The Experimental Taxonomy of <u>Stachys ambigua</u> <u>Sm.</u>,

<u>S. palustris</u> L., and <u>S. sylvatic</u>a L. ~~in Britain.~~
                                    SYLVATICA

A Thesis submitted for the Degree of Doctor of
Philosophy in the University of London

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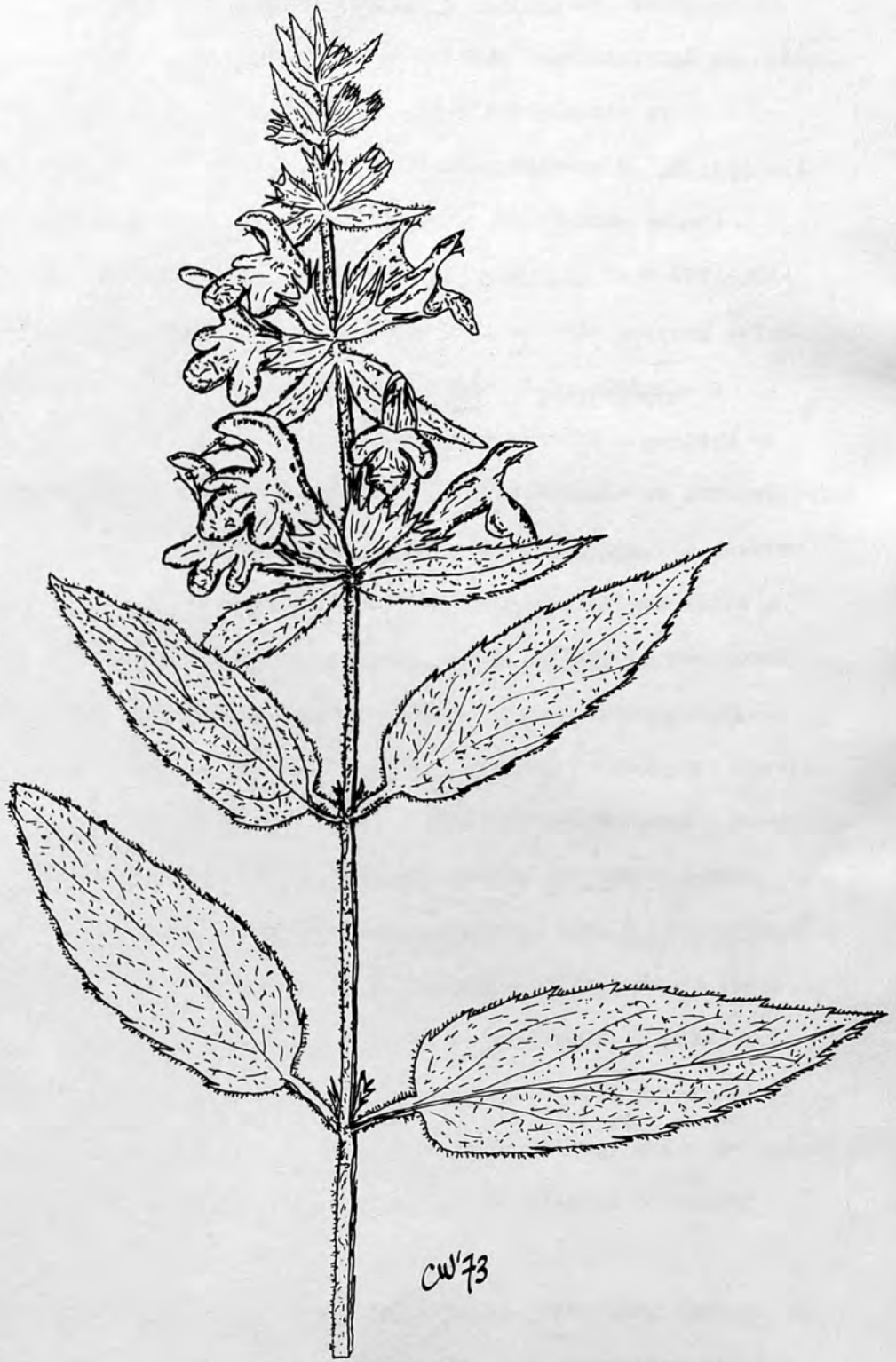
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ABSTRACT

The putative hybrid, Stachys x ambigua Sm, is shown to overlap with S. palustris L. in all the morphological characters studied and in flower pigments. A discontinuity in morphological variation usually exists between S. ambigua and S. sylvatica L. in these characters. Chromosome counts /S. sylvatica 2n = (62)-66-(68), S. palustris 2n = (97)-102-(103) and S. ambigua (78)-84-(86)/ and meiotic pairing relationships have confirmed the hybrid origin of S. ambigua.

No field populations were discovered with a pattern of morphological variation indubitably attributable to introgressive hybridization between S. ambigua and S. palustris. A scatter diagram, based on leaf data, of all the populations shows an intergradation between S. ambigua and S. palustris previously interpreted as introgression. Morphological intergradation occurs because 1. S. palustris is a highly polymorphic species exhibiting a high degree of both within - and between - population variability, and 2. F_1 S. ambigua plants are not intermediate but morphologically closer to S. palustris than S. sylvatica.

The pollen fertility of S. ambigua populations is shown to be low ($<$ 5%) but this is unlikely to be itself an effective barrier to backcrossing. Pollinators show no discrimination between the three taxa, and rare backcrosses may exist in nature. There is, however, no evidence of the occurrence of hybrid swarms.

Competition experiments between the three taxa through two seasons of growth show that eliminations of competing genotypes are density-dependent. Interspecific competition is not likely

to be the most important factor causing the absence of one or both parents from localities where the hybrid occurs. Accidental or desired distribution of rhizome fragments of the hybrid by man and the disappearance of S. palustris from marshland as a result of drainage are considered to be more important causal factors.

Chromatographic spot patterns from flower samples of Stachys populations were associated by computer using three different coefficients of association - simple matching, Jaccard and Dice coefficients. These were evaluated by their application to the data of Moore et al (1970). The simple matching coefficient used in conjunction with the weighted variable group clustering procedure is shown to be the most effective in reflecting the relationships between both the Stachys and the Empetrum rubrum samples.

