

INCOMPATIBILITY STUDIES IN THE GENUS *GIBASIS* SECTION *HETEROBASIS* D. R. HUNT (COMMELINACEAE)

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SUMMARY

Two species in the genus *Gibasis* section *Heterobasis* have been examined to confirm their incompatibility reaction and to determine its genetic control. Five individuals of *G. oaxacana* ($2x = 16$) from a single Mexican population (Hunt 8175) were found to be self-incompatible. Three accessions of tetraploid cytotypes ($4x = 32$; Hunt 7012, 7072 and 7160) and two accessions of hexaploid cytotypes ($6x = 48$; Jones 72 and Bacigallipo 711257) of *Gibasis geniculata* were found to be self-compatible. Analysis of an F_1 population raised from an intercross between two heterozygous genotypes of *G. oaxacana* was consistent with an hypothesis that self-incompatibility is controlled by a single multi-allelic gene acting gametophytically in the pollen.

Unilateral incompatibility was found in crosses between *G. oaxacana* and *G. geniculata*.

1. INTRODUCTION

THE purpose of this study is to confirm the existence of self-incompatibility in *Gibasis oaxacana* and to determine its genetic control. An attempt is made to demonstrate the relationship between species in the section *Heterobasis* of the genus particularly with regard to the evolution of the breeding system.

For several years at Kew considerable effort has been focused on the cytology and cytotaxonomy of the *Tradescantia* family, Commelinaceae (Jones and Colden, 1968; Jones, Colden and Hunt, 1969; Jones and Kukkonen, 1971; Jones and Jopling, 1972; Jones, Papes and Hunt, 1975; Jones, 1974, and unpub.). Not surprisingly these results have often influenced taxonomic decisions (*e.g.* Hunt, 1975) as well as giving support to particular taxonomic treatments. Chromosome data for example supports the taxonomic treatment of Rohweder (1956) in separating the genus *Gibasis* (with at least 14 species) from *Tradescantia*. The species of the genus *Gibasis* ($x = 4, 5$) examined by Jones and Jopling (1972) were unlike *Tradescantia sensu stricto* in basic number and all, apart from diploid *G. pulchella* ($2n = 10$), had asymmetric chromosome complements.

Gibasis still embraces a remarkable variation in chromosome number, size and shape but it can be partitioned into a number of groups on the basis of karyotype (Jones, Papes and Hunt, 1975) and has been divided broadly into two sections, *Gibasis* and *Heterobasis* (Hunt, 1975). *Heterobasis*, the concern of this paper, includes two species both with relatively small asymmetric chromosomes and a basic number of $x = 8$. *G. oaxacana* is self-incompatible and diploid ($2x = 16$), *G. geniculata* is highly self-fertile and two cytotypes have been found, *viz.* a tetraploid ($4x = 32$) and a hexaploid ($6x = 48$; Jones, Colden and Hunt, 1969).

Although self-incompatibility is widespread among species in the family (Owens, unpub.) attempts to discover its genetic control have been focused exclusively on North American species of *Tradescantia*. Anderson and Sax (1934) in an analysis based upon aceto-carminic smears of self- and cross-pollinated styles in three *Tradescantia* species, viz. *T. edwardsiana*, *T. gigantea* and *T. humilis*, decided that their results were in broad agreement with the oppositional factor hypothesis (incompatibility of the gametophytic type). This conclusion received support from the work of Brewbaker (1957, 1967). The mature pollen grain of species in the genus *Tradescantia* is of the binucleate type (containing a single vegetative and a single undivided generative nucleus) and Brewbaker (l.c.) showed that the gametophytic type of incompatibility system was almost always associated with species which had mature binucleate pollen grains. The most complete analysis has been that of Annerstedt and Lundquist (1967) in *T. paludosa*. From a cross between two self-incompatible individuals obtained from different sources they raised an F_1 progeny of 200 plants. Ten were selected and intercrossed in a diallel together with the two parents, and fell into four intra-incompatible, inter-compatible groups with respect to mating behaviour. All were reciprocally cross-compatible with the parents. The remaining 190 F_1 individuals when crossed with selected single plants from each of the four groups were incompatible with one and totally compatible with the remaining three. From their results Annerstedt and Lundquist (l.c.) favoured an interpretation involving a single multi-allelic locus operating gametophytically in the pollen. They could not, however, completely exclude an interpretation involving a second locus. This latter hypothesis appeared improbable since it required the genotype of the original parents to contain identical alleles at one locus, e.g. $S_{1,2} Z_{5,5}$ and $S_{3,4} Z_{5,5}$, a situation considered to be most unlikely when the sources of the original material were so different.

