The identification and origin of *Stachys* × *ambigua* Sm.

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ABSTRACT

The origin of *Stachys* × *ambigua* Sm., *pro sp.*, by hybridization between *S. palustris* L. and *S. sylvatica* L. is confirmed. Chromosome numbers for the three taxa have been determined from British material.

The level of pollen inviability in population samples of *S. palustris* and *S. sylvatica* was found to be generally less than 10%, though male-sterile plants and populations of *S. palustris* occur. *S.* × *ambigua* population samples have a pollen inviability of more than 10% and frequently more than 50%. The degree of outbreeding is greater in *S. palustris* than in *S. sylvatica*, though there are considerable differences between genotypes of each species. F_1 hybrids are difficult to produce artificially.

The diagnostic characters, distribution and habitat preferences in Britain of the three taxa are given.

INTRODUCTION

In *English Botany* (t.2089) J. E. Smith (Sowerby & Smith 1810) described a new labiate, *Stachys ambigua* Sm. He noted the similarity of some forms of the new species to *Stachys sylvatica* L. and of others to *S. palustris* L. The first published suggestion that *Stachys ambigua* may have originated by hybridization between *S. sylvatica* and *S. palustris* was made by Syme (1867).

The chromosome numbers previously published for the three taxa are presented in Table 1 and, although variable, suggest a significant difference between *S. palustris* and *S. sylvatica*. The work of L. S. Gill and J. K. Morton (*fide* Morton 1973) indicates that in N. America *S. palustris* may be represented by two chromosome races of a polyploid series.

The only previously published chromosome count for *S.* × *ambigua*, $2n = 83$ (Morton 1973), is from British material which Morton assumed to have arisen from the hybridization of *S. palustris*, $2n = 102$, and *S. sylvatica*, $2n = 64$. Other possible chromosome numbers for *S.* × *ambigua*, based on the reported counts from European material of the putative parents, are $2n = 84, 75$, *c* 65 and *c* 56.

Some experimental crosses between central European *S. sylvatica* and *S. palustris* produced a number of mature hybrid plants (Lang 1940), although the percentage germination was low and only one plant was obtained from crosses with *S. sylvatica* as the maternal parent. These synthesized F_1 hybrids showed 33 or more bivalents at metaphase I of pollen-mother-cell meiosis. Morton (1973), however, reported 83 univalents at diakinesis in British *S.* × *ambigua*.

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TABLE 1. REPORTED CHROMOSOME NUMBERS OF *STACHYS PALUSTRIS*, *S. SYLVATICA* AND *S.* × *AMBIGUA*

Stachys palustris L.	$2n = c$ 64	Wulff (1938)	Germany
	102	Lang (1940)	Germany
	?102	Löve (1954)*	?
	96	Gill & Morton, *in litt.*	N. America
	64	Gill & Morton, *in litt.*	N. America
	102	Morton (1973)	Britain
Stachys sylvatica L.	$2n = c$ 66	Scheerer (1939, 1940)	Germany
	66	Lang (1940)	Germany
	48	Löve & Löve (1942)*	Sweden
	48	Delay (1947)	France
	66	Pólya (1950)	Hungary
	66	Gadella & Kliphuis (1963)	Netherlands
	64	Gill (1970)	Himalayas
	64	Morton (1973)	Britain
Stachys × *ambigua* Sm.	$2n =$ 83	Morton (1973)	Britain

* A. & D. Löve appear to have withdrawn their 1942 count for *S. sylvatica* as it does not appear in Löve & Löve (1961). A. Löve's (1954) count for *S. palustris* was cited in Löve & Löve (1961) as $2n = 102$. However, in the text of the 1954 paper he recorded $2n = 64$ and $2n = 48$ for *S. palustris* and *S. sylvatica* respectively but with no other details.

Regular meiotic pairing of chromosomes in *S. sylvatica* was reported by Lang (1940) and Gill (1970), although the numbers of bivalents are slightly at variance (33 and 32 respectively). *S. palustris*, according to the work of Lang (1940), exhibits 51 bivalents or, occasionally, 49 bivalents and 1 quadrivalent. Approximately 4% of cells have quadrivalents.