1. INTRODUCTION

In 1810, J.E. Smith figured (t.2089) a new Labiate species, Stachys ambigua, in his English Botany. The following description accompanied the figure:-

'The specimen in our plate was gathered in the Orkneys, where this plant is very abundant in potato fields and other cultivated ground, by Mr. W. Borrer and Mr. W.J. Hooker, who also found the same near Loch Arron and in Glen Ely in the North of Scotland, in September 1808. We have received in August 1809 a specimen, gathered by Mr. J.R. Weatherhead in a boggy place at the foot of one of the Pentland Hills near Edinburgh, of the same plant in a less luxuriant state, about a foot high, with much narrower and more silky leaves and a scarcely spotted flower. This latter approaches nearer to S.palustris, (t.1675), from which however it differs in its stalked leaves, which are not dilated at their base, and in having a faint degree of the peculiar foetid smell of S. sylvatica. Mr. Hooker's and Mr. Borrer's specimens more approach this latter, but the stem is hollow, (as Mr. Weatherhead well observes in his), not filled up with pith as in sylvatica; the leaves are oblong, not rounded, though slightly heartshaped at the very base. The root is white and creeping. Hairs on the stem more or less deflexed. Corolla with a variegated lip in general, though sometimes very slightly so.'

Smith, in this description, indicates some of the variation to be encountered within S. ambigua and the affinities of some forms with Stachys palustris and of others with S. sylvatica.

While general botanical opinion was still unfavourable

towards the notion of hybrids, botanists during the next ten
years began to recognise the intermediate nature of <u>Stachys ambigua</u>
between <u>S. palustris</u> and <u>S. sylvatica</u>. Hooker (1821) writes
that <u>S. ambigua</u> 'appears intermediate between the preceeding
/<u>S. sylvatica</u>/ and the following /<u>S. palustris</u>/ but more
approaching the latter.' He continues to give <u>S. ambigua</u>
specific status in the early editions of his English Flora
(1830   ) but by 1842, and the fifth edition, the similarity
of <u>S. ambigua</u> to <u>S. palustris</u> Hooker first noted in 1821 has
lead him to add the concluding remarks to his description,
'probably only a variety of the latter' /<u>S. palustris</u>/. This
treatment is followed in the seventh edition of Withering's
Botany (1848), but some authors still consider the taxon to
warrant specific status while accepting its intermediary, for
example Deakin (1845).

By 1858, Bentham is able to be more precise in his treat-
ment of <u>S. ambigua</u> Sm. In the description of <u>S. palustris</u> he
writes, 'a variety with rather broader and longer-stalked leaves,
and a rather longer tube to the corolla, has been distinguished
under the name of <u>S. ambigua</u> (Eng. Bot. t.2089), but it appears
to be connected with the common form by too close a chain of
intermediates to be separable from it.'

However, closer investigation, together with an increasingly
more flexible view of the species concept, led Syme in 1867, and
almost 60 years after Smith's first description, to suggest that
<u>S. ambigua</u> was probably a hybrid. This is almost certainly the
first published report that <u>S. ambigua</u> may have originated by
hybridization. Syme proposed this view on two accounts. First,
because of its intermediate morphology between <u>S. palustris</u> and

S.sylvatica and secondly because it never appeared to 'perfect its seeds', a fact either not previously noted or ignored. In the same description after stating the distribution of the hybrid he warned, 'it has been reported from many other localities, but the subpetiolate forms of S. palustris are so often mistaken for it, that localities not confirmed by specimens cannot be relied upon'.

But controversy continued and in a review of the Flora of Somerset (1896), Dunn observed in 1897 that 'it is satisfactory to see the query before S. ambigua Sm, as a synonym for Stachys palustris x sylvestris /sic/. Smith's plant was described and figured from a form plentiful in Orkney, and was doubtless one of the dry ground conditions of S. palustris.'

At the end of the century the taxonomic treatment of Smith's taxon in Britain was still inconsistent; with specific hybrid or varietal status being variously adopted. European opinions, as reviewed by Clos (1889) and Bornmüller (1920) were just as divergent.

In J.D. Hooker's revision (6th edition: 1892) of Bentham's Handbook of the British Flora, S. ambigua is treated under the description of S. palustris as 'a hybrid with S. sylvatica), after being considered by Bentham in the first edition as inseparable from S. palustris. Babington (1904) treats it as a variety of S. palustris (β ambigua) and adds in parenthesis, 'considered a hybrid between S. palustris and S. sylvatica'.

During the first three decades of the twentieth century, Smith's Stachys ambigua became fairly well established in the literature as a hybrid being cited as such by Druce (1932). Clapham, Tutin and Warburg's (1952) comments after descriptions

of the two putative parental species are terse but definite.
'S. palustris x sylvatica = Sxambigua Sm. has oblong shortly-
petioled leaves and is normally sterile. It is widespread and
not uncommon with the parents'. S. palustris is mentioned to
occur occasionally in arable land but the problems of the close
association of this form with Stachys ambigua, the existence of
intermediates between these two species mentioned by Bentham,
and the degree of similarity between the Scottish S. ambigua and
S. palustris originally noted by Smith himself have all
evaporated.

The extensive fieldwork undertaken by botanists during the
1950s to produce the Atlas of the British Flora in 1962 must have
uncovered some of the old problems, as Perring (1962) gave some
hints on the determination of Stachys ambigua. 'This hybrid may
be recognised by its linear-lanceolate shortly petioled lower
leaves, which are 2-4 times as long as broad, and broadest about
the middle. Sterile. Not uncommon throughout the British Isles
and sometimes in the absence of S. sylvatica e.g. in the north
and west.' Later, Perring revised his views on the sterility of
Stachys ambigua and wrote in the Critical Supplement (1968),
'there is considerable overlap between S. palustris and
S.xambigua /in lamina breadth:length ratio/ which suggests that,
though the hybrid is said to be sterile, occasional backcrossing
to S. palustris does occur.' The existence of intermediate forms
between S. palustris and S. ambigua, first noted by Bentham over
100 years earlier are proposed to originate by introgressive
hybridization.

