

Experimental studies of relationships in the genus *Jatropha*. I. *J. curcas* x *integerrima*

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RUPERT, E. A., B. DEHGAN, and G. L. WEBSTER. (Dept. of Botany, Univ. of Calif., Davis). Experimental studies of relationships in the genus *Jatropha*. I. *J. curcas* x *integerrima*. Bull. Torrey Bot. Club 97: 321-325. 1970. A vigorous interspecific hybrid has been obtained from cross-pollinations between two tropical woody species of *Jatropha*, *J. curcas* and *J. integerrima*, which belong to different sections of the genus. Although the hybrid is effectively self-sterile, it can be backcrossed with *J. integerrima* and the progeny show segregation for leaf characters. The high percentage of normal pollen (66%) and ease of backcrossing were unexpected since the parents are rather distantly related according to taxonomic standards. Apparently morphological variation and geographical separation have not been accompanied by the formation of strong reproductive barriers.

Jatropha is a large genus of the Euphorbiaceae with an interesting geographical distribution: nearly two-thirds of the 150 known species are American, while most of the remainder are tropical African, with a few species in Arabia and India. The genus is so diverse in both vegetative and floral structure that it has been variously split or subdivided by taxonomists. Two of the most recent general treatments, those of Pax and Hoffmann (1931) and McVaugh (1945), present radically different considerations of the species.

The two American species of interest here, *J. curcas* and *J. integerrima* Jacq., are not closely associated taxonomically in the systems of any previous workers. Pax (1910) and Pax and Hoffmann (1931) placed *J. curcas* in subgenus *Curcas* because of its coherent petals, while *J. integerrima* was referred to subgenus *Adenoropium* because of its separate petals. McVaugh (1945) criticized Pax's treatments as unnatural, and abandoned the formal recognition of subgenera. However, he also separated the two species and placed *J. integerrima* in section *Polymorphae* among the taxa characterized by having free red petals and calyx lobes not distinctly imbricate. In contrast, he put *J. curcas* in subsect. *Eucurcas* of section *Mozinna*; this section falls in the group of taxa characterized by distinctly imbricate calyx-lobes and a corolla with yellowish or greenish to white petals which are so firmly coherent as to simulate a gamopetalous condition.

Indeed, *J. curcas*, with its small yellowish-green, pseudo-gamopetalous corol-

las, palmately lobed leaves, and drupaceous fruits, has a strikingly different appearance from *J. integerrima*, which has bright red separate petals, obovate unlobed leaves, and capsular fruit. Furthermore, the species differ in geographical origin, *J. curcas* having been originally confined to Mexico and Central America (Wilbur, 1954), while *J. integerrima* is endemic to the Greater Antilles. The taxonomic dispositions of Pax and of McVaugh therefore seem reasonable, and one would not anticipate that such morphologically divergent plants would be very closely related genetically or interfertile. However, in 1968 three viable seeds were obtained from a series of crossing experiments originated by one of us (B.D.) involving the pollination of *J. curcas* by *J. integerrima*. These seeds produced two striking plants obviously intermediate between the two parental species in a number of characteristics (Figs. 1, 2, and Table 2). The surprising success of this rather wide cross-pollination prompted us to investigate and compare the morphological and cytological characteristics of the hybrid and its parental species.

Materials and methods. The following source materials were used: 9 individuals of *J. curcas* obtained from seeds and cuttings of UCD Accession No. B 63.017; 30 individuals of *J. integerrima* obtained from seeds and cuttings of UCD Accession No. B. 67.280; and 5 hybrids obtained from cuttings of the two hybrids noted above. Even so, the total number of pollinations

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was limited by the scarcity of pistillate flowers on *J. curcas*.

All pollinations were made in an insect-free greenhouse. Ten pistillate flowers of *J. integerrima* and 6 of *J. curcas* were tagged and left without hand-pollination as a test for accidental pollination. Self- and cross-pollination consisted of brushing the stigma of a pistillate flower with dehiscent anthers from the selected staminate flower. Cheesecloth bags were attached later in development to prevent loss of seeds from the explosive fruits. A summary of attempted self- and cross-pollinations with their results is given in Table 1.

Somatic chromosome counts were made from acetocarmine smears of young leaves

Table 1. Pollinations of *J. curcas*, *J. integerrima*, and their hybrids, numbers of flowers pollinated, seeds obtained and surviving plants, 1968-1970.