2. MATERIALS AND METHODS

Plants of *G. oaxacana* and *G. geniculata* were taken from the collection of Commelinaceae held at Kew and are listed in table 1. All individuals were grown in greenhouses (temperature range 13-20°C) in isolation from other *Gibasis* species during the winter period when insect pollinators were entirely absent.

(i) *Self-pollinations and intraspecific crosses*

Self- and cross-pollinations were made in the morning and for each pollination a single anther was removed from a flower with forceps and pollen dusted on to the recipient stigmas. (Flowers were not emasculated.) Each pollinated flower was marked by sticking a coloured adhesive spot to a petal and careful records were kept of each cross performed. An average of four (range 1-10) flowers was used to determine the compatibility of self- or cross-pollinations. All pollinations were made during the period, 7th November 1972 to 23rd February 1973 inclusive.

Seed capsules from compatible pollinations could be scored after about 1 week from pollination. In incompatible matings flowers dropped from the inflorescence 3-4 days after pollination.

Controlled crosses between two self-incompatible genotypes of *G.*

oaxacana (8175G × H) yielded 11 capsules and 63 seeds from which 31 F_1 hybrid individuals were raised. F_1 plants were intercrossed in a 21 × 21 diallel.

Pollen tube growth was observed in stigmatic tissues by means of the callose fluorochrome reaction (de Crehu, 1968). For the assessment of pollen tube growth self-pollinations were performed and recorded as above.

(ii) *Interspecific crosses*

Although the flowers of *G. geniculata* open in the afternoon, cross-pollinations are possible in the morning. Flowers which are due to open in an afternoon are recognisable in the morning because the white petals begin to show as the sepals separate. Flowers in this condition were forced open with forceps, emasculated and pollinated with pollen from *G. oaxacana*. Often pollen was already being shed in the closed bud (cleistogamy) but buds in this state were not used as females in crosses. Pollen from these buds was, however, used in the reciprocal with *G. oaxacana*. Seed set and pollen tube growth were observed and recorded as above.

3. RESULTS

(i) *Self-pollination*

The results of selfing are shown in table 1. Three tetraploid accessions and two hexaploid accessions of *G. geniculata* were self-compatible. Five individuals of the diploid *G. oaxacana* collected from a single site were self-incompatible. Pollen tubes were inhibited in the stigmatic tissues (plate 1 (a) and (b)).

(ii) F_1 analysis

The mating behaviour of the 21 F_1 progeny is shown in table 2. These plants were all self-incompatible (as their parents) and could be grouped into four intra-incompatible, inter-compatible classes containing three, seven, two and nine individuals respectively. The numbers of plants found in each of the four pollination groups do not differ significantly from the expected 1 : 1 : 1 : 1 ratio, since $\chi^2_{(3)} = 6.238$, $P = 0.2-0.1$. This latter result accords with an hypothesis that control is by a single multi-allelic locus operating gametophytically in the pollen. It also confirms that the parents were heterozygous for alleles at the S locus and had no alleles common.