Wilcock (19 69 72) produced a scatter diagram of the three taxa
based on leaf characters analyzed from material at the British

Museum and Kew herbaria (see fig.22). The overlap between
S. palustris and S. ambigua is clearly evident. At the same
time S. ambigua appears to be isolated from S. sylvatica by
a large morphological discontinuity.

To resolve the taxonomic problem posed by the plants more
or less intermediate between S. palustris and S. sylvatica, it
is necessary to understand the factors isolating the species in
nature. Distinctions between the three taxa will remain
uncertain while the notion of S. ambigua as a hybrid, and the
intermediates as introgressants, remains putative.

This investigation is an attempt to determine the status of
both Stachys ambigua Smith and the so-called 'dry ground' petiolate
form of S. palustris. The origin of the natural variation
observed in the three taxa has also been studied.

2. REPRODUCTIVE ISOLATION

INTRODUCTION

The role of isolating mechanisms as barriers to
hybridization has been discussed by many authors and are now
well documented (see Davis and Heywood, 1963 and Stebbins, 1966).
A lack of internal isolation between sympatric species undergoing
cross-pollination will lead to the production of F_1 hybrids and
subsequent backcrosses, provided that the F_1 does not itself
become isolated from its parents (for example by polyploidy or
the acquisition of a new pollinator). The intrinsic isolating
mechanisms preventing different gamodemes from interbreeding
have been reviewed by Solbrig (1968).

In most cases of hybridization, the hybrids are normal in
their vegetative development, but show some kind of reduction
in fertility (Stebbins 1950, 1958). Most frequently this is
the result of haploitic (chromosomal) sterility and is due to
numerical and/or structural differences in the chromosomes of
the parental species. Reduction in fertility in hybrids may be
recognised by the production of a high proportion of absortive
pollen grains, low pollen fertility and irregularities in
chromosomal pairing during meiosis. These features may there-
fore be used in the identification of hybrids. Wagner (1968)
considers that, 'the pattern of hybridity in plants is now so
well-known that it is entirely sufficient merely to establish
(1) intermediary /in morphology/ and (2) changes in the
reproductive system' to establish a hybrid - diagnosis for a
plant or population in nature.

Other intrinsic isolating mechanisms studied in this

investigation were gametic isolation as exemplified by pollen incompatibility in interspecific crosses and the inviability of hybrid embryos in set fruit. The aim of these studies was to determine the barriers, if any, to hybridization and to detect the existence of hybrids in natural populations.

The reported chromosome numbers of Stachys palustris and S. sylvatica, although variable, suggest a significant difference between the two species (see table 1). The work of Morton et al, in press, suggests that in N. America S. palustris may be represented by two chromosome races of a polyploid series, possibly based either on $x = 16$ (with $4x = 64$ and $6x = 96$) or $x = 8$ or 4.

One somatic chromosome count for S. ambigua has been obtained by Morton (in press) giving $2n = 83$ and based on S. palustris $2n = 102$ and S. sylvatica $2n = 64$. Other expected somatic numbers for S. ambigua might be $2n = 56, 64, 65, 72, 75, 80, 81$ and 84 depending on the chromosome number of the parental plants involved based on the numbers given in table 1 . Counts obtained from European material give S. palustris $2n = 102$ or C.64 and S. sylvatica $2n = 66$, or possibly $2n = 48$. Stachys ambigua in Europe is therefore more likely to possess the somatic number $2n = 84, 75,$ c.65 or c.56.

Meiotic chromosome studies of the three species have been very few. Both Gill (1970) and Lang (1940) report regular meiotic pairing in S. sylvatica, although the numbers of bivalents are slightly at variance, 32IIs and 33IIs respectively. S. palustris, also from the work of Lang (1940), exhibits 51IIs or occasionally 50IIs and 1IV. In S. ambigua, Morton (in press) reports 83 univalents at diakinesis. Lang reports, in synthesized F_1 hybrids between S. palustris and S. sylvativa, 33 bivalents

Table 1. Reported chromosome numbers of <u>Stachys palustris</u>,
S. <u>sylvatica</u> and <u>S. ambigua</u>.

				Origin of Material
<u>Stachys palustris</u> L. 2n =	c.	64	Rohweder 1937	Germany
	c.	64	Wulff 1938	Germany
		102	Lang 1940	Germany
		102	Love 1954*	?
		96	Gill and Morton, in press	N. America
<u>Stachys sylvatica</u> L. 2n =	c.	66	Scheerer 1939, 1940	Germany
		66	Lang 1940	Germany
		48	Love and Love 1940*	Sweden
		48	Delay 1947	France
		66	Pólya 1950	Hungary
		66	Gadella and Kliphuis 1963	Netherlands
		64	Gill 1970	Himalayas
<u>Stachys ambigua</u> Sm. 2n =		83	Morton, in press	Britain

*Love and Love appear to have withdrawn their 1942 count for

<u>S. sylvatica</u> as it does not appear in Chromosome Numbers of

Central and N.W. European Plant Species (Love and Love, Opera

Botanica, <u>5</u>, 1961). A. Love's count for <u>S. palustris</u> cited in

the same publication has not been traced. In the 1954 paper he

gives the numbers 2n = 64 and 2n = 48 for <u>S. palustris</u> and

<u>S. sylvatica</u> respectively.

or more at metaphase I.

In an intensive survey of the inter-relationships of the tribes and species within the genus Stachys, Lang (1940) has studied self-pollination and hybridization in S. palustris and S. sylvatica. The results he obtained were, as follows:-

	No. of flowers	No. of fruits	% set
Self-fertilization			
S. palustris	80	134	42
S. sylvatica	186	445	60
Experimental crosses			
S. sylvatica (♀) x S. palustris	81	110	33.5
S. palustris (♀) x S. sylvatica	45	49	26

On germination of the fruits obtained with S. sylvatica as the maternal parent, only one seedling developed. However, with S. palustris as the maternal parent he obtained a number of viable hybrid plants, although the percentage fruit set was less than the reciprocal cross. The percentage germination is given as 'poor' with over 50% of the fruits being empty and several simply developing green, undifferentiated tissue.