Species combinations	No. flowers	Seeds obtained	Surviving plants
<i>J. curcas</i> , selfs	5	9	not planted
<i>J. integerrima</i> , selfs	10	30	" "
<i>J. curcas</i> , not pollinated	6	0	0
<i>J. integerrima</i> , not pollinated	10	0	0
♀ ♂			
<i>J. curcas</i> × <i>integerrima</i>	8	8	3
<i>J. integerrima</i> × <i>curcas</i>	19	0	0
<i>J. hybrid</i> × <i>hybrid</i>	58	0	0
<i>J. "</i> × <i>curcas</i>	15	0	0
<i>J. "</i> × <i>integerrima</i>	17	0	0
<i>J. curcas</i> × <i>hybrid</i>	4	9	9
<i>J. integerrima</i> × <i>hybrid</i>	10	15	5
<i>J. integerrima</i> × backcross	12	11	9

(Baldwin, 1939). Meiotic divisions and pollen stainability were examined in acetocarmine anther smears from buds of various sizes fixed in a 3:1 ethanol/acetic acid solution. Pollen diameters were determined by averaging the measurements of 50-100 fresh grains suspended in distilled water; randomization was achieved by mixing pollen from several flowers and moving a mechanical stage at two millimeter intervals. Obviously aberrant grains were not measured.

Morphological comparisons of flower, leaf and stem characters were made from living plants and are summarized in Figs. 1, 2, and Table 2.

Results. Both cuttings and seedlings of the *Jatropha* species flowered within 12-18

months as small shrubs, even though at maturity they are classified as large shrubs or small trees.

From 143 cross-pollinations among *J. curcas*, *J. integerrima* and their progeny, 26 plants of varying degrees of hybridity were obtained. No seeds were obtained from non-pollinated flowers of either *J. curcas* or *J. integerrima*, and it was concluded that crosses could be kept safely without bags. Seeds developed freely, however, on hand-pollinated, selfed plants (Table 1).

Six seeds (3 of which germinated) were obtained from crossing pistillate *J. curcas* with staminate *J. integerrima* in 1968. Two of these hybrid seeds have grown into vigorous, freely flowering intermediates. The cross was repeated in 1970, yielding two seeds, one of which germinated. From the 58 self-pollinations of pistillate hybrid flowers made between 1968 and 1970 no seeds were obtained, although capsule enlargement was often observed.

No seeds were obtained from 19 attempts to make the reciprocal cross, *J. integerrima* × *J. curcas*, although the capsules enlarged for several days and immature seeds were found to contain partially developed embryos but no endosperm.

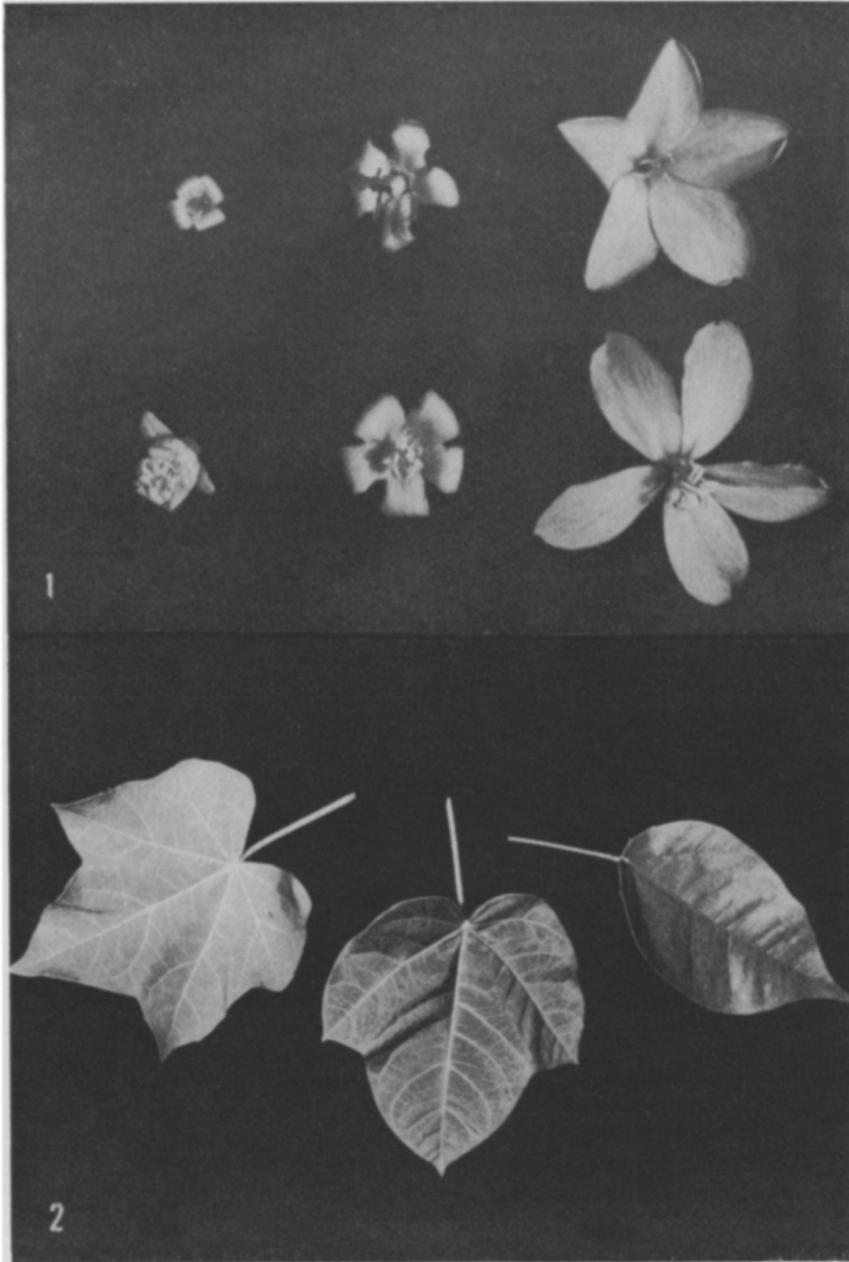
Nine seedlings from 4 backcrosses of *J. curcas* × hybrid were obtained. These are still too young for comparative study. From 10 backcrosses of *J. integerrima* × hybrid 5 seedlings are distinctly segregating for leaf form and texture. One plant from this backcross flowered precociously and was used to pollinate *J. integerrima*, with which it produced 9 seedlings.

