut. C. dentata generally had good form, fast growth, high tannin content in the bark, and wood which was valued for its resistance to rot fungi. Superficially there appears to be little reason why resistance to the blight fungus (Endothia parasitica) found in C. mollissima and C. crenata could not be transferred into a prototype of the American chestnut. A more formidable task, though, would be that of synthesizing a tree fully competitive with the native vegetation and one also capable of satisfactorily reproducing itself under natural conditions. Of the specific traits considered in this paper male-sterility might be used to advantage in setting up seed orchards to obtain large quantities of hybrid seed, particularly if the desire was to eliminate the possibility of self-fertilization or if one had several male-sterile trees to be crossed by a single male-fertile tree.

Most important in considering the chestnut tree as a source of food for game and wildlife would be nut quantity, as measured in terms of size and number, and consistant productivity from year to year. Fortunately chestnut trees are unique among the native nut trees in that fruit production is not strongly cyclical. For this reason the chestnuts are valuable when planted with oak, beech, and hickory to tide over the lean food years. Recombination of large nut size and blight resistance, prevalent in some of the Asiatic trees, with the native prolific Chinkapin species would offer possibilities of a high food yielding tree. Some selections of the Chinese chestnut have already shown promise in conservation work, but by crossing these selections with a native species, particularly one of the Chinkapins, greater adaptability and higher production could be expected. Tree form need not be of primary concern unless the trees are eventually to be cut for timber. As with a timber tree the ability to compete favorably with native vegetation would be desirable.

For orchard nut production emphasis need not be placed on competitiveness or tolerance of the tree to native vegetation. Important factors here are fruiting at a young age, large nut size, nut quality, and maximum production year after year. C. seguinii and the Chinkapin species are good sources for several of these traits, i.e. flowering when only two or three years old, prolificness, and dwarfness or limited vegetative growth. The possibility of recombining these characters with the blight-resistant, large fruiting traits of some of the Chinese and Japanese chestnuts is very promising, and in fact some progress has already been made in this direction with the cross of C. mollissima × C. seguinii. A tree not dependent on out-crossing for nut production, i.e. one capable of undergoing self-fertilization or apomixis, would be desirable because there would be no need to grow more than a single clone in any one orchard. As yet trees are not known which are freely self-compatible or highly apomixtic. Male-sterile trees might be used advantageously as orchard trees since they may have additional carbohydrate reserves and thus have a potential for greater nut production. On the other hand, the positive effect of the male parent on nut size might be utilized if obligate cross-pollinating trees are used or, if the proper genome constitution can be obtained, self-pollination might also be satisfactory. Nuts placed on the commercial market should have good storage qualities and long dormancy requirements so that they will not germinate readily. The short dormancy requirements of some of the Chinkapin species and their effect on other species when used as pollen parents has already been discussed.

In using chestnut trees as ornamentals and shade trees in landscape gardening any of the three types already discussed might be satisfactory, but several other traits could be used to advantage. Complete sterility would be valuable for trees planted along streets or in yards where debris resulting from the dropping of the catkins, burs, and nuts was undesirable. However, complete female-sterility is virtually unknown, but it may yet be found to occur in some interspecific crosses or it could be produced by radiation. Dwarfness, characteristic of some of the Seguins, and long, pendant catkins, typical of some Japanese × American hybrids, would be additional useful attributes to be considered.

Even though the American chestnut was destroyed by the blight fungus and the European chestnut is being threatened by the same fungus, as well as a root disease, there is still reason to believe that new chestnut trees tailored for various needs are within the reach of the geneticist and plant breeder.

APPENDIX

BURIED-INARCH TECHNIQUE FOR ROOTING CHESTNUT CUTTINGS

Introduction

Vegetative propagation of chestnut by rooting is difficult. To date the use of misting systems, plastic tents, bottom heat, and other special conditions combined with auxin treatments have not met with much success in rooting cuttings. The only satisfactory technique of asexual propagation has been grafting, but with this method the possible effect of the stock on scion growth is always present.

