HYBRIDIZATION AND GRAFTING IN SPECIES OF *THEOBROMA* WHICH OCCUR IN AMAZONIA

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Introduction

Beginning in 1945, the authors have carried out a series of experiments on hybridization of species of *Theobroma* which occur in the basin of the Amazon. The cultivated cacao tree (*Theobroma cacao*) has been used frequently in these experiments, the original purpose having been an attempt to transfer various desirable characters of the wild species into the cultivated form, as suggested by Posnette (1945), who first succeeded in obtaining some cross-pollinations between species of *Theobroma*. Experiments have also been made on reciprocal grafting between the various species. These experiments have revealed an interesting parallelism between the behavior of some species in hybrids and in grafts. The purpose of the present article is to present a summary of the results obtained in our *Theobroma* work to date.

The Parental Forms

In his revision of the genus *Theobroma*, Ducke (1940) recognizes nine species which occur in Amazonia. Considered morphologically, these species fall into several groups. Thus, *T. Mariae* and *speciosum* have flowers on the trunks only, *T. microcarpum, grandiflorum, subincanum, obovatum,* and *bicolor* produce flowers only on branches, while *T. cacao* and *Spruceanum* produce them both on trunks and on branches. Seeds of *T. Mariae* germinate in 30 or more days, while those of other species take only 15 days to germinate. The branching of most species is trichotomous, but in *T. Mariae* it is monopodial, and in *T. cacao* it is in fives. Axillary buds on the branches are absent in *T. speciosum, Spruceanum, bicolor* and *cacao,* and present in the other species. In *T. speciosum, Spruceanum, bicolor* and *cacao,* the stem arises from buds below the branching points while in the other species it arises from axillary buds on the branches. *T. cacao* is somewhat variable with respect to the above trait, since some branches develop buds which give rise to a secondary stem. Furthermore, *T. cacao* is the only species which is capable of base sprouting, even in adult trees.

The leaves are digitate in *T. Mariae,* while the leaves of the other species are entire. Young plants of *T. bicolor* have cordiform leaves. Young leaves are green in *T. Mariae, speciosum, Spruceanum, bicolor* and *microcarpum,* and either green or purple in the other species. The hybridization experiments have shown that the green color is a single recessive trait, purple being dominant. Accordingly, we have whenever possible used plants with green foliage as female parents, and those with purple foliage as male parents. This permits ready detection of the possible contaminations. Leaves of *T. Mariae, subincanum, obovatum* and *grandiflorum* are hairy; the adult leaves of *T. Mariae* have the further characteristic of not losing their hairs when rubbed against human hands.

The morphological and gross physiological traits thus shed relatively little light on the relationships of the nine species considered. It is clear only that *T. Mariae* is a very distinct form, and indeed it is placed in a separate subgenus or section Herrania (Ducke, 1940), which some authors consider to be a separate genus (Shultes, 1945).

The Species Crosses

The techniques used in the hybridization experiments have been described elsewhere (Addison and Tavares, 1952). Here it will be sufficient to note that different individual trees of the same species may vary with respect to the ease with which they produce hybrids with other species when used as female parents in crosses. Thus one tree of *T. cacao* growing in the garden of the Museum Paraense "Emilio Goeldi," at Belém, proved most favorable for our purpose, and was subsequently used more than were other trees.

The results of the cross-pollination of the species of *Theobroma* are summarized in table 1. In this table the name of the species used as the mother is always given first, that of the father second. It is clear at a glance that most combinations of species produce no offspring. It should be noted that this inability to produce hybrids is not due to failure of germination of the pollen or to failure of the pollen tube growth, as has been verified by special observations. An attempt to improve matters by application of 0.1% to 0.25% alfanaphtalene acetic acid lanoline at the point of abscission of the flowers gave no positive results.

The only species which, when used as a female, is able to produce hybrid seeds with most of its congeners is the cultivated *T. cacao*. Only the cross *cacao* × *Spruceanum* has consistently failed, and *cacao* × *bicolor* produced a single ripe fruit with few seeds. On theoretical grounds, it is perhaps suggestive that the isolating mechanisms which exclude interspecific crosses should be weakest in the

TABLE 1. *Numbers of pollinations made, of fruits obtained, of seeds in the fruits, and the frequency of seeds which germinated in various interspecific crosses in the genus* Theobroma