This paper attempts to confirm the putative origin of *Stachys* ×*ambigua* Sm., *pro sp.*, and gives notes for its identification.

MATERIALS AND METHODS

A list of the population samples used as the source of material in the experiments is presented in Table 2.

Somatic chromosome counts were made from metaphases of root-tip mitosis. Root-tips were pretreated for one hour in 0.1% colchicine at room temperature and fixed in Newcomer's fluid (2-propanol:propionic acid:petroleum ether: acetone:dioxan 6:3:1:1:1 by volume). Roots were hydrolyzed in N HCl for nine minutes at 60°C, stained in Feulgen reagent and squashed in 1% aceto-carmine. Meiotic preparations were obtained from fresh buds, squashes of single anthers being mounted in acetic-orcein.

The level of pollen inviability of a number of natural populations was estimated in the field from pollen freshly obtained from the anthers of 10–15 plants and mounted in cotton-blue in lactophenol.

Selected genotypes of the three taxa were studied in self- and cross-pollination experiments. In the latter, only a small proportion of the flowers in an inflorescence were emasculated, the remaining buds being removed. Inflorescences were isolated by means of a ventilated, clip-on polythene bag. This was used in preference to muslin to maintain a high humidity and prevent dehydration of the exposed stigma and style. The stigmas were pollinated two or three days after emasculation. Ripe nutlets were collected and attempts were made to germinate them approximately four months after ripening.

TABLE 2. HABITAT, SITE AND GRID REFERENCE FOR *STACHYS* POPULATION SAMPLES

The prefixes of the population sample numbers refer to populations of *S. palustris* (P), *S. sylvatica* (S) and *S.* × *ambigua* (H).

Population sample number			Habitat and site	Grid reference
—	—	H1A	Streamside, Silverburn, Isle of Man, v.c. 71	24/266.682
—	—	H2	Marshland surrounding stream and by edge of pond, Billown Mooar, Isle of Man, v.c. 71	24/263.697
P5	—	H4	Marshland by side of R. Derwent, Grange-in-Borrowdale, Cumberland, v.c. 70	35/253.170
P7	—	—	Wet ground by road, near Bothel, Cumberland, v.c. 70	35/203.364
—	S10	H5	Roadside verge, near Inverary, Argyll, v.c. 98	27/114.098
P9	S12	H6	Margins of ditch, Glendaruel, Argyll, v.c. 98	26/033.906
P10	S13; S14	H7; H8; H9	Wet fields, Dalmally, Argyll, v.c. 98	27/167.275
P11	—	—	Marshland and nearby field, Elphin, W. Sutherland, v.c. 108	29/214.116
—	—	H10	By railway bridge and roadside, Crianlarich, Mid Perth, v.c. 88	27/383.254
—	—	H11	Neglected garden, White Glen, Hoy, Orkney, v.c. 111	N30/243.024
—	S15	H12	In ridge of ayre, Loch of Carness, Mainland, Orkney, v.c. 111	N30/465.138
—	—	H13	Garden, Netherhouse Farm, Mainland, Orkney, v.c. 111	N30/372.185
—	—	H14	On rubbish tip in mire by field, near Netherhouse Farm, Mainland, Orkney, v.c. 111	N30/374.185
—	S16	—	Shady bank near stream, Kirk Burn Bu', Hoy, Orkney, v.c. 111	N30/235.046
—	S18	—	Near Ayre, Loch of Scockness, Rousay, Orkney, v.c. 111	N30/449.331
—	—	H15	Neglected garden, Rousay, Orkney, v.c. 111	N30/445.320
P13	—	—	Streamside, Waulkmill Bay, Mainland, Orkney, v.c. 111	N30/386.062
P14	—	—	In ditch at edge of field, Wideford Hill, Mainland, Orkney, v.c. 111	N30/397.117
P20	—	—	Sugar beet field, Aylmerton, E. Norfolk, v.c. 27	63/184.400

RESULTS

CHROMOSOME NUMBERS

The somatic chromosome numbers obtained are given in Table 3 together with the ranges for preparations from which it was not possible to obtain definite counts. Three distinct groupings occur among the plants studied. The group with low chromosome numbers ($2n$ = 62–68) is composed of plants identified morphologically as *S. sylvatica.* The group with high numbers ($2n$ = 97–103) comprises plants identified morphologically as *S. palustris.* The numbers between $2n$ = 79 and 86 refer to plants with an intermediate morphology. The morphological variation of the three taxa is described elsewhere (Wilcock 1973, and in preparation).