As part of the research program at The Connecticut Agricultural Experiment Station it is becoming increasingly important to have a means of vegetative propagation by which the question of stock-scion interaction is avoided.

The technique described below has been successful in producing root tips for cytological studies, and will be used in the future to reproduce vegetatively some of the more promising hybrids so that they can be tested thoroughly under varying conditions. The method described is referred to as the "buried-inarch" technique and involves the rooting of a scion through the aid of a young seedling tree. A similar technique has been used to root other woody plants, such as apple (Kemmer, 1958), but it had not been tried with chestnuts.

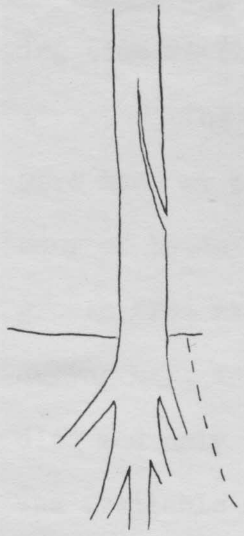
Description of the Buried-Inarch Technique

Scions from the last year's growth of the desired trees were collected in March and stored in damp sphagnum moss at 4 C until normal spring grafting time. At that time, the end of April and beginning of May in Connecticut, the scions were cut into six to eight inch lengths and wedge shaped on the upper end in preparation for the graft (Figure 52, a and b). Meanwhile a small hole about six inches deep was dug at the base of the seedling tree. Above this hole and approximately two inches above ground level a diagonal cut was made in an upward direction in the trunk of the seedling (Figure 51). The wedge shaped end of the scion was fitted into this slit and the lower three to five inches of the scion buried in the ground. The graft union was then bound with suitable material and coated with grafting wax (Figure 53). With proper sized stock two or three scions can be grafted into the same seedling.

Care has to be taken when making the graft to expose at least one bud above ground level, for this bud develops into the shoot. Once the shoot has developed and roots are formed on the base of the scion, the scion can be cut free of the stock. The result is an independent plant.

Results

Callus began to form at the base of the scions shortly after grafting. In some cases this callus became very large and by the end of the summer measured up to two inches in diameter. During the two years this technique was used roots were formed within the callused area in August and September (Figure 54). Limited testing



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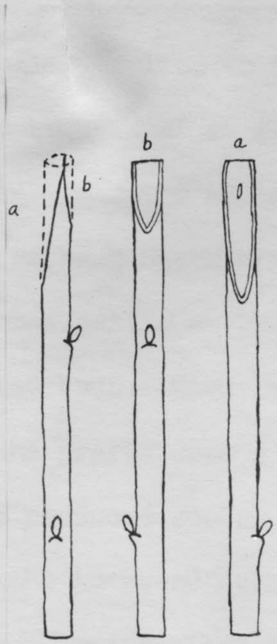

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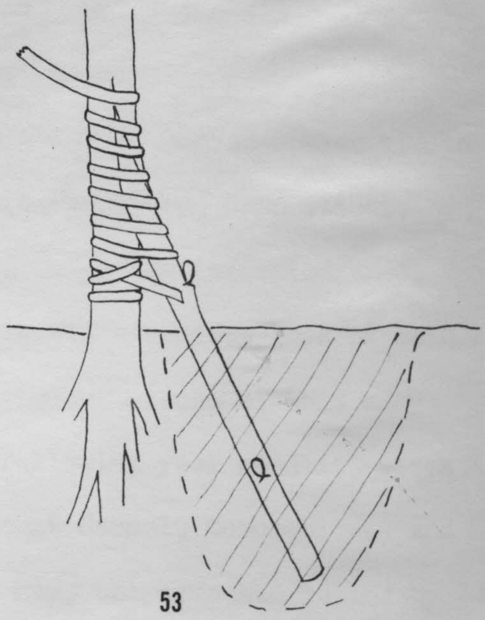