The hybrid was found to have the same somatic chromosome number of 22 noted in *J. curcas* and *J. integerrima* by Miller and Webster (1967), but identification of individual chromosomes in *Jatropha* is hindered by their small size—from less than two to about four microns. Few meiotic divisions were seen. *Jatropha* is evidently among those groups with a very short time lapse between initiation of the reduction divisions and tetrad formation. Although the hybrid is sterile, neither univalents nor anaphase bridges were observed; their possible occurrence, however, is not excluded. Percentage comparisons of pollen stainability indicate some reduction of fertility in the hybrid. While 96.8% of the pollen released from *J. curcas* and 98.8% of that from *J. integerrima* appeared normal, the

percentage fell to 66.2% in the hybrid, more than enough, however to provide an adequate supply of male gametes.

As can be seen in Table 2 and Figs. 1 and 2, morphological intermediacy is more apparent among floral than foliar charac-

ters. The pink flower of the hybrid is clearly intermediate between petal colors in the parents; reflexing of the petals, hirsuteness, petal length and coherence are also intermediate. Both species show considerable variation in leaf shape among na-



Figs. 1-2. Flowers and leaves of *J. curcas*, *J. curcas* × *integerrima*, and *J. integerrima*.—Fig. 1. Pistillate (upper row) and staminate (lower row) flowers. ×1.—Fig. 2. Typical leaves. ×0.5.

tural populations; however, the hybrid leaves are clearly intermediate in lobing. Average pollen diameter in the hybrid is greater than in either parent, an increase which could result from either unequal divisions during microsporogenesis or from the increased vigor of the hybrid.

Discussion. Numerous morphologically distinguishable taxa deserving specific rank have evolved in the genus *Jatropha*. In *J. curcas* and *J. integerrima*, the accumulation of differentiating mutations has been sufficient to remove them to separate sections of the genus. Nevertheless, reproductive isolation has not accompanied their morphological differentiation, and the production of more than 65% normal pollen by their hybrid suggests that allelic muta-

florescence, whereas self-pollination is more likely at a later stage. Thus, in nature as in the laboratory, there should be no formidable barriers to the transfer of pollen from a hybrid to parental or non-parental forms.

Natural hybridization and introgression as sources of plant variation and speciation have been clearly discussed by Anderson (1949) and Stebbins (1950, 1969). Hybrids frequently occur between species when spatial barriers between habitats are removed. Less frequently these hybrids or their segregants find habitats in which they can competitively establish a permanent population. Ashton (1969) concluded that this lack of suitable terrain in tropical regions has minimized the influence of hybridiza-

Table 2. Morphological comparisons of *Jatropha curcas*, *J. integerrima* and *J. curcas* × *integerrima*.