Species	Pollinations	Fruits	Seeds	Germination
cacao × *speciosum*	375	24	224	0
cacao × *Mariae*	397	52	191	0
cacao × *microcarpum*	489	7	11	20%
cacao × *obovatum*	674	45	few	0
cacao × *grandiflorum*	999	285	548	40%
cacao × *subincanum*	375	24	few	0
cacao × *bicolor*	381	1	few	0
cacao × *Spruceanum*	2,239	0	0	0
bicolor × *speciosum*	293	0	0	0
bicolor × *Spruceanum*	556	0	0	0
bicolor × *obovatum*	520	0	0	0
bicolor × *subincanum*	250	0	0	0
bicolor × *cacao*	2,440	0	0	0
bicolor × *Mariae*	641	0	0	0
grandiflorum × *cacao*	745	0	0	0
grandiflorum × *bicolor*	462	0	0	0
grandiflorum × *Spruceanum*	743	0	0	0
grandiflorum × *speciosum*	541	0	0	0
grandiflorum × *microcarpum*	654	0	0	0
grandiflorum × *Mariae*	531	0	0	0
grandiflorum × *obovatum*	465	37	701	77%
grandiflorum × *subincanum*	317	39	514	72%
subincanum × *microcarpum*	259	0	0	0
subincanum × *Mariae*	550	0	0	0
subincanum × *speciosum*	690	0	0	0
subincanum × *Spruceanum*	505	0	0	0
subincanum × *grandiflorum*	189	42	756	65%
subincanum × *obovatum*	515	136	1,632	High

TABLE 1.—*Continued.*

Species	Pollinations	Fruits	Seeds	Germination
obovatum × *cacao*	246	0	0	0
obovatum × *Mariae*	194	0	0	0
obovatum × *microcarpum*	581	0	0	0
obovatum × *Spruceanum*	527	0	0	0
obovatum × *grandiflorum*	506	0	0	0
obovatum × *subincanum*	539	0	0	0
speciosum × *cacao*	1,011	0	0	0
speciosum × *bicolor*	1.264	0	0	0
speciosum × *Mariae*	1,896	0	0	0
speciosum × *grandiflorum*	660	0	0	0
speciosum × *Spruceanum*	437	72	1,406	63%
speciosum × *obovatum*	25	0	0	0
Spruceanum × *cacao*	159		0	0
Spruceanum × *bicolor*	97	0	0	0
Spruceanum × *microcarpum*	102	0	0	0
Spruceanum × *grandiflorum*	102	0	0	0
Spruceanum × *Mariae*	13	0	0	0
Spruceanum × *subincanum*	101	0	0	0
Spruceanum × *obovatum*	102	0	0	0
Spruceanum × *speciosum*	41	32	62	52%
microcarpum × *cacao*	395	0	0	0
microcarpum × *bicolor*	161	0	0	0
microcarpum × *grandiflorum*	500	0	0	0
microcarpum × *Mariae*	490	0	0	0
microcarpum × *obovatum*	500	0	0	0
microcarpum × *subincanum*	187	0	0	0
microcarpum × *speciosum*	176	0	0	0
microcarpum × *Spruceanum*	1,786	0	0	0
Mariae × *microcarpum*	353	0	0	0
Mariae × *Spruceanum*	426	0	0	0
Mariae × *speciosum*	235	0	0	0
Mariae × *obovatum*	1,214	0	0	0

most extensively cultivated species. Hybrid seeds are produced in the crosses *subincanum* × *grandiflorum* and the reciprocal, *speciosum* × *Spruceanum* and the reciprocal, *grandiflorum* × *obovatum*, and *subincanum* × *obovatum*. Reciprocals of the two last named crosses have so far failed to give hybrid seeds. It must however be noted that *obovatum* is, in contrast to other species with the exception of some *cacao* strains, self-fertile, and this fact makes the hybridization work with this species especially difficult. *T. bicolor*, when pollinated by *cacao*, *speciosum*, and *Spruceanum*, gives fruits which do not reach maturity but fall off after growing to about 10 cm. in length. It may be noted that the abscission of the fruits is a characteristic of the species *bicolor*, but in *bicolor* it takes place when the fruits are ripe. In the reciprocal crosses, the fruits attain no more than 3 cm. in size, and die off.

Obtaining hybrid seeds does not insure production of hybrid plants because of the deficient germination of the seeds, as indicated in table 1. The seeds of the pure species give, in most cases, between 90% and 100% germination.

THE F_1 HYBRIDS

As shown above, only some of the many species combinations have produced viable

hybrid seeds. The characteristics of the hybrids obtained are as follows.