The degree of inviable pollen in flowers, when high in hybrids, is generally a useful guide to hybridization in natural populations (Valentine, 1950) and this was studied in the Stachys populations. While quick assessments of stainable/non-stainable pollen give no information on pollen fertility they do indicate the level of male sterility in populations. Pollen germination tests on sucrose agar give a more definitive

indication of the degree of pollen fertility and these were performed on some plants.

MATERIALS AND METHODS

Chromosomes were investigated at metaphase of mitosis to obtain somatic counts and at prophase stages of first division of meiosis to study pairing relationships. The best mitotic results were obtained using root tips pretreated for one hour in 0.1% colchicine at room temperature. The material was then fixed in Newcomer's fluid . *ie.* (isopropyl alcohol $\overline{/2}$-propano$\underline{1}\overline{/}$, propionic acid, petroleum ether, acetone and dioxan in the volume ratios 6:3:1:1:1 respectively). Prior to hydrolysis, the root tips were washed twice in NHCl.. The hydrolysis was effected by treating for 9 minutes in NHCl at 60°C and the root tips were then transferred to cold NHCl.. Staining followed in Feulgen solution for 2-3 hours. Root tip squashes were mounted in 1% acetocarmine. Individual counts were difficult and several cells were counted from different root preparations as accurately as possible. In some cases roots from a number of individual plants were examined in an attempt to obtain one entirely reliable count. Meiotic preparations were obtained from fresh buds. Squashes of single anthers were mounted in aceticorcein. No permanent preparations were made.

The breeding relations of the three taxa were investigated using selected genotypes of each species. In outcrossing experiments flowers were emasculated prior to anthesis and pollen transferred by means of a thin brush or wooden splint. Only a small portion of the flowers in an inflorescence were used, the others being removed. Each inflorescence was isolated

by means of a ventilated clip-on polythene bag. These were used in preference to muslin to keep the humidity high and prevent the exposed stigma and style from dehydration. The stigmas were pollinated two or three days after emasculation (cf. Lang, 1940 where pollination was performed the next day). Ripe fruit was collected and attempts were made to germinate the seeds obtained.

To determine the best conditions for germination, seven treatments were given. They were:-

Treatment	Conditions
1. Control	Untreated fruits/Light/Room temperature
2. Scoured	Scoured fruits/Light/Room temperature
3. High temperature	Untreated fruits/Light/c.20°C
4. Gibberellic acid	Fruits soaked in GA$_3$/Light/Room temperature
5. Dark	Untreated fruits/Dark/Room temperature
6. Vernalization	Vernalized fruits/Light/Room temperature
7. Low temperature	Untreated fruits/Dark/4°C

Fruits were scoured by rubbing them carefully between two pieces of fine glass paper. The high temperature was effected by placing fruits in a cabinet fitted with a 100W light bulb (pearl) placed 6 inches above the petri dishes to give a 9 hour day. The gibberellic acid treatment was obtained by soaking fruits in an 0,04% aqueous solution of GA$_3$ with one drop of a wetting agent for 12 hours. The fruits were not bathed in gibberellic acid solution during the test period (cf. Thompson, 1969). Fruits to be vernalized were first soaked in water, then placed in petri-dishes covered in foil and maintained for 14 days at 4°C. Dark treatments were obtained by covering the petri-dishes with aluminium foil. The light condition was

provided by daylight, except in the high temperature treatment where the 100W electric light bulb was enhanced by a 13W fluorescent lamp as the light source.

Fruits of a Lake District collection of S. palustris (P7) and an Orkney collection of S. sylvatica (S15) were used in the experiment. 20 mericarps were placed on seed testing paper (thick) dampened with distilled water in 9 cm petri dishes. Each treatment was replicated 4 times for each species. The seed paper was maintained in a moist condition throughout the duration of the experiment by periodic additions of distilled water. All fruits obtained in the breeding experiments were germinated employing the gibberellic acid treatment.

The degree of pollen inviability, expressed as a percentage of the total pollen count (100-200 cells), was obtained for plants studied in the field from mature anthers mounted and gently squashed in cotton blue in lactophenol. The mean percentage pollen inviability for a population falls within one of the following categories: $<$10, 10-30, 30-50, 50-70, 70-90, $>$90. An inviable pollen grain was taken as one without contents (unstained) and/or distorted in shape. The results were obtained during August 1970. The date may be important as Ockenden and Walters (1970) have produced some evidence indicating that in Linum species pollen inviability increases towards the end of the flowering season.

Pollen germination was tested by tapping pollen from anthers at anthesis onto slides coated with sucrose agar (40g sucrose, 6g agar in 500 mls distilled water). 0.5 mls of boric acid solution (1.4g in 500 mls distilled water) was added to 25 mls of the agar before coating the slides. The slides were maintained

in a humidity chamber at 20°C and after three hours the pollen
tubes were stained with cotton blue in lactophenol. Counts of
the germination and length of the pollen tubes were obtained
from 100 germinated grains/slide. Individual flowers from 10
different populations of each species collected throughout
Britain and grown at Royal Holloway College were tested.

Appendices 1 and 2 give a key to the field populations and
those genotypes used in the experiments.

RESULTS AND DISCUSSION

From the somatic chromosome numbers obtained, three distinct
groupings occur within the plants studied (see table 2). The low
chromosome-numbered group (62-68) were obtained from plants whose
morphology had already allowed them to be determined as S. sylvatica.
The high-numbered group (97-103) were obtained from plants
already putatively determined as S. palustris. The intermediate
numbers (78-86) occur in populations with intermediate morphology.
Determination of the populations, on the basis of a combination
of several characters, are fully described in the General
Discussion, p. 136

The mean of each group is 64.4, 82.85 and 99.55. These
figures are based on the mean of total scores for each somatic
number within the group. Where a count is only accurate to two
or more chromosomes and is given as a range this scores 1
for each number falling within it.

If S. ambigua is a hybrid between S. palustris and S. sylvatica
then its expected chromosome number is 2n = 84, 75, c.65 or c.56
(based on European counts of the putative parents). 2n = 48,
one of the somatic counts for S. sylvatica, is outside the range
of numbers observed in this investigation and therefore has

Table 2. Somatic chromosome numbers of _Stachys_ determined from root tip squashes.