Character	<i>J. curcas</i> (B 63.017)	Hybrid (B 68.422)	<i>J. integerrima</i> (B 67.280)
Pollen diameter, μ	76-87, av. 83.5	76-93, av. 89	67-78, av. 73
Flower color	greenish-white	pink	scarlet
" cup diam.	5-7 mm	12-15 mm	26-32 mm
Petal configuration	coherent	intermediate	separate
" attitude	reflexed	intermediate	straight
" pubescence	hirsute over much of petal	intermediate	hirsute only at base
" length	6-8 mm	12-14 mm	14-17 mm
Calyx lobes	imbricate	less imbricate	separate
" length	4-5 mm	4-5 mm	3-4 mm
Disc glands ♂	prominent	intermediate	reduced
Leaf blades	deeply lobed	shallowly lobed	unlobed
Trunk	stout	stout	flexible

tion rather than chromosomal rearrangement has been primarily responsible for speciation. Preliminary results with other cross-pollinations indicate that additional interspecific combinations are possible.

As Ornduff (1969) emphasizes, the use of hybridization in determining relationships is of value only if qualified by understanding of the varying expressions of the compatibility systems, genetic or other, and of the pollination mechanisms, involved in their reproductive patterns. Although a solitary self-sterile hybrid cannot originate an immediate new evolutionary line, if it can backcross with either parental type it provides an introgressive bridge between species. The monoecious *Jatrophas* appear to be a facultatively autogamous-xenogamous group: the carpellate flowers usually mature before the staminate on a particular plant or in a particular inflorescence; therefore, cross-pollination is likely in the early stages of expansion of a cymose in-

tion on tropical genera: a hybrid swarm may flourish during a temporary disturbance of the climax vegetation but is likely to disappear as the original flora reinvades. However, Siebert (1947) concluded that the natural hybridization which has occurred many times in *Hevea* has been responsible for a proliferation of intergrading taxonomic entities. Hybrid complexes have been described among many other tropical and subtropical genera, i.e. *Acacia*, *Eucalyptus*, *Rhododendron*, but little is known of their persistence in the flora or of the extent of backcrossing which may have occurred during the ephemeral existence of a hybrid complex.

Partly because of inaccessibility, and partly because of the prolonged zygote-fruit interval in woody plants, few controlled experimental studies have been made among tropical groups. The evidence from this cross of tropical species of *Jatropha* indicates that wide morphological

differences do not preclude introgression as a source of variability in the species complex whether or not a hybrid population persists.

Further studies of reproductive systems in the family and genus are in progress.

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Scanning electron microscopy of pollen as an aid to the systematics of *Vernonia* (Compositae)

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JONES, S. B. (Univ. Georgia, Athens). Scanning electron microscopy of pollen as an aid to the systematics of *Vernonia* (Compositae). *Bull. Torrey Bot. Club.* 97: 325-335. 1970.—The pollen grains of 24 species of *Vernonia* were examined by scanning electron microscopy. Micrographs of the pollen grains are presented for representative species from among those studied. The pollen of *Vernonia* is sufficiently complex to permit recognition of a large amount of variation in the surface sculpture of the exine. It is possible, therefore, to deduce phylogenetic trends in the genus using palynological and biosystematic evidence together.

The advantages of the scanning electron microscope in the study of pollen morphology have been demonstrated by Echlin (1968) and Heslop-Harrison (1968). Scanning electron micrographs resolve features of the pollen grain wall that cannot be accurately measured, or even seen with light microscopy. There is a greater increase in the depth of the field, a relative ease of specimen preparation, and a wider range of magnifications. The net result is the opportunity for a new look at the surface sculpture of pollen grains.

The pollen of *Vernonia* possesses an elaborate system of ridges exhibiting an unusual amount of variation in the surface sculpture. The admirable researches of Wodehouse (1928) with light microscopy revealed *Vernonia* as an ideal genus in

which to study pollen by means of scanning electron microscopy. He found that it was possible to trace phylogenetic trends in the genus from palynological evidence. Wodehouse's investigation was confined to the four sections of the genus found in North America. As cited by Gleason (1922), these sections are: *Stenocephalum*, *Stengelia*, and *Tephrodes*, each with one species, and *Lepidaploa* with over 100 species.

Wodehouse (1928) observed both a wide range in the types of pollen grain surface in the North American species of *Vernonia*

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