

Research

## <sup>1</sup>Cytology and hybridization in *Ipomoea triloba* L. complex (Convolvulaceae) and its taxonomic consequences

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### Abstract

Hybrids of two extreme morphologically variable populations of *Ipomoea triloba* were produced with 12.5% success. Bagged flowers set 100% fruit showing the species is strongly self-compatible and only facultatively outcrossing (10-20%). Nevertheless, fruit and seed development, germination, growth and development, pollens and chromosomes of  $F_1$  hybrids were normal. Parents and  $F_1$  plants were diploid ( $2n=30$ ). Hybrids morphologically resembled a wider spread of vigorous natural population. The taxonomic implication of the relationship is discussed.

**Keywords:** Chromosomes, hybridization, *Ipomoea triloba*, anthesis, taxonomy

### Introduction

*Ipomoea triloba* L. (Convolvulaceae) is a complex diverse species of roadside outgrowths, abandoned and waste land, drainages, streams and riverbanks. The highly variable nature of the species has led to the description of many intraspecific species, vars. *genuina* Meissn., *glaberrima* Meissn. and *eustachiana* Jacq., (Meissner, 1869; Eggers, 1879).

The breeding systems of any group of plants determine the effectiveness of gene exchange, variation and pattern of recombination of genetic variability, fitness, adaptability and survival in critical and precarious periods (Allard, 1960; Davis & Heywood, 1963; Ugborogho, 1973). Thus, breeding systems are concerned with both the genetic systems and evolutionary potentialities of a species or group which, to some extent, are reflected in the phenotype with resultant consequences for taxonomy. Variations in breeding systems are controlled by genetic and environmental factors (Davis & Heywood, 1963; Wyatt, 1983; Ledig, 1986; Charlesworth & Charlesworth, 1987). Fryxell (1957) recognized five such major factors: entomological, geographical, climatic, genetic

and experimental.

The ecology, taxonomy and evolution of many plant groups have been impacted significantly by hybridization and the structure of genetic diversity (Grant, 1981; Arnold & Bennett, 1993; Arnold, 1993; Bachmann, 1994; Rieseberg, 1995; Smith & Pham, 1996; Cruzan, 1998; Cole & Kuchanreuther, 2001). Hybridization may result in formation of new derivative species (Gallez & Gottlieb, 1982), a change in the adaptive norms of either or both parental taxa (Potts, 1986; Levin & Bulinska-Radomska, 1988) and or fusion of parental taxa into a polymorphic taxon (Grant, 1963).

Intraspecific hybridization of two extremely variable populations of *I. triloba* has been undertaken to clarify the pattern of genetic control and inheritance of characters, the breeding systems inherent in the species, the basis of its variability and the taxonomic implications.

### Material and methods

#### Anthesis

Ten flower buds were tagged per population and the

anthers observed for the time of opening at 90 minutes interval, 24 hours before the flowers opened.

#### Self-compatibility test

Mature flower buds were bagged in nets to prevent insects from making contact with the stigmas of opened flowers. They were then observed for fruit-set. Ten flower buds were bagged per population.

#### Cross-compatibility test

Flowers were emasculated before full anthesis and bagged. Pollen from another flower of the same plant and of other plants of the same population were transferred on to the stigmas of the emasculated flower at intervals of 3 hours until flower opened. 15 flowers were considered per population.

#### Agamospermy

Ten matured flower buds were emasculated and bagged to assess the possibility of obligate agamospermy in a population.

#### Fruit-set

Twenty randomly selected flowers per population were tagged each day for five days and observed for fruit set. The percentage fruit-set was calculated from the formula:  $\frac{X}{N} \times 100$

where X, represents the number of flowers that set fruit and N, the total number of flowers considered.

#### Hybridization experiments

The techniques of Scott-Emuakpor and Ugborogho (1980) were adopted. Ten randomly selected flower buds were emasculated 12 hours before the flowers opened and bagged. Freshly collected pollen from flower of the desired population was applied to the stigma of the emasculated flower with forceps every 3 hours until the flowers opened. The same procedure was followed for the reciprocal crosses. The cross-pollinated flowers were bagged to prevent contamination of stigma by foreign pollen. The treated flowers were observed for fruit-set.

#### Hybrid analysis

Seeds obtained from successful hybridization were

sown. Pattern of inheritance of morphological characters by hybrids were observed and assessed.