S10 67$\pm$1	H1A 79$\pm$1	H7 85+1	P6 102!
S12 64+1	H1A 81$\pm$1	H7 85$\pm$1	P11 98$\pm$1
S13 62$\pm$1	H1A 81!	H8 81$\pm$1	P11 97+1
S13 63$\pm$1	H2 81+1	H8 83!	P13 103!
S14 62+1	H2 82$\pm$1	H8 85!	P14 99$\pm$2
S14 63$\pm$1	H2 84$\pm$1	H9 80$\pm$1	P14 102!
S14 63+1	H2 85+1	H10 84$\pm$1	
S15 66$\pm$1	H2 85$\pm$1	H10 83+1	
S15 65+1	H2 84$\pm$1	H11 84!	
S18 64!	H4 85!	H12 82!	
	H4 85!	H13 83!	
	H6 83!	H13 84$\pm$1	
	H6 84!	H18 84$\pm$1	
	H6 85!	H18 83+1	

10 μm
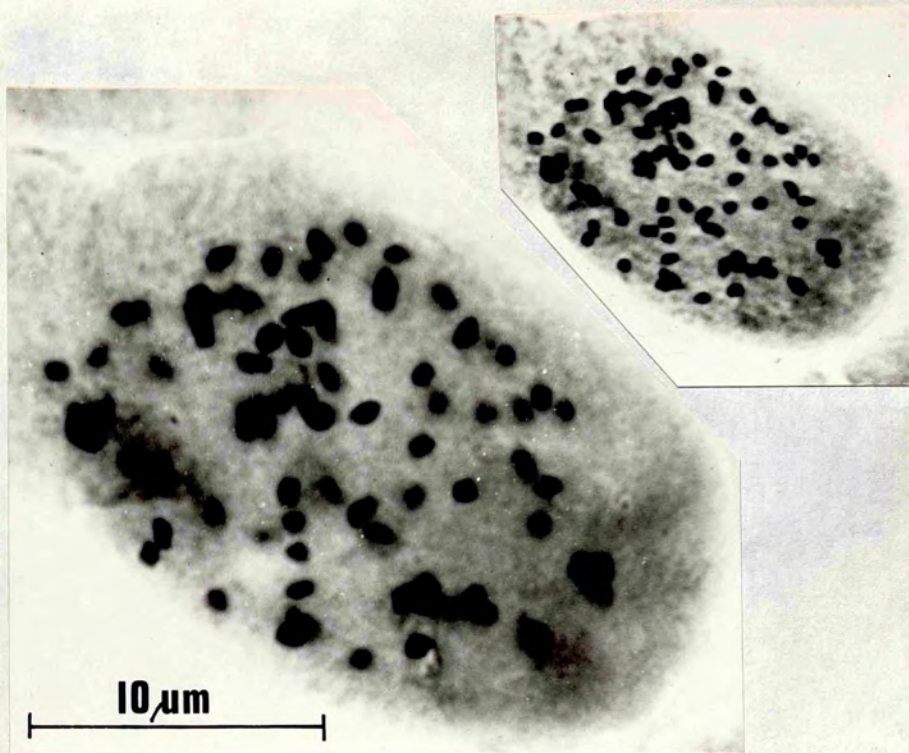
Fig. 1. H9. 2N=80±1. <u>Inset</u> the same cell at a lower magnification.

10 μm
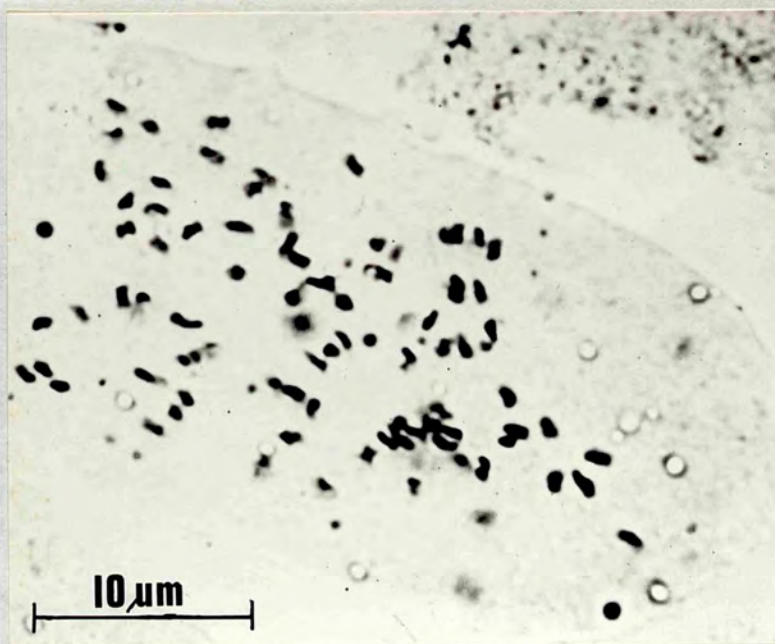
Fig. 2. H2. 2n=85±1. (Two chromosomes wholly out of focus).

Figs. 1 and 2. Chromosomes of <u>Stachys ambigua</u>.