(iii) *Interspecific crosses*

The results of the interspecific crosses (table 3) show that the pollen of the self-compatible *G. geniculata* is inhibited on the stigma of self-incompatible *G. oaxacana* while the reciprocal cross at either ploidy level is compatible. Inhibition of pollen tubes in incompatible matings is in the stigmatic tissue and is therefore the same site as for the self-incompatible reaction. In compatible matings pollen tube growth was normal although the rate of growth was not recorded.

TABLE I
The incompatibility system of two species in the section Heterobasis D.R. Hunt

Species	Accession no.	Collector	Chr. no.	Reference	Seed set.	Pollen tube growth on self-pollination
<i>Gibasis geniculata</i> (Jacq. Rohw.)	466-69.03799	Hunt 7012	4x = 32	Jones, Papes and Hunt, 1975		+
<i>Gibasis geniculata</i> (Jacq. Rohw.)	473-69.03875	Hunt 7072	4x = 32	Jones, Papes and Hunt, 1975		+
<i>Gibasis geniculata</i> (Jacq. Rohw.)	504-69.04119	Hunt 7160	4x = 32	Jones, Papes and Hunt, 1975	+	
<i>Gibasis geniculata</i> (Jacq. Rohw.)	163-72.01519	Jones 72	6x = 48	Jones, Papes and Hunt, 1975	+	
<i>Gibasis geniculata</i> (Jacq. Rohw.)		Bact. 711257	6x = 48	Jones, unpub.		+
<i>Gibasis oaxacana</i> D. R. Hunt	337-71.03052/3	Hunt 8175 A, C, D, G and H	2x = 16	Jones, Papes and Hunt 1975	-	-

+ Self-compatible.

- Self-incompatible.

