The artificial synthesis of *Solanum* × *procurrens* Leslie (*S. nigrum* L. × *S. sarrachoides* Sendtn.)

J. M. EDMONDS

Botany School, Downing Street, Cambridge

ABSTRACT

The artificial synthesis of the hybrid *Solanum* × *procurrens* Leslie from the parental species *S. nigrum* L. and *S. sarrachoides* Sendtn. is described, and a comparison made between the natural and the artificially derived hybrid. The fertility of the original parental populations, and the possible occurrence of natural amphiploids, are discussed, and additional records of the hybrid are given.

INTRODUCTION

In the Autumn of 1975 A. C. Leslie found several plants of a putative *Solanum* hybrid in mixed populations of *S. nigrum* L. subsp. *nigrum* and *S. sarrachoides* Sendtn. at Gamlingay, Cambridgeshire. These plants were later confirmed as interspecific hybrids, and formally described as *Solanum* × *procurrens* Leslie (Leslie 1978).

The hybrid nature of these plants was established from living and herbarium specimens collected from the mixed Gamlingay populations. The progeny grown from seeds collected from the plants of *S. nigrum* and *S. sarrachoides* proved to be hexaploid ($2n = 72$) and diploid ($2n = 24$) respectively, and both the original plants and their offspring displayed high pollen stainability and seed set values (Table 1). The hybrid plants were morphologically intermediate, tetraploid ($2n = 48$) and completely sterile. In contrast to the parental taxa, potential pollen fertility was extremely low and, though the plants developed large numbers of berries, these contained abortive ovules (Leslie 1978; Table 1). The contents of over 30 berries were examined, and only one potentially well-developed seed was found, which proved to be inviable. Although such data confirmed the probable hybrid origin of these plants, it was thought that the artificial synthesis of similar progeny would verify their hybridity beyond dispute.

ARTIFICIAL SYNTHESIS OF THE HYBRID

METHODS

The methods used for berry and seed harvesting, seed sowing, pollination, pollen stainability assessments, and cytological preparations follow those given in Edmonds (1977). The pollen stainability data were recorded from 500 grains per accession, and the numbers of seeds/berry were averaged from five berries per accession (Table 1).

Seeds harvested from the parental taxa at Gamlingay in 1975 were sown in the spring of 1976 following pre-treatment with 2000 p.p.m. gibberellic acid. When mature, plants of *S. nigrum* (C115) and *S. sarrachoides* (C114) were transferred to an insect-proofed glasshouse where, following emasculation, they were reciprocally pollinated.

RESULTS

When *S. nigrum* was used as the maternal parent (i.e. C115 × C114), 75% of the pollinations were successful, with the resultant berries setting 12–32 ($\bar{x}$ 25·3) seeds/berry. All pollinations were successful when *S. sarrachoides* was used maternally (i.e. C114 × C115), but the resultant berries only contained abortive seeds.

TABLE 1. FERTILITY DATA OF PARENTAL AND HYBRID ACCESSIONS

	Chromosome number	Germination No.	Germination %	% Pollen stainability	Seed set Range	Seed set Average
Original collections						
S. nigrum (Edmonds 41)	—	—	—	88·6	5–29	17·0
S. sarrachoides (Edmonds 40)	—	—	—	98·8	17–19	17 8*
Hybrid	2n = 48	—	—	0·6–1·6†	0	0‡
Experimental accessions						
S. nigrum (C115)	2n = 72	23/28	82·1	69·0	42–58	48·2
S. sarrachoides (C114)	2n = 24	17/26	65·4	99·6	16–23	19 8*
Artificial hybrid C115 × C114 (M1473)	2n = 48	17/25	68·0	4·8	0	0

* +1–2 sclerotic granules   † Average of three plants 0·96%   ‡ 30 berries examined.