Squash preparations of metaphase of root tip mitosis. Chromosomes pretreated with colchicine, stained in Feulgen and mounted in acetocarmine.
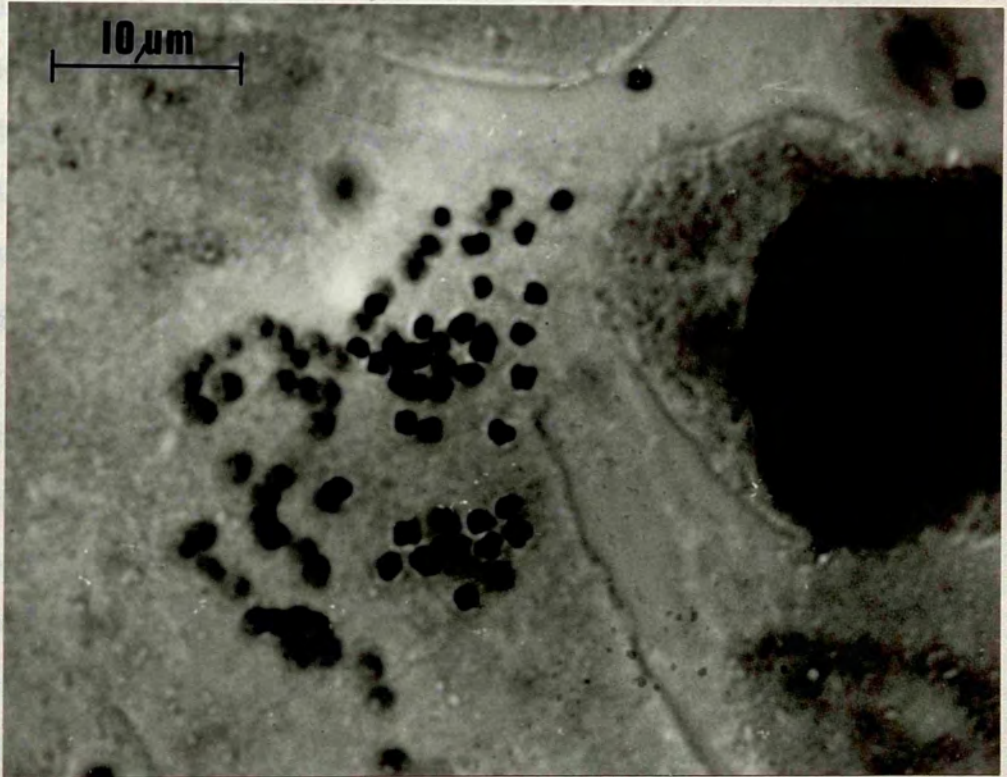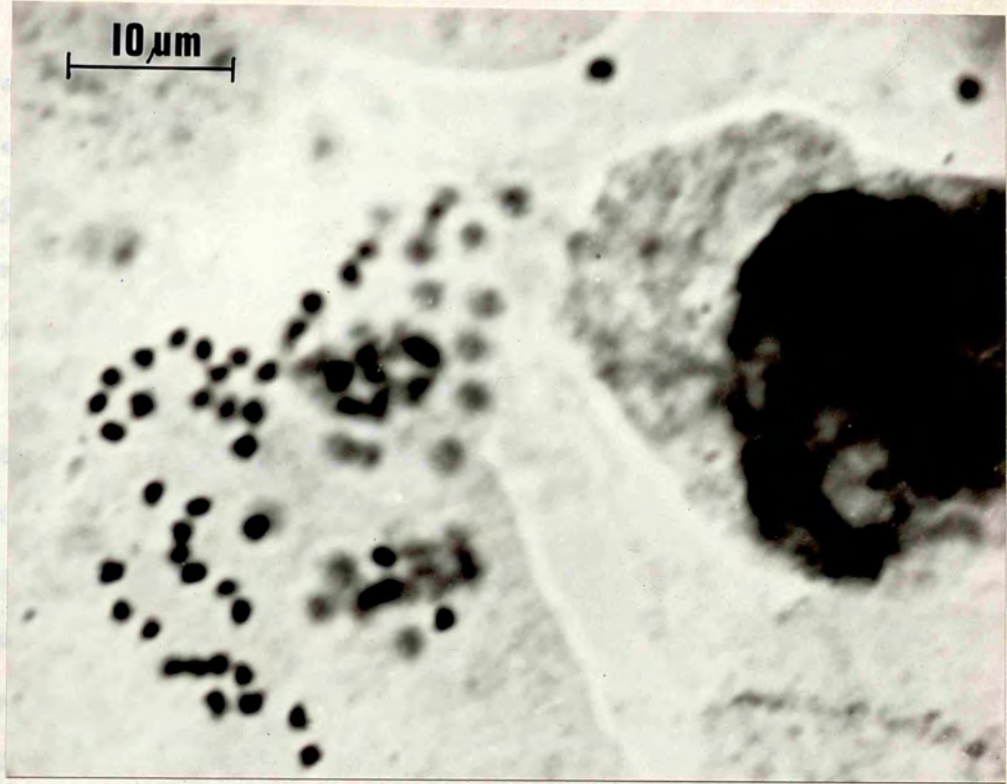
Fig. 3. **Chromosomes** of <u>Stachys ambigua</u> (H18).

2n = 83+1 (two levels of focus). *One chromosome out of view.*

Squash preparation of metaphase of root tip mitosis.
Chromosomes pretreated with colchicine, stained in
Feulgen and mounted in acetocarmine.

still to be reported from the British Isles. The highest
expected number for S. ambigua (based on 2n = 66 for
S. sylvatica and 2n = 102 for S. palustris) does, however, fall
within the range of the intermediate group of counts, and
indicates a hybrid origin for the taxon.

The mean numbers for both S. sylvatica and S. ambigua
correspond closely with those given by Gill (1970) and Morton
(in press) respectively. The mean number for S. palustris is
not in agreement with any published count. With published
numbers of 2n = 64, 96 and 102, though, it seems likely that a
wide range of chromosome numbers exists within the species. If
2n = 64 and 2n = 83 are correct somatic numbers for S. sylvatica
and S. ambigua then involvement of S. palustris with 2n = 102
is indicated. However, the report of 2n = 66 for S. sylvatica
has been obtained several times by different authors and cannot
be ignored. With 2n = 83 for S. ambigua, hybridization in this
instance would involve a second parent with 2n = 100. This,
indeed, lies close to the mean obtained in this study for
S. palustris.

The somatic chromosome numbers for S. ambigua show a range
lower than would be expected in a situation where backcrossing
to S. palustris was occurring: backcrosses would give rise to
aneuploid numbers ranging between 84 and 102. The somatic
number for P11 (2n = 97-98) may perhaps be considered a back-
cross to S. palustris, although it is treated here as pure
S. palustris for the reasons of chromosomal variability within
the species already discussed.

While further work is needed on chromosome numbers within
the group, this investigation has shown that cytological

evidence indicates that the morphologically intermediate (H number) plants may be F_1 hybrids between S. palustris and S. sylvatica.

The pairing relations of the four S. ambigua plants studied at meiosis (see table 3) suggest that between 34 and 36 bivalents may be formed. This agrees with Lang's (1940) findings in synthesized F_1 hybrids where he reports that the same number of bivalents found in S. sylvatica /i.e. 33/ or more may occur. However, these results are in conflict with the work of Morton (in press) who found no bivalents at diakinesis at all in the hybrid.