TABLE 3. SOMATIC CHROMOSOME NUMBERS OF *STACHYS PALUSTRIS, S. SYLVATICA* AND *S.* × *AMBIGUA* DETERMINED FROM ROOT-TIP SQUASHES

The population sample numbers refer to those in Table 2.

S10	66–68	H1A	78–80	H7	85 or 86	P6	102
S12	64 or 65	H1A	80–82	H7	84–86	P11	97–99
S13	61–63	H1A	81	H8	80–82	P11	97 or 98
S13	62–64	H2	81 or 82	H8	83	P13	103
S14	62 or 63	H2	81–83	H8	85	P14	97–101
S14	62–64	H2	83–85	H9	79–81	P14	102
S14	63 or 64	H2	85 or 86	H10	83–85		
S15	65–67	H2	84–86	H10	83 or 84		
S15	65 or 66	H2	83–85	H11	84		
S18	64	H4	85	H12	82		
		H4	85	H13	83		
		H6	83	H13	83–85		
		H6	84	H14	83–85		
		H6	85	H18	83 or 84		

Pollen-mother-cell meiosis of four plants of intermediate morphology (Table 4) showed between 9 and 14 univalents. Quadrivalents occurred, and the number of bivalents was between 33 and 37. Lang (1940) reported 33 or more bivalents in synthesized F_1 hybrids between *S. sylvatica* and *S. palustris.* However, these results conflict with those of Morton (1973), who found no bivalents in three British plants of *S.* × *ambigua.*

TABLE 4. PAIRING RELATIONS OF CHROMOSOMES AT MEIOSIS IN *STACHYS PALUSTRIS* (P14) AND *S.* × *AMBIGUA* (H2, H6)

The population sample numbers refer to those in Table 2.

	Univalents	Bivalents	Trivalents	Quadrivalents	Total
H2	12–9	34–36	0	0	80–81
H2	11	36–37	0	0	83–85
H6	14	36	0	0	86
H6	10–12	33–37	2	1	86
P14	0	51	0	0	102

The cytological evidence indicates that a group of plants with intermediate morphology, intermediate chromosome numbers and irregular meiosis occurs in nature.

POLLEN INVIABILITY

An inviable pollen grain was taken as one without contents (unstained) and/or distorted. The true level of inviability is probably higher than recorded, as germination on sucrose-agar always showed a significant proportion of round, stainable pollen grains which failed to germinate. The degree of pollen inviability was expressed as a percentage of the total pollen count (100–200 grains) for each plant. The percentage pollen inviability among the plants sampled in each population generally fell within one of the following arbitrary categories: <10, 10–30, 30–50, 50–70, 70–90. The results, shown in Table 5, were obtained during August 1970. The date may be important as Ockendon & Walters (1970) have produced some evidence indicating that in *Potentilla anserina* L. pollen inviability increases towards the end of the flowering season. In these three taxa high pollen inviability (>10%) generally characterises plants with intermediate chromosome numbers and morphology.

TABLE 5. MEAN POLLEN INVIABILITY SCORES FOR 29 FIELD POPULATIONS OF *STACHYS PALUSTRIS*, *S. SYLVATICA* AND *S.* × *AMBIGUA* SAMPLED DURING AUGUST 1970

Where the population range falls outside one category the complete range is shown in parentheses.
The population sample numbers refer to those in Table 2.