The F$_1$ hybrids (M1473) derived from the successful crosses were grown in 1977 (see Plates 3 & 4). These plants were tetraploid and sterile, as expected; their germination, pollen stainability, berry and seed set data are recorded in Table 1. Artificial selfing of these plants failed to result in any berry set, but 50% of the sib-mated pollinations were successful, though the resultant berries again only contained abortive ovules.

The plants exhibited the following characters. Stems sprawling in excess of 2m, ascending to c. 60cm, moderately pubescent with ascending or appressed and occasional glandular multicellular (> 3-celled) hairs. Leaves lanceolate to ovate-lanceolate, sinuate-dentate with 2–6 lobes. Inflorescences simple extended cymes with 7–9 flowers, generally extra-axillary; peduncles to 20mm, many subtended by small leaves; pedicels to 10mm. Corolla c. 7mm diameter, white to pale purple with conspicuous yellow and brown basal star. Anthers c. 2·3mm long, pollen 20·7–35·4 (x̄ 26·1) μm diameter; filaments c. 1·9mm long. Styles c. 3·9mm. Berries purple/black, dull, broadly ovoid, c. 4 × 3mm, parthenocarpic, with 1–2 sclerotic granules, on patent to reflexed pedicels, bases surrounded by adherent/accrescent calyces, shed with pedicels.

A table comparing the more important characters exhibited by the parents with those of the hybrid is given in Leslie (1978).

DISCUSSION

Morphologically, the artificially derived tetraploid plants were virtually identical with the natural hybrids collected at Gamlingay (Plates 3 & 4). Plants of the artificial hybrid grown in the experimental field displayed the vigorous sprawling habit observed in the natural populations (see Leslie 1978). Though some of the features described above differ slightly from those recorded by Leslie (1978), natural hybrid plants transplanted from Gamlingay to the University Botanic Garden displayed very similar characters and dimensions, and the differences are probably due to personal interpretation of the more subjective characters. The leaf margins of the experimental plants were all sinuate-dentate, whereas this character varied from plant to plant in the natural populations, those of some plants being sinuate rather than sinuate-dentate. This reflects the variability of this character in both parental taxa (see Leslie 1978), and probably demonstrates the polytopic origin of the hybrid plants at Gamlingay.

The ease with which this hybrid could be synthesized confirmed previous experimental work, where, out of 25 attempts to cross accessions of S. nigrum and S. sarrachoides, 23 were successful, resulting in prolific seed set (Edmonds 1977, 1979 and unpublished). Some of these successful crosses involved S. nigrum as the maternal parent and others S. sarrachoides. They also included many morphological variants of S. nigrum, involving accessions with different habits, leaf margins and berry colours of both subsp. nigrum and subsp. schultesii (Opiz) Wess. The morphology of the hybrid progeny derived from all these crosses was extremely similar to that observed in M1473 and, when the eglandular-haired subsp. nigrum was used parentally, the pubescence of the resultant progeny was identical with that

PLATE 3. Artificial synthesis of *Solanum × procurrens*. A. *S. nigrum* (C115), female parent. B. Sterile tetraploid derivative (M1473). C. *S. sarrachoides* (C114), male parent.
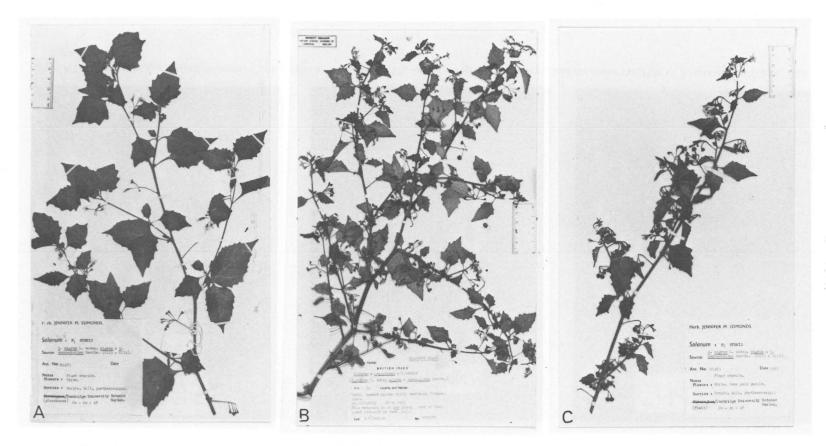
PLATE 4. A. Glasshouse herbarium specimen of M1473. B. Part of holotype plant of natural *Solanum* × *procurrens*. C. Field herbarium specimen of M1473.