#### Cytology

##### Mitotic Chromosomes

Root tips (~4mm long) were cut from 2-3 day old radicle of germinating seeds and pre-fixed in Paradichlorobenzene for 2 hours. The root tips were washed thoroughly in distilled water and transferred into 1:3 acetic-alcohol for 24 hours. Hydrolysis was carried out in IN-HCl at 60°C for 10 minutes. The root tips were again washed in distilled water, thoroughly. Squash preparations were made in 2% acetocarmine on a glass slide to spread out the cells. A gentle but firm pressure was applied by the thumb over the cover slip to make the cell contents prominent. Slides of screened, well-spread somatic metaphase chromosomes were made. Chromosome number was determined from metaphase plates. Chromosome plate was photographed under oil-immersion (1000x) phase contrast Mka 5 Wild compound Light Microscope.

#### Results

##### Anthesis

The anthers dehisced longitudinally to release the pollen before the flower buds opened. Full anthesis occurred from 1400-1500 hours in *I. triloba*, about 16 hours before flowers opened (0600-0730hours). The flowers remained opened for 3½ to 5 hours. Closure of flowers started from 1000 through 1200 hours depending on the population.

##### Compatibility test

Evidence from compatibility experiment suggested that *I. triloba* was strongly self-compatible. Thus, there was a very low degree of success in cross pollinated flowers (10-20%) as opposed to the high degree success of fruit-set in bagged flowers (100%), Table 1. There were no fruit-set in emasculated flowers precluding agamospermy.

##### Hybridization

Only two of the 16 reciprocal crosses made set fruit (12.5%). Fruit development was normal. The seeds were normal and germinated in soil. Growth was

Table 1: Compatibility test and fruit set from open and controlled pollination of *I. triloba*

Treatment/Taxon	Open				Bagged				Crossed				Emasculated			
	a	b	c	d	a	b	c	d	a	b	c	d	a	b	c	d
<i>I. triloba</i> -population I	120	118	3-4	98	15	15	3-4	100	15	3	3-4	20	10	0	0	0
population II	107	102	3-4	95	12	12	3-4	100	10	1	3-4	10	10	0	0	0

Open: flowers observed on the field for open pollination

Bagged: Bagged flowers observed for spontaneous self-pollination

Crossed: Controlled hand cross pollinated flowers

Emasculated: Emasculated flowers observed for spontaneous fruit development

a: Number of flowers observed

b: Fruit set

c: Number of seed per fruit

d: Percentage fruit-set

vigorous and normal. Apart from the stem and petiole colour, most of the vegetative features of the hybrid were similar to those of the male parent (population I) particularly the glabrous and herbaceous appearance. Conversely, the reproductive features resembled the female parent (population II) with purple corolla colour, hairy ovary and fruit, and even-seed ornamentation. The detailed analysis of the parents' and F<sub>1</sub> hybrids' characteristics is presented in Table 2.

Cytological characteristics of the hybrid were normal. Pollen formation was normal. The pollens were spherical and highly fertile. The hybrid and parents were diploid (2n=30), Fig. 1a-c.

The notable characteristics of the F<sub>2</sub> plants were the appearance of white (male parent) and purple (female parent) flower individuals as well as early maturing plants (male parent). All were glabrous.

## Discussion

The diploid nature of the hybrid and the parents indicated that meiosis was probably normal in the parents, and the hybrids were likely formed from reduced haploid gametes.

The pattern of character inheritance showed

that purple flower colour was dominant over white in the F<sub>1</sub> while both colours reappeared in the F<sub>2</sub> progenies. Early maturity transmitted via the male parent also reappeared in some individuals in the F<sub>2</sub>. Intermediate individuals for varying characteristics (pubescence, reproductive age, and leaf, petiole, stem colour and texture) have been observed in natural populations thus implicating natural hybridization.

The rate of hybrid formation was low from controlled pollination and probably in natural population due to high degree of selfing and perhaps drawbacks to mating (Arnold, 1997), making hybridization only a chance occurrence. Such chance hybridization between pure line individuals introduces variations into populations. Hybrid vigour may equip hybrid populations with genetic mechanisms for better adaptation to changing and new environment than the parental form. Taxonomic complications arise from bred true homozygous pure lines which appear as morphologically varied population and unique taxonomic entities. Johnson *et al.* (1998) attributed such events to the description of over 80 species originally in *Apocynum* which were later reduced to seven.