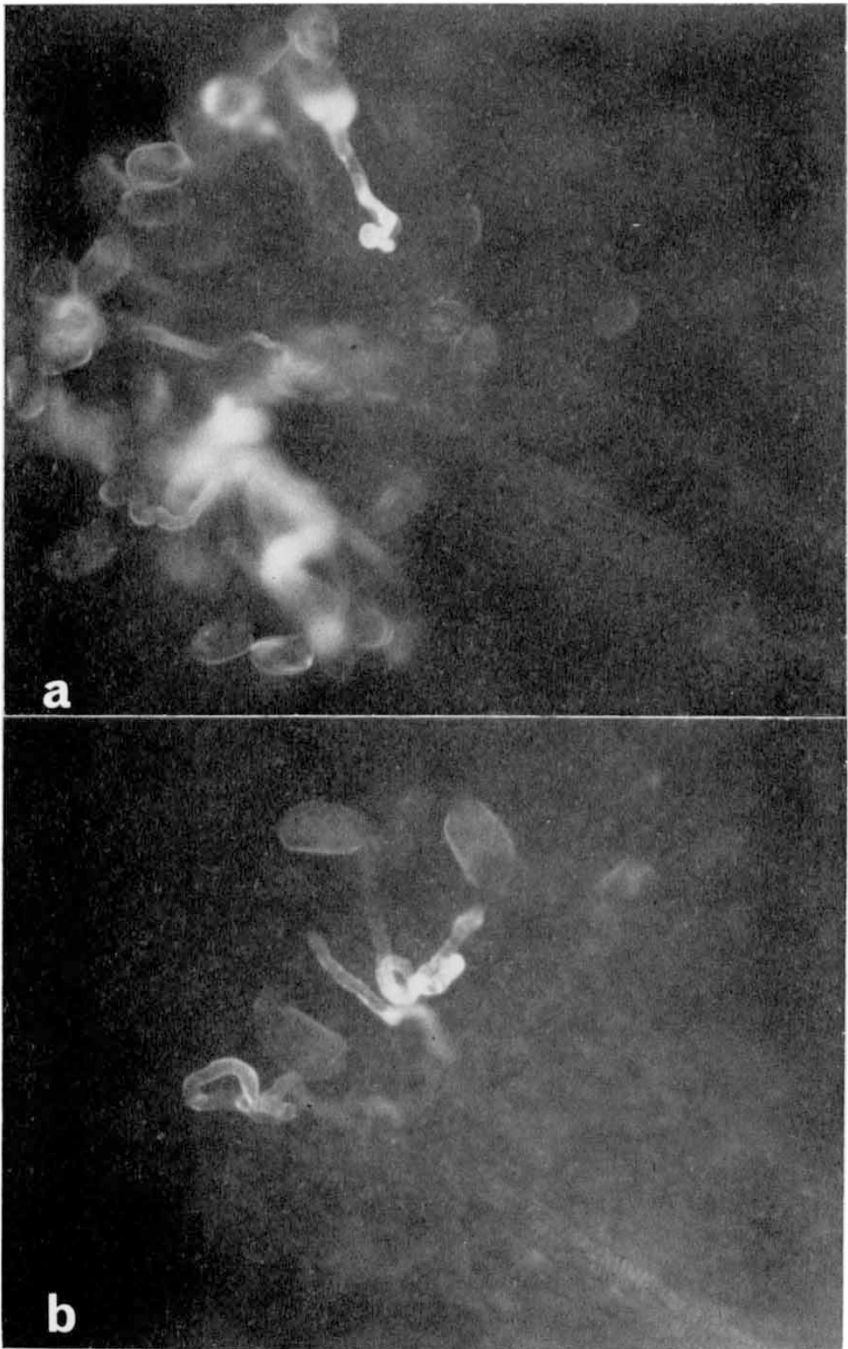


PLATE I(a) and (b). Inhibition of pollen tubes in the stigma tissues of *G. oaxacana* after self-pollination.

TABLE 2

The results of intercrossing 21 F_1 plants of *G. oaxacana* arranged into four inter-compatible, intra-compatible groups, A-D

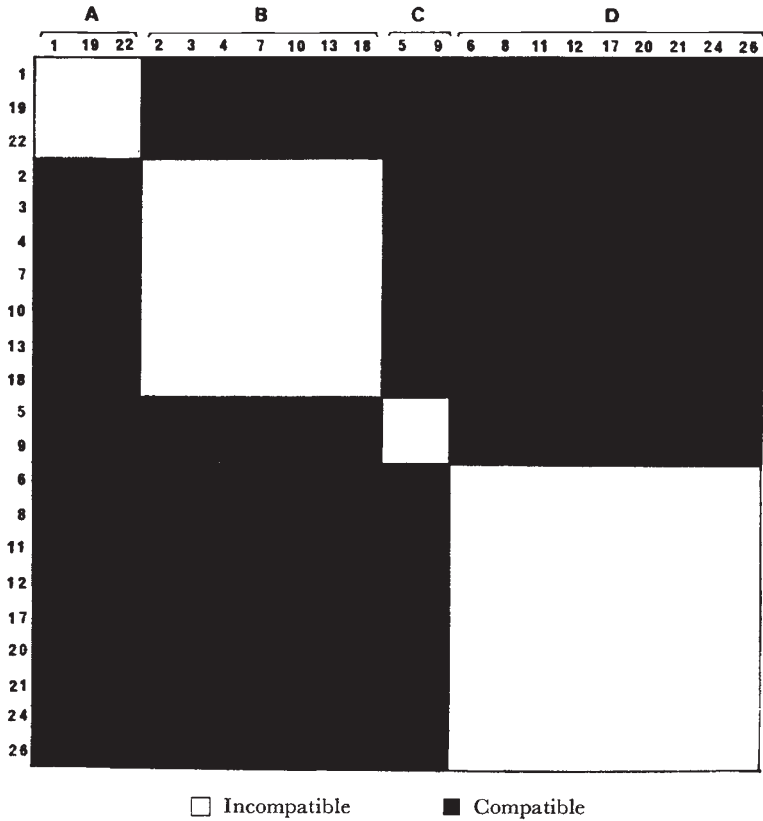


TABLE 3

Pollen tube growth in crosses between *G. oaxacana* and *G. geniculata*

♀	♂		
	<i>G. oaxacana</i>	<i>G. geniculata</i> 4x	<i>G. geniculata</i> 6x
<i>G. oaxacana</i>	-	○	-
<i>G. geniculata</i> 4x	+	+	○
<i>G. geniculata</i> 6x	+	○	+