observed in the Gamlingay hybrids. The pubescence of hybrids derived from the use of the glandular-haired subsp. *schultesii* parentally was generally much denser, with the majority of the multicellular hairs being glandular and spreading.

The two failures encountered during these previous attempts at inter-specific hybridization both involved *S. nigrum* subsp. *nigrum* maternally, and neither cross resulted in any berry set. The development of berries with abortive ovules when *S. sarrachoides* was used maternally was therefore rather unexpected. Genome disharmony, largely caused by the incorrect balance in ploidy levels between the embryo and endosperm, was most probably responsible for these empty berries. Although such disharmony is probably more easily overcome when the female parent is of the higher ploidy level, as is the case here, this does not explain my earlier results where two crosses involving the hexaploid *S. nigrum* maternally failed completely, and where the reciprocal crosses involving *S. sarrachoides* maternally were successful (see Edmonds 1977, 1979). It must be assumed that the Gamlingay populations of *S. sarrachoides* constituted a strain of this species that was more genetically isolated from *S. nigrum* than the accessions of this species that had been previously used successfully as the maternal parents in similar crosses. Although these observations might suggest that the Gamlingay hybrids had arisen through the pollination of *S. nigrum* by *S. sarrachoides*, there is no proof that these naturally-occurring hybrids had arisen in this way; it is possible that they arose from the reciprocal cross, or from a combination of both types of crosses.

The plants of *S. nigrum* at Gamlingay did not seem to be as fertile as expected from previous work on this species. Although the pollen stainability of the plant collected at Gamlingay (*Edmonds 41*) was 88·6%, this fell to 69·0% the following year in the plants (C115) raised from the seed extracted from this plant. Such a low value was unexpected in this species, where pollen stainability values are usually in excess of 90% (see Edmonds 1977). Similarly, the potentially viable seed numbers/berry were low for typical *S. nigrum*, ranging from 5–29, and the berries also contained many empty seeds – an extremely unusual occurrence in this species. However, in the following year, the plants obtained by selfing C115 set berries which contained potentially viable seed numbers that were more typical of this taxon (ranging from 42 to 58 ($\bar{x}$ 48·2) in experimentally selfed berries). Moreover, the pollen stainability values of the plants grown on from this selfed seed in 1978 increased to 94·8%. Repeated selfing therefore seemed to restore the fertility of these *S. nigrum* plants. The fertility of *S. sarrachoides*, on the other hand, remained stable, setting characteristic numbers of seeds/berry, with the pollen stainability even increasing slightly in the experimental accession (Table 1).

It is possible that the pollen from *S. sarrachoides* and/or from the tetraploid hybrid (where the occasional grain is potentially functional) was 'contaminating' the *S. nigrum* population at Gamlingay. Such mixed pollination might have caused the low seed numbers and the development of occasional empty seeds observed in *S. nigrum*, and the dramatic increase in viable seed set in the experimentally-selfed berries of this species (Table 1) might be considered to support this hypothesis. However, it is difficult to explain the low pollen fertility recorded in the offspring (C115) of the original plant (*Edmonds 41*) collected at Gamlingay. Unfortunately, the sample size was much too small for any conclusions to be drawn, and a satisfactory explanation would require extensive field sampling of *S. nigrum* at Gamlingay.