T. grandiflorum × *T. obovatum.* In 1949, several hundred hybrid seeds germinated in baskets with sandy soil. Many of the seedlings were transferred in a lath house. Of these, 7 died, 7 are at present (1952) dwarfs or semi-dwarfs, and 22 grew normally. Other seedlings (328) were transplanted in the field; only 50 of them are alive at present. The slow growing hybrids, and some of the normally growing ones, show a characteristic necrosis of the tips of the leaves, and sometimes of as much as a half of the leaf blade. Only 14 hybrid plants appear to be normal both with respect to the growth and of the condition of the leaves.

Seven pollinations were made using the F_1 hybrids as mothers and *T. obovatum* as father. No fruits were obtained. A total of 103 pollinations of the hybrids by the pollen of *T. grandiflorum* gave 63 fruits, 20 of which matured and produced 354 seeds, most of which germinated successfully. Of the 135 pollinations with hybrid pollen on *T. grandiflorum* stigmas, only 8 gave fruits with a total of 161 seeds. These seeds also germinated successfully. The backcross to *T. obovatum* as mother has not matured yet.

The fruits produced by the hybrid plants are, on the average, 78 mm. long and 44 mm. in diameter; in *T. obovatum* the corresponding dimensions are 51 × 34 mm., and in *grandiflorum* 158 × 92 mm. A hybrid fruit contains, on the average, 12 seeds, while the parents have respectively 6 and 38 seeds. The taste of the hybrid fruit is likewise intermediate between the parents. So is the external appearance of the surface of the fruits. A trait which is dominant rather than intermediate in the hybrids is the falling off of the mature fruits from the tree, which also occurs in *grandiflorum.*

T. grandiflorum × *T. subincanum.* This cross produces seeds which germinate well and give seedlings that are normally viable. In 1949, a total of 45 hybrid seedlings were planted in a lath house. They grow quite normally, and do not show the necrosis of the leaves observed in the *grandiflorum* × *obovatum* hybrids (see above). The hybrids, like the *subincanum* parent, have the young stems flexible and nodding until hardened: the *grandiflorum* parent does not show this trait. These hybrids have flowered in 1952. The appearance of the flowers in the hybrids is intermediate between the parental species.

T. subincanum × *T. grandiflorum.* These hybrids are not appreciably different from the preceding ones.

T. subicanum × *T. obovatum.* This cross also produces good seeds and viable seedlings. In 1949, 56 seedlings were planted in the lath house. The hybrids are about as small as the *obovatum* parents of corresponding age. Some of the hybrid plants show disturbances in the formation of the leaves but others appear to be perfectly normal. Only three hybrid trees have flowered to date; their pollen grains appeared to be normal in the iodine solutions. Attempts to obtain a backcross progeny by pollinating the hybrids with *subincanum* pollen gave, so far, no fruits. Some fruits have nevertheless been obtained by using the pollen of a third species, *grandiflorum.*

T. Spruceanum × *T. speciosum.* The 35 seedlings which germinated in 1949 were planted in a lath house and gave rise to apparently healthy trees. The parental species are rather similar in appearance of the leaves and in their general habitus; with respect to whatever differences can be noted between them, the characteristics of *speciosum* seem to be dominant in the hybrids. Thus far 5 hybrids have flowered; the flowers are intermediate between the parents. Pollination with *Spruceanum* pollen gave rise to many fruits which, however, failed to grow beyond about 2.5 cm. in length. This may be due to the youth of the hybrids. It may be noted that *Spruceanum* flowers at the age of about 1½ years, while *speciosum* reaches flowering when 5 or more years old. The early flowering trait is then, dominant in the hybrids.

TABLE 2. *The numbers of grafts made (the upper figures in each cell), of grafts that took (the lower figures), and development of the scions in species of Theobroma*

N—the scion grows normally; SN—subnormal growth of the scion; M—the scion becomes about 30 cm. long and then dies off; F—the scion develops some leaves and then dies; D—the scion dies soon after the beginning of growth.

Stock ↓ / Scion →	*cacao*	*bicolor*	*grandiflorum*	*speciosum*	*subincanum*	*Spruceanum*	*microcarpum*	*Mariae*	*obovatum*
cacao	20	20	34	50	34	20	30	20	34
	16	9	20	28	18	15	20	2	18
	N	M	F	M	F	M	SN	D	M
bicolor	35	20	35	40	35	20	35	35	40
		19	11	22	20	15		15	4
	D	N	D	SN	D	SN	D	D	D
grandiflorum	24	20	20	40	23	20	20	20	23
	4	4	8	10	8	9	6		9
	F	F	N	SN	N	F	D	D	N
speciosum	35	20	35	40	35	20	35	35	39
		17	7	31	3	19		9	
	D	N	D	N	D	N	D	D	D
subincanum	24	25	20	25	20	25	24	25	25
	24	23	17	22	15	16	13	13	11
	F	D	N	M	N	M	D	D	N
Mariae	20	20	20	20	20	20	20	20	20
	3	3	15	20	13	10		20	18
	D	F	F	F	F	F	D	N	F
obovatum	20	20	20	20	20	20	20	20	20
	18	16	17	6	19	13	10	2	13
	D	F	N	F	N	M	D	D	N

T. speciosum × *T. Spruceanum.* These hybrids were planted in the field and developed into trees which resemble those of the preceding cross which grow in the lath house. Thus far only a single tree has flowered.