<10		10–30	30–50	50–70	70–90
P5	S10	H12(10–50)	H8(30–90)	P6	P13
P7	S12			H1A	H6
P9	S13			H2	H9(50–90)
P10	S14			H4	H10(50–90)
P11	S15			H5	H11
P14	S16			H7(50–90)	H13(50–90)
P20	S18				H14(50–90)
					H15(50–90)

Two population samples (P6, P13) have the chromosome number and morphology of *S. palustris* but show a high level of pollen inviability. They are male-sterile populations of *S. palustris*; P13 produces significantly smaller flowers than is usual, a feature known to be associated with male-sterility in the Labiatae (Willis 1891, Hedge 1968).

BREEDING EXPERIMENTS

The overall results of the crossings (Table 6) conceal considerable inter-genotypic differences. In the selfing experiments with *S. sylvatica* and *S. palustris* a range of from 2 to 59% nutlet-set was obtained. The mean nutlet-set for all *S. palustris* genotypes is less than half that of *S. sylvatica*, suggesting that the former species is less self-compatible than *S. sylvatica*. There is also a difference in seed-viability between the two species: only *c* 50% germinate in *S. palustris* compared with over 80% in *S. sylvatica*. Inter-genotypic crosses within the two species show a greater percentage nutlet-set in *S. palustris* than in *S. sylvatica*, and a greater proportion of the seeds are viable. *S. palustris* therefore outcrosses more readily than *S. sylvatica*.

Experimental hybridization between the two species is apparently more successful with *S. sylvatica* as the maternal parent but, when sown on filter paper, the majority of these nutlets showed no signs of germination and were found to be empty. A few produced green, undifferentiated outgrowths which eventually became infected with fungus. Others gave rise to recognizable seedlings which died on transference to soil. The reciprocal cross was totally unsuccessful.

Lang (1940) obtained only one seedling on germination of the nutlets obtained from hybridizations with *S. sylvatica* as the maternal parent, but his reciprocal crosses gave rise to a number of viable hybrid plants, even though the percentage nutlet-set was much lower. The percentage germination was given as 'poor' with over 50% of the nutlets being empty and several simply developing green, undifferentiated tissue. The mature F_1 plants strongly resembled their maternal parent (*S. palustris*) in morphology. The lack of nutlets obtained from *S. palustris* (female) × *S. sylvatica* (male) crosses in the present investigation may be due to the nature of the genotypes used, as has been shown, for example, in *Primula* by Valentine (1947) and in *Potentilla* by Matfield (1972).

IDENTIFICATION AND DISCUSSION

Wagner (1968) considered that 'the pattern of hybridity in plants is now so well known that it is entirely sufficient merely to establish (1) intermediacy (in morphology) and (2) changes in the reproductive system' as a hybrid diagnosis for a wild plant or population. From our results it is clear that plants intermediate in morphology between *S. palustris* and *S. sylvatica* show chromosome numbers, pairing relations and levels of pollen inviability consistent with a hybrid origin.

The difficulty found by us and by Lang (1940) in synthesizing the F_1 hybrid has been confirmed by J. K. Morton (pers. comm. 1971). The F_1 plants obtained by Lang more closely resembled *S. palustris* than *S. sylvatica* (a feature we noted in our plants), and showed chromosome numbers and pairing relations at meiosis similar to the British plants studied in this investigation. As far as can be ascertained, the F_1 hybrids obtained by Lang were referable to *S.* × *ambigua* Sm.

The morphology of these taxa throughout Britain has been studied both in the field and from samples in cultivation. A list of the most reliable diagnostic characters is presented in Table 7 together with the habitat preferences and known distribution of each taxon in Britain.

Field identification is not usually difficult as at a single site the range of variation is not wide; a combination of the first two characters in Table 7 will generally be sufficient. Critical specimens may be confirmed by the level of pollen inviability but the only entirely reliable character so far discovered is chromosome number.