The species of the section *Solanum* are largely autogamous, but cross pollination can and does occur in this species group and natural inter- and intra-specific hybrids have now been reported for a number of the component species (Edmonds 1979). That cross pollination was probably occurring at Gamlingay is demonstrated by the fact that artificial selfing of the experimental hybrid failed to produce any berry set, whereas 50% of the sib-mated flowers resulted in berry set. Many of the natural hybrid plants bore abundant berries, which could well have arisen through cross pollination.

Although Leslie (1978) failed to trace any published records of this hybrid, it is probably quite widespread in mixed populations of *S. sarrachoides* and *S. nigrum*. *Solanum sarrachoides* sometimes occurs as a component of wool shoddy, which is locally spread on light sandy agricultural soil in south-eastern Britain. New strains of this species are therefore probably being introduced to these areas from its native habitats in South America. The species can be highly successful on these soils, and has become an established alien in a few places. It also occurs more widely as a casual alien on refuse tips and other waste places. In all areas where the distribution of this species overlaps with that of the native *S. nigrum*, hybridisation is likely, though not inevitable. According to Leslie (pers. comm.), large mixed populations of these two species can produce abundant hybrids (as at Gamlingay), a few hybrids, or none at all (as in some Surrey populations).

In addition to the records listed by Leslie (1978), this hybrid has now been positively identified from the following sites (Leslie pers. comm.):

Market garden field , Maulden, Beds., v.c. 30, GR 52/062.374, September 1977, H. J. M. Bowen, comm. J. G. Dony ('One large plant').
Market garden field, between Broom and Biggleswade, Beds., v.c. 30, GR 52/182.435, 23 October 1977, E. J. Clement ('very numerous', 'both parents abundant').
Sugar beet field, Milton, Cambs., v.c. 29, GR 52/472.631, 17 November 1977, G. M. S. Easy, conf. A. C. Leslie ('a single, large plant' ; 'mixed populations of parents quite common about Milton').
Market garden field, near Flitwick, Beds., v.c. 30, GR 52/026.358, Summer 1978, C. G. Hanson and B. Wurzell ('quite frequent', 'both parents abundant', 'field record').
Dumped soil by road, Kennett, Cambs., v.c. 29, GR 52/701.682, 21 September 1979, G. M. S. Easy, conf. A. C. Leslie (**herb. A. C. Leslie**), ('a single plant', 'only *S. nigrum* present').

It is highly probable that the critical examination of *Solanum* collections in various European herbaria will reveal specimens of *Solanum* × *procurrens*, as yet unrecognized. The only positive herbarium record that I have so far encountered was collected in Sweden, as long ago as 1958 (*A. Nilsson s.n.*: *S. nigrum* L. × *S. nitidibaccatum* Bitter 'Flora Suecia; Scania, Saxtorp. Flygeltofta. 1 planta bland föräldraarterna.' [one plant among the parents]. 26.7.1958, **H.**).

The discovery of this hybrid at Gamlingay was of considerable interest, since my previous experimental work on the derivation of *S. nigrum* had demonstrated that this hexaploid might contain a set of genomes from the diploid *S. sarrachoides* (Jardine & Edmonds 1974; Edmonds & Glidewell 1977; Edmonds 1977, 1978, 1979). This hypothesis was partly based on the fact that although *S. sarrachoides* seemed to be completely isolated genetically from all other species in the section *Solanum*, it could be easily hybridized with *S. nigrum*. Moreover, amphiploidy had been readily induced in a similar tetraploid hybrid between *S. nigrum* subsp. *nigrum* and *S. sarrachoides* (Edmonds 1979). The resultant octoploid plants were extremely vigorous, and moderately fertile, spontaneously setting large numbers of berries. These berries contained moderate numbers of seeds in the F_2 generations, but relatively few in the F_3 generations.