Table 2: Comparison of Parents and F<sub>1</sub> hybrids of *Ipomoea triloba*

Character/Taxa	♂ <sup>†</sup> Population I	*F <sub>1</sub> hybrid	♀ <sup>‡</sup> Population II
Stem colour	Green - light brown	Brown - purple	Brown - red - purple
Stem indumentum	Glabrous - rarely sparsely hairy	Glabrous	Scabrid
Internode length (cm)	10.6 ± 2.3	9.7 ± 1.9	8.1 ± 1.4
Leaf shape	Cordiform/Trilobate	Cordiform/Trilobate	Cordiform/Trilobate
Leaf margin	Entire	Entire	Entire-ciliolate
Leaf texture	Herbaceous/fleshy	Herbaceous	Papery
Leaf colour	Glaucous green	Green	Green turning purple
Leaf indumentum	Glabrous	Glabrous	Glabrous-ciliolate
Leaf length x breadth (cm)	6.3 x 4.9	6.9 x 4.7	6.2 x 4.5
Petiole colour	Green-light brown	Brown - purple	Brown - red - purple
Petiole indumentum	Glabrous	Glabrous	Scabrid
Petiole length (cm)	5.2 ± 1.9	3.2 ± 1	3.7 ± 1.4
Inflorescence	Cyme	Cyme	Cyme
Pedicel length (mm)	5 ± 0.5	-	5 ± 1
Sepal indumentum	Pilose-ciliate	Pilose-ciliate	Villous-ciliate
Sepal colour	Yellowish green	Green	Green
Sepal shape	Lanceolate / oblanceolate	Lanceolate/oblanceolate	Lanceolate / oblanceolate
Sepal length x breadth (mm)	7 x 3	8 x 3	9 x 4
Corolla colour	White	Pale purple - purple	Pink - purple
Corolla length x limb diam. (cm)	2 x 1.5	2.5 x 2.2	2.1 x 1.7
Stamen length (cm)	1.1 ± 0.1	1.5 ± 0.1	1.4 ± 0.1
Ovary indumentum	Glabrous-sparsely hairy	Densely hairy	Densely hairy
Pistil length (cm)	1.3 ± 0.1	1.5 ± 0.05	1.5 ± 0.1
Fruit shape	Oval	Oval	Oval
Fruit indumentum	Glabrous - sparsely hairy	Pilose	Pilose
Fruit length x diam. (mm)	5 x 5	5 x 4.5	4 x 5
Seed shape	Deltoid	Deltoid	Deltoid
Seed ornamentation	Dotted	Even	Even
Seed indumentum	Glabrous	Glabrous	Glabrous
Seed length x diam. (mm)	3 x 2.5	3 x 2	3 x 2

Ogunwenmo 110<sup>†</sup>, 93<sup>‡</sup>, 109<sup>‡</sup>, 150<sup>‡</sup> (Abeokuta, Idi-aba, Oremeji area), 146<sup>‡</sup> (Lagos, Akoka, Univ. of Lagos, Biological garden)

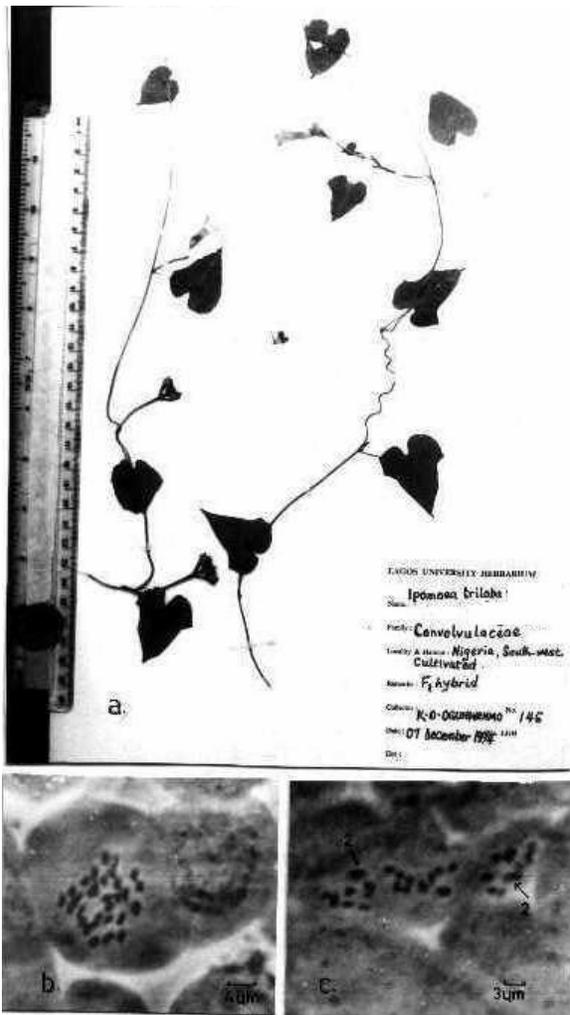


Fig. 1: Flowering shoot and chromosomes of *I. triloba*. a.  $F_1$  hybrid (*I. triloba* population I x population II), b-c. Somatic metaphase chromosomes of parent and  $F_1$  hybrid ( $2n = 30$ ), b. *I. triloba* population I, c.  $F_1$  hybrid.