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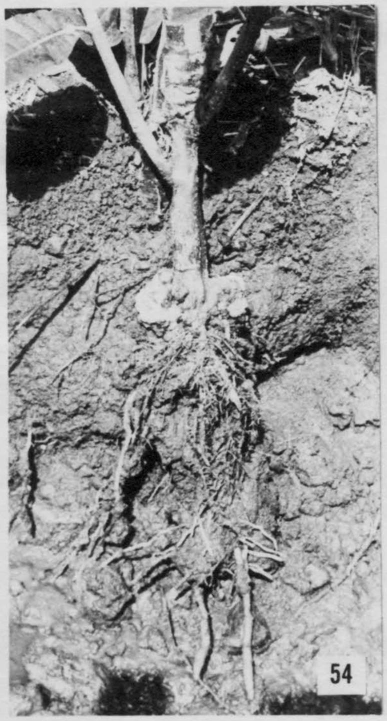

54

BURIED - INARCH

A grafting technique for obtaining rooted seedlings from scions of hard to root species. Root tips were of importance for chromosome counts.

Figs. 51 and 52 Preparation of the stock and scion.

Fig. 53 Graft completed, wrapped and ready for waxing.

Fig. 54 Four months after grafting. Note shoots above soil level and extensive root development below large callus.

indicated that auxins applied to the basal end of the scion at grafting time might be beneficial in inducing rooting.

The first year the method was tried 67 buried-inarch grafts were made at the Pachaug State Nursery in Voluntown, Connecticut. Many of these formed callus at the graft union and initiated shoot growth from exposed buds. However, there was a serious lack of rain during May, and the grafts were not watered. Because of this many died and only two produced roots. The following year seedling stock was available at the Lockwood Farm in Mount Carmel, Connecticut, and of 36 buried-inarch grafts attempted in May, thirteen had rooted by October 4. Eighteen of these grafts were with scions of C. sativa mailed from France, and these had been received in a poor condition. If these poor scions are eliminated then it can be said that 50% of the buried-inarch grafts rooted. Root tips for cytological studies were obtained from these rooted scions in September.

Arthur H. Graves tried a few buried-inarch grafts in conjunction with this author and his results support those reported here (Personal communication).

Conclusions

The results have been very encouraging, and there is good reason to believe that the percentage of rooting of buried-inarch grafts will be increased by using only stocks and scions in good condition and by applying auxins such as indolebutyric acid. The technique described is little known, but it warrants consideration in the rooting of other hard-to-root woody plants, particularly those where grafting has been demonstrated to be no problem.

If a true rooting hormone occurs in plants, e.g. rhizocaline, and is produced in the leaves and transported down the stem, then the method described here would take full advantage of this material by collecting it at the base of the inarched scion. Perhaps this is the reason why chestnut and apple scions root when the buried-inarch technique is used.

Summary

A method is described by which chestnut material can be rooted. It is basically a grafting technique, and involves the rooting of a scion through the aid of a young seedling tree. The procedure has been important in obtaining root tips for cytological studies. This technique will be used to reproduce asexually some of the best chestnut hybrids.

Literature Cited

Kemmer, E. 1958. Stadienbeobachtungen an Kernobstgehölzen. Der Züchter 28: 367-377.

Table 17. FEULGEN SCHEDULE USED IN STAINING ROOT TIPS

Steps	Conditions	Time
1	Fix in acetic acid-alcohol solution of 1 part glacial acetic acid to 3 parts 95% ethyl alcohol	24 or more hours
2	Rinse in distilled water	2 minutes
3	Hydrolyze in 1N HCl preheated and maintained at 60 C	12 minutes
4	Cool in 1N HCl at room temperature	1 minute
5	Rinse in distilled water	2 minutes
6	Stain in Feulgen reagent A	25-30 minutes
7	Rinse in Feulgen reagent B	2 minutes
8	Mount in 45% acetic acid	-