In 1976, spontaneous fertile branches arose on two otherwise sterile plants of another artificial tetraploid hybrid between *S. nigrum* and *S. sarrachoides*, though, this time, the maternal parent was the glandular-haired subsp. *schultesii* (Edmonds unpublished). Morphologically the F_2 and F_3 generations subsequently raised from this spontaneous amphiploidy were very similar to the artificially-induced octoploids. The stem and leaf hairs were denser, and largely glandular and spreading, however, thereby reflecting the indumentum-types of the two parents.

The fertility of these spontaneous octoploid plants was generally higher than that recorded from their artificially-induced counterparts. This was particularly true of the F_3 generation, where germination, pollen stainability and seed set values remained comparable to those recorded from the F_2 generation plants. The decrease observed in the pollen and seed fertility of the artificially-derived octoploids was provisionally attributed to the duplication of similar genomes in the hybrid, causing meiotic disturbances in both the pollen mother cells and the embryo sac mother cells; these unbalanced segregations possibly became accentuated in the F_3 generations through successive inbreeding (Edmonds 1979). This hypothesis may need partial revision in view of the higher fertility observed in the spontaneous octoploid progeny, as reported here. The increase in fertility noted in the subsequent generations of these plants could be due to the involvement of the glandular-haired *S. nigrum* subsp. *schultesii*. However, since this is probably closer genetically to the glandular-haired *S. sarrachoides* than is the eglandular-haired subsp. *nigrum*, which had been used as the maternal parent in the artificially-induced octoploids, more meiotic disturbances might be expected in such progeny. It would obviously be of interest to study the fertility of artificially-induced octoploids derived from hybrids between the subsp. *schultesii* and *S. sarrachoides* in order to clarify this point.

It is possible that such spontaneous amphiploidy may occur naturally, and that similar fertile branches may occasionally arise on otherwise sterile plants of *S.* × *procurrens*. Any seed germinating from such a source could give rise to vigorous octoploid plants, which would probably be mistaken for *S. nigrum*, and especially for the subsp. *schultesii*. The general morphology of the artificially-derived octoploids is illustrated in Edmonds (1979), where details of flowering and fruiting inflorescences are also included. Though such amphiploids may establish themselves as natural octoploid populations, they would probably be at a competitive disadvantage to the parental species, since their overall

fertility is comparatively low. Moreover, since *S. nigrum* subsp. *nigrum* is much more common than subsp. *schultesii*, especially in Britain, any octoploids would probably be similar to the less fertile artificially-derived hybrids described above.

The only anomaly in the suggestion that the hexaploid *S. nigrum* might contain a set of genomes from *S. sarrachoides* arose from the apparent reproductive isolation of this species from all other diploids in the section *Solanum*. However, this anomaly has recently been overcome, following the successful hybridization of *S. sarrachoides* with the diploid *S. douglasii* Dunal (Edmonds unpublished). The fact that *S. sarrachoides* can spontaneously hybridize with *S. nigrum* in areas where the distributions of these species overlap, despite the differences in their ploidy levels, lends considerable support to the common genome hypothesis (see Edmonds 1979).

The question of the correct name for the diploid parent of *Solanum* × *procurrens* still remains a problem (see Leslie 1976, 1978). Leslie showed that most British alien material named *S. sarrachoides* Sendtn. is, in fact, *S. nitidibaccatum* Bitter, whilst *S. sarrachoides* Sendtn. emend. Bitt. is a much rarer plant, and that if these two taxa are subsequently recognized as distinct species then *S.* × *procurrens* strictly refers to the hybrid between *S. nigrum* subsp. *nigrum* and *S. nitidibaccatum* (Leslie 1978). I have recently obtained seed of *S. sarrachoides* Sendtn. emend. Bitt. from two British localities. This seed appears to be viable, unlike previous accessions of this taxon that I obtained. It is therefore hoped that hybridization studies between this material and *S. nitidibaccatum* Bitter, together with additional work on the variation of the South American representatives of these taxa, will help to resolve their taxonomy in the near future.

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