A few herbarium specimens exist which possess a bewildering combination of *S. palustris* and *S.* × *ambigua* characters. Perring & Sell (1968) considered these to be back-crosses, but without further information on the population structure, variability and chromosome numbers of the plants, this supposition cannot be confirmed. In particular, some specimens are morphologically close

TABLE 6. SELF- AND CROSS-POLLINATION IN *STACHYS PALUSTRIS*, *S. SYLVATICA* AND *S.* × *AMBIGUA*

Lang's (1940) results are given in parentheses.

Female	Male	No. of flowers pollinated	No. of ripe nutlets	Mean % nutlet-set and range	No. germinated	% germinated
S. sylvatica selfed		129(196)	219(445)	42(60):59–4	181	83
S. sylvatica ×	*S. sylvatica*	122	127	26:37–4	95	75
S. palustris selfed		125(80)	85(134)	17(42):31–2	44	52
S. palustris ×	*S. palustris*	110	209	47·5:63–21	189	90
S. sylvatica ×	*S. palustris*	144(81)	140(110)	24(33·5):44–0	10*	7*
S. palustris ×	*S. sylvatica*	135(45)	0(49)	0(26)	0	0
S. palustris ×	*S* × *ambigua*	115	12	3:12–0	0	0
S. × *ambigua* ×	*S. palustris*	31	0	0	0	0
S. sylvatica ×	*S.* × *ambigua*	45	0	0	0	0
S. × *ambigua* ×	*S. sylvatica*	27	0	0	0	0

* All died at the seedling stage.

TABLE 7. DIAGNOSTIC CHARACTERS, HABITAT PREFERENCES AND DISTRIBUTION OF *STACHYS PALUSTRIS*, *S.* × *AMBIGUA* AND *S. SYLVATICA* IN BRITAIN

	S. palustris	*S.* × *ambigua*	*S. sylvatica*
1. Petiole: total leaf-length	0·02–0·09	0·09–0·16	0·30–0·44
2. Nutlet production	Mature nutlets usually produced	Mature nutlets rarely produced	Mature nutlets always produced
3. Level of pollen inviability	Usually < 10%, but male-sterile plants and populations occur	> 10% and generally > 50%	< 10%
4. Somatic chromosome number ($2n$)	(97–)102(–103)	(78–)84(–86)	(62–)66(–68)
5. Corolla colour	Usually pale pink	Usually bright red	Dark mauve
6. Habitat preferences in Britain	Marshland, banks of canals and rivers; sometimes as a weed in dry places	By streams and rivers, but often in disturbed ground by road-sides, etc.; very common in gardens in N.W. Scotland and on islands off the N. and W. coasts of Scotland	Generally dry habitats in hedgerows, thickets, edges of woods and gardens
7. Distribution in Britain	Common and widely distributed, but decreasing as a result of drainage	Frequent in N. and W. Scotland, Lake District and Isle of Man; becoming rare towards S. and E. England	Very common and widely distributed; absent or rare on islands off the N. and W. coasts of Scotland

to *S.* × *ambigua* but show a high percentage nutlet-set, while others are morphologically close to *S. palustris* but have a very low nutlet-set. Specimens of the second type may be F_1 plants, back-crosses, or male-sterile plants of *S. palustris* which had no nearby source of viable pollen. Herbarium specimens of both the above types cannot always be determined with certainty.

The barriers to hybridization which exist between *S. palustris* and *S. sylvatica* are:

1. The species usually occur in different habitats and, in some regions of Britain, are geographically separated. Furthermore, the hybrid commonly occurs in the absence of one or both parents.

2. Interspecific hybrids and back-crosses are difficult to produce artificially, indicating strong internal barriers to hybridization. Different chromosome num-

bers in the parents may be one of the important internal barriers which lead to irregular pairing at meiosis and low levels of pollen germination in the hybrid. *S.* × *ambigua* plants show an extremely low level of nutlet-set and attempts to germinate the few nutlets obtained have failed.

ACKNOWLEDGMENTS

This research was undertaken while one of us (C.C.W.) was recipient of a Tutorial Research Studentship awarded by Royal Holloway College, and it was presented as part of a Ph.D. thesis in the University of London. We gratefully acknowledge the assistance of Mr J. R. Colman with the population studies.

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(*Accepted December* 1973)