T. cacao × *T. grandiflorum.* As shown in table 1, this cross gives rise to many hybrid seeds, although the number of seeds per fruit is lower than that in pure *cacao.* The germination of the hybrid seeds is nevertheless poor, and few seedlings reach as much as 15 cm. in height. Those hybrid seedlings which grow push the cotyledons out of the soil, resembling in this respect the *cacao* parent. No hybrids have grown anywhere near the flowering stage.

GRAFTING

Experiments were made grafting the buds from the stem of one species to that of another individual of the same or of a different species. The results are summarized in table 2.

It can be seen that all the intraspecific grafts that take develop normally. Among the interspecific grafts much variation is observed. The three species *grandiflorum, obovatum,* and *subincanum* can be grafted reciprocally with ease. The scion of *grandiflorum* and *subincanum* gave dwarf trees when grafted on *obovatum* stocks, since *obovatum* is the smallest of the three species. *Speciosum* can also be grafted on *grandiflorum*; one of the grafts has attained 120 cm. in height, but died there-

after. Another well defined group are *bicolor, speciosum,* and *Spruceanum,* although *bicolor* is less closely related to the other two than the latter are to each other. When *bicolor* is used as scion and *speciosum* as stock, some grafts develop a callosity at the locus of the graft, which has a tendency to sprout roots. *Spruceanum* is a smaller plant than its two relatives, and when it is grafted on *speciosum,* the scion grows more vigorously than it does on its own roots. The scions of *microcarpum* develop fairly normally on *cacao.*

Discussion

The data presented above demonstrate the existence, in *Theobroma,* of a far reaching, although by no means perfect, correlation between the outcomes of hybridization and of grafting experiments. In general, the species which produce hybrid progenies, or at least hybrid seeds, can be grafted successfully onto each other. Vice versa, where the interspecific grafts die off hybridization is usually impossible. Thus, the three species, *T. subincanum, grandiflorum,* and *obovatum,* can exchange grafts freely, all nine possible combinations of scions and stocks giving normal growth (table 2). *Subincanum* and *grandiflorum* also produce viable hybrids, the reciprocal crosses being alike; *obovatum* crosses freely with the two other species when used as a male parent, but not as a female (table 1). Normal or subnormal growth is also observed in reciprocal grafts of the three species *speciosum, Spruceanum* and *bicolor.* Of these, *Spruceanum* and *speciosum* can be crossed in either direction, but *bicolor* gives no hybrid seeds with either of the two other species. The scions of *microcarpum* grow reasonably well on *cacao,* and the cross *cacao* × *microcarpum* does not give a viable progeny, as the reciprocal one.

Whether the success of hybridization on the one hand, and the outcome of grafting on the other, have a common physiological basis is a matter of speculation. However that may be, the data at our disposal permit the following division of the nine *Theobroma* species into four groups:

I	II	III	IV
subincanum	*cacao*	*speciosum*	*Mariae*
grandiflorum	*microcarpum*	*Spruceanum*	
obovatum		*bicolor*	

This classification, based on experimental data, agrees with, or at least is not contradicted by, the morphology of the species concerned.

In conclusion, it may be pointed out that the species of *Theobroma* are remarkable by the completeness of the reproductive isolation which prevents the gene exchange between them. Hybridization of these species in their natural habitats is less probable than that of species of many other genera of plants. The rather extensive field work of the authors and of their colleagues in different parts of Amazonia has thus far failed to detect any natural hybrids between species of *Theobroma.*

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Summary

Experiments have been made intercrossing the nine species of *Theobroma* which occur in *Amazonia.* Most cross pollination gives no normally maturing fruits, but some crosses result in production of hybrid seeds. Hybrid seeds germinate in some cases with difficulty, and the seedlings often show a lowered viability. Only some adult hybrids have been obtained, but these have proved at least partly fertile. Buds of some species when grafted onto others fail to develop, while in other combinations of species the scions grow normally. The degrees of success of interspecific hybridization and of grafting show a strong positive correlation. A classification of the nine species based on the experimental data is proposed.

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