+ = Normal pollen tube growth in style.
 - = Pollen tubes inhibited in top 1 mm of style.
 ○ = Cross not made.

4. DISCUSSION

Self-incompatibility in *G. oaxacana* has been found to be controlled by a single locus gametophytic system, the most common system found in

Angiosperm families. This result is in complete agreement with the interpretation of Annerstedt and Lundquist (1967) for diploid *T. paludosa*. The two genotypes of *G. oaxacana* which were crossed, 8175G and H, may therefore be designated $S_{1,2}$ and $S_{3,4}$ respectively, the genotypes of the four F_1 classes being $S_{1,3}$, $S_{2,3}$, $S_{1,4}$ and $S_{2,4}$.

Unlike the species of *Tradescantia* investigated (Anderson and Sax, 1934; Annerstedt and Lundquist, 1967) the related polyploid *G. geniculata* is not self-incompatible. Indeed it is highly self-fertile. The results obtained from crosses between *G. oaxacana* and *G. geniculata* are those expected for crosses between self-incompatible and self-compatible species. Indeed, these results extend the rule of Lewis and Crowe (1958) that unilateral incompatibility is of universal occurrence regardless of the type of control of self-incompatibility. *G. geniculata* would appear to be of the SC type (Lewis and Crowe, l.c.) and the self-fertility therefore of ancient origin. This conclusion is given added support by the reduction in size of all floral parts in *G. geniculata* in comparison to those of *G. oaxacana* (Jones, Colden and Hunt, 1969; Hunt, 1972) and to the occurrence of cleistogamous flowers in the former species, both strong adaptations to inbreeding.

It is tempting to suggest that the self-compatibility in *G. geniculata* arose because of the change in chromosome number from diploid to tetraploid and hexaploid. This situation, although rare in monocotyledons (see below), is well known for diploid and tetraploid species of several members of the dicotyledons which possess a gametophytic incompatibility system (e.g. see Lewis, 1954). An increase in the level of ploidy leads to either partial or full self-compatibility because of allelic interaction in the pollen which results in both alleles failing to produce their effect. An hypothesis which explains the origin of self-compatibility in *G. geniculata* cannot, however, be erected on the basis of results in this study for two chief reasons. Firstly the relationship of the two species in the section *Heterobasis* although close is not simply one of a change in ploidy levels. External morphology including leaf and floral characters (Hunt, 1972) as well as anatomical features (Stant, 1973) clearly distinguish the species and although they are similar in both chromosome size and asymmetry of the chromosome complement, differences in karyotype morphology support their separation. Their distributions are also different. *G. geniculata* is distributed widely in lowland tropical America whilst *G. oaxacana* is known only from one locality in the state of Oaxaca in Mexico (Hunt, 1972). It is highly unlikely from several points of view, therefore, that the polyploid arose directly from the diploid. Secondly polyploid species in the other section, *Gibasis*, of the genus are self-incompatible (Owens, unpub.) as are the polyploid species of *Tradescantia* (Anderson and Sax, 1934; Annerstedt and Lundquist, 1967). Indeed the lack of allelic interaction in polyploid species appears to be a characteristic of the Monocotyledons as a whole (Sagawa, 1958; Collins, 1961; Murray, 1974; Lundquist, 1975) and is thought to be a prerequisite for the development of the two-locus gametophytic system found in the family Gramineae (Murray, 1974; Lundquist, 1975). This latter situation will be discussed more fully in a later publication.

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