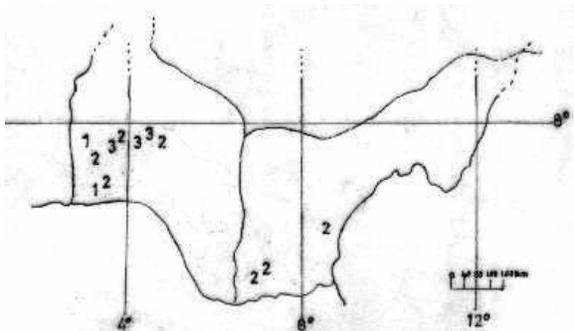


Fig. 2: Distribution of *I. triloba* in Nigeria - Parents [*I. triloba* population I (1) & population II (3)] and  $F_1$  hybrids (2)

The experimental  $F_1$  hybrids bore morphological resemblance to an intermediate natural population whose growth was vigorous and more widespread than either parent (Fig. 2). The relative fitness and success of hy-

brids with respect to their parents have been recognised generally in plants (Stebbins, 1984; Bennett & Grace, 1990; Arnold & Hodges, 1995; Arnold, 1997). Taxa of hybrid origin may themselves constitute the foundations upon which diversification occurs through ordinary divergent speciation (Eckenswalder, 1998). Natural hybridisation may again account for the high degree of morphological variability observed in *I. triloba* complex. The maintenance of such genetic variability and their sufficiency within and among populations are critical to long term survival of the species particularly in a changing environment with challenges of new selective pressure (Barrett & Kohn, 1991). Hybridization may be accompanied by introgression (Rieseberg & Wendel, 1993) leading to greater morphological differentiation and preponderance of morphological intermediates (Grant, 1981). Rieseberg (1995) considered hybrids as more than mere intermediates but a composite taxonomic entity and carriers of parental, intermediate and extreme characters. The high occurrence of extreme characters in the hybrid data presented showed that hybridization engenders variability which acts as bedrock for selection.

Taxonomic recognition of the variations in this complex is plausible since each of the populations maintained a measure of genetic integrity and morphological differentiation which are sometimes inclusive due to occurrence of intermediate individuals. Lewis (1959) had contended that genetic integrity could subsist despite genetic interchange among species. Three subspecies of *Sida rhombifolia* L. (subsp. *rhombifolia*, *retusa* and *alnifolia*) were described with clearly defined morphological differences (Ugborogho, 1980) despite intraspecific hybridisation and gene exchanges among them (Ugborogho, 1982). Moreso, a puzzling chance hybridisation between clonally propagated putative parents (*Apocynum cannabinum* L. and *A. androsaemifolium* L.) did not inhibit nor invalidate the specific recognition of *A. x-floribundum* Greene, the hybrid origin and taxonomic entity of which were verified by allozymes and morphological studies (Johnson *et al.* 1998).