The results of the crossing experiments (table 4) conceal considerable intergenotypic differences. In the selfing experiments with S. sylvatica and S. palustris the range is from 2-59% fruit set. The mean fruit set for all S. palustris genotypes is less than half that of S.6sylvatica, suggesting that this species is more outbreeding than S. sylvatica. Lang (1940) also found a lower fruit set in S. palustris than S. sylvatica with self-pollination, although the difference is not so marked. This is also allied in the present studies to a difference in the viability of the fruits produced. Only c.50% germinate as compared with over 80% in S. sylvatica. In the inter-genotypic crosses within the two species, S. palustris produces a greater percentage fruit set than S. sylvatica and the seeds are largely viable. This further confirms the difference in breeding system of the two species.

Experimental hybridization between the two species is apparently more successful with S. sylvatica as the maternal parent but, when sown, the majority of these fruits showed no

Table 3. Pairing relations of chromosomes at meiosis in
S. palustris (1) and S. ambigua (4).

Plant	I	II	III	IV	TOTAL
H2/1	12-9	34-36	–	–	80-81
H2/4	11	36-37	–	–	83-85
H6/3	14	36	–	–	86
H6/4	12	37	–	–	86
	10	33	2	1	86
P14	–	51	–	–	102
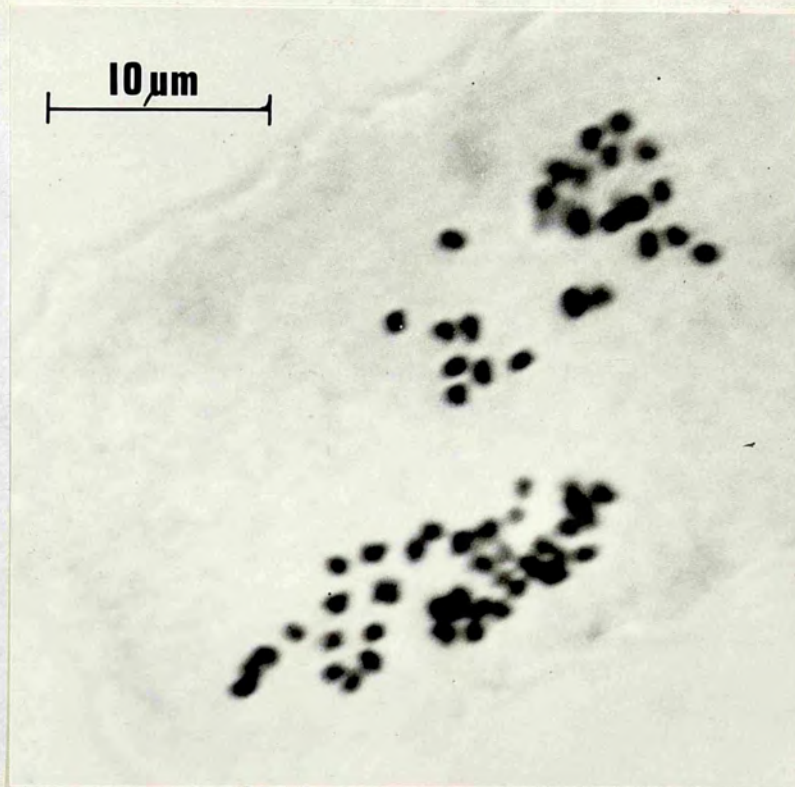
Fig. 4. Chromosomes of <u>Stachys sylvatica</u> (S15). $2n = 66\overset{+}{-}1$.

Squash preparation of metaphase of root tip mitosis.
Chromosomes pretreated with colchicine, stained in
Feulgen and mounted in acetocarmine.
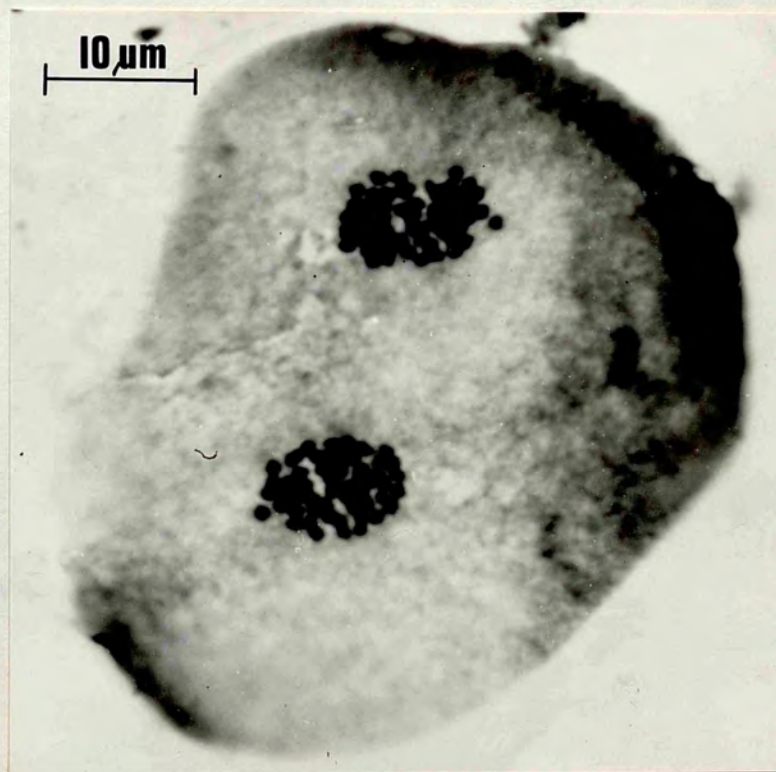
Fig. 5. Chromosomes of <u>Stachys palustris</u> (P14).