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## References

- Allard, R.W. 1960. *Principles of Plant Breeding*. Japan: Wiley & Sons, 485pp.
- Arnold, M. L. 1993. *Iris nelsonii* (Iridaceae): Origin and genetic composition of a homoploid hybrid species. *Am. J. Bot.* **80**: 577-583.
- Arnold, M. L. 1997. *Natural Hybridization and Evolution*. Oxford: Oxford University Press.
- Arnold, M. L. & Bennett, B. 1993. Natural hybridization in Louisiana irises: Genetic variation and Ecological determinants. In: *Hybrid zones and the Evolutionary process*, R. G. HARRISON (ed.). Oxford: Oxford University Press, p. 115-139.
- Arnold, M. L. & Hodges, S.A. 1995. Are natural hybrids fit or unfit relative to their parents? *Trends in Ecology and Evolution* **10**: 67-71.
- Bachmann, K. 1994. Molecular markers in plant ecology. *New Phytologist* **126**: 403-418.
- Barret, S. C. H. & Kohn, R. 1991. Genetic and evolutionary consequences of small population size in plants: implications for conservation. In: D. A. FALK & K. E. HOLSINGER (eds.), *Genetic conservation of rare plants*, p. 1-30. New York.
- Bennett, B. D. & Grace, J. 1990. Shade tolerance and its effect on the segregation of two species of Louisiana *Iris* and their hybrids. *Am. J. Bot.* **77**: 100-107.
- Charlesworth, D. & Charlesworth, B. 1987. The effect of investment in attractive structure on allocation to male and female function in plants. *Evolution* **41**: 948-986.
- Cole, C. T. & Kuchenreuther, M. A. 2001. Molecular markers reveal little genetic differentiation among *Aconitum noveboracense* and *A. columbianum* (Ranunculaceae) populations. *Am. J. Bot.* **88**: 337-347.
- Cruzan, M. B. 1998. Genetic markers in plant evolutionary ecology. *Ecology* **79**: 400-412.
- Davis, P. H. & Heywood, V. H. 1963. *Principles of Angiosperm Taxonomy*. –New Jersey.
- Eckenwalder, J. E. 1998. Hybridization as Evolutionary creation. *Am. J. Bot.* **85**: 1043-1045.
- Eggers, H. F. A. Von 1879. Flora of St. Croix and the Virgin Islands. *Bull. U. S. Natl. Mus.* **13**: 71
- Fryxell, P. A. 1957. Mode of Reproduction of Higher Plants. *Bot. Rev.* **23**:135-233.
- Gallez, G. P. & Gottlieb, L. 1982. Genetic evidence for the Hybrid origin of the diploid plant *Stephanomeria diegensis*. *Evolution* **36**: 1158-1167.
- Grant, V. 1963. *The Origin of Adaptations*. New York: Columbia University Press.
- Grant, V. 1981. *Plant speciation*. New York: Columbia University Press.
- Johnson, S.A., Bruederle, L. P. & Tomback, D. F. 1998. A mating system conundrum: Hybridization in *Apocynum* (Apocynaceae). *Am. J. Bot.* **85**:1316-1323.
- Ledig, F. T. 1986. Heterozygosity, heterosis and fitness in outbreeding plants. In: *Conservation Biology: The Science of Scarcity and Diversity*, M. E. SOUTE (ed.). Sunderland, MA: Sinauer, p. 77-104.
- Levin, D.A. & Bulinska – Radomska, Z. 1988. Effects of Hybridization and Inbreeding on fitness in *Phlox*. *Am. J. Bot.* **75**: 1632-1639.
- Lewis, H. 1959. The Nature of Plant Species *J. Arizona Acad. Sci.* **1**: 3-7.
- Meissner, C. F. 1869. Convolvulaceae. In: *Flora Brasiliensis* **7**, K. F. P. Martius (ed.).
- Potts, B. M. 1986. The population dynamics and regeneration of a hybrid zone between *Eucalyptus risdonii* Hook. f. and *E. amygdalina* Labill. *Austral. J. Bot.* **34**: 305-329.
- Rieseberg, L. H. 1995. The role of hybridization in evolution: Old wine in new skins. *Am. J. Bot.* **82**: 944 - 953.
- Rieseberg, L. H. & Wendel, J. 1993. Introgression and its consequences in plants. In: *Hybrid zones and the evolutionary process*, R. G. HARRISON (ed.). Oxford: Oxford University Press, p. 70-109.
- Scott-Emuakpor, M. B. & Ugborogho, R. E. 1980. Cytogenetic studies on *Sida* species in Nigeria: *S. acuta* complex, *S. garckeana* and *S. scabrida*. *Bol. Soc. Brot.* **53**:443-463.
- Smith, J. F. & Pham, T. V. 1996. Genetic diversity of the narrow endemic *Allium aaseae* (Alliaceae). *Am. J. Bot.* **83**: 717-726.
- Stebbins, G. L. 1984. Polyploidy and the Distribution of the Arctic-Alpine Flora: New evidence and a new approach. *Botanica Helvetica* **94**: 1-13.
- Ugborogho, R. E. 1973. North American *Cerastium arvense* L. I. Cytology. *Cytologia* **38**: 559-566.
- Ugborogho, R. E. 1980. The Taxonomy of *Sida* L. (Malvaceae) in Nigeria. II. The *S. rhombifolia* complex *Bol. Soc. Brot.* **54**: 65-85.
- Ugborogho, R. E. 1982. Cytogenetic studies on the *Sida rhombifolia* complex in Nigeria. *Cytologia* **47**: 11 - 20.
- Wyatt, R. 1983. Pollinator-Plant interactions and the Evolution of Breeding systems. In: *Pollination Biology*, L. A. Real (ed.). Orlando: Academic Press, 51 - 95.