Squash preparation of metaphase II of meiosis with two
groups of 51 Is.
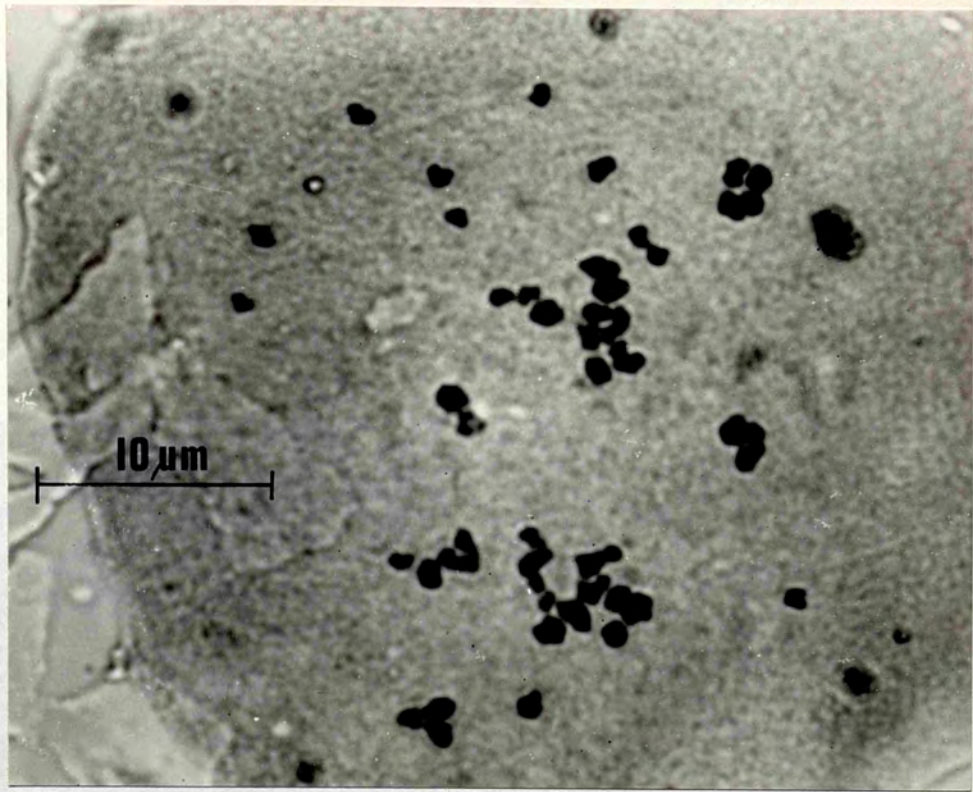
Fig. 6.   H6/3 with 33 IIs and 14 Is.
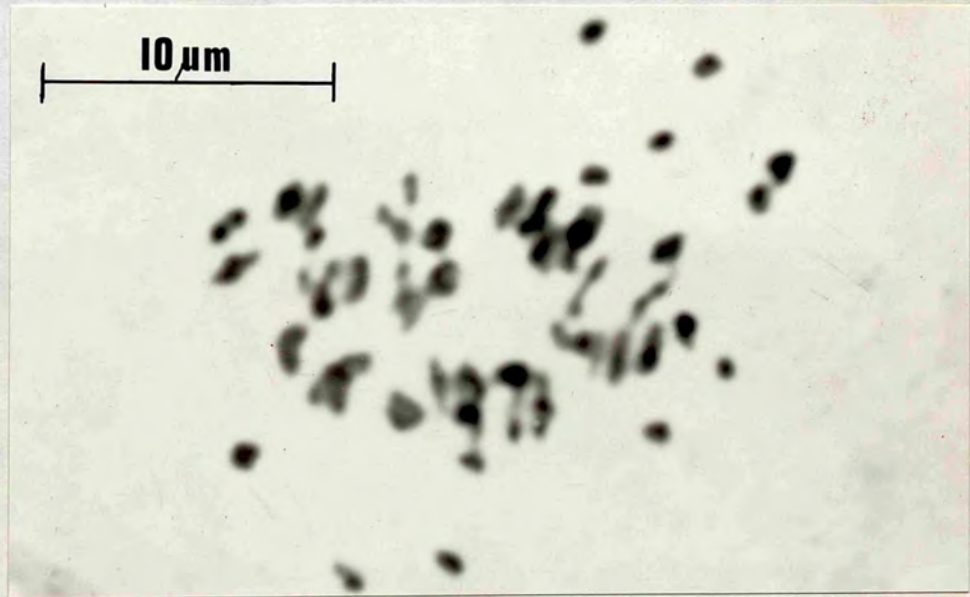
Fig. 7.   H2/4 with 36 or 37 IIs and 11 Is.

Figs. 6 and 7.   Chromosomes of Stachys ambigua.

Squash preparations of metaphase I of meiosis.
Chromosomes stained with acetocarmine.

Fig. 8. Chromosomes of <u>Stachys ambigua</u>.

H6/4 with 37 IIs and 12 Is or 33 IIs, 10 IIs, 2 IIIs
and 1 IV. The trivalents and quadrivalent are arrowed.

Squash preparation of metaphase I of meiosis. Chromosomes
stained with acetocarmine.

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 normal seedlings but died on transference to soil. The reciprocal cross was unsuccessful.

Lang's (1940) figures show a similar difference in the ease of fruit production on experimental hybridization, dependent on the maternal parent. The majority of fruits in both crosses were found to be empty but while he was able to raise only one hybrid from the crosses involving S. sylvatica as the maternal parent, in the reciprocal cross he obtained a number (unspecified but 'percentage fruit set less than in selfed plant.' Translated from the original German.) of plants. The morphology of these F_1 hybrids he reports as most closely resembling their maternal parent (S. palustris).

The backcrosses were almost totally unsuccessful with only 21 ripe fruits produced. These, however, failed to germinate and were found to be empty. Clearly, the production of mature fruits may be independent of embryo development in all three taxa.

The difficulty found in synthesizing the F_1 hybrid in this investigation and by Lang has also been confirmed by Morton (personal communication). The synthesis of F_1 hybrids between some species has been shown to be dependent on the choice of genotype used (Valentine, 1947 and Matfield 1972) and this may be the case with S. ambigua.

The seed germination tests, given in table 5 show a significant effect of treatment with gibberellic acid on germination. This is in agreement with the work of Thompson

Table 4. Self- and cross-pollination in Stachys palustris, S. sylvatica and S. ambigua. Lang's (1940) results in brackets.

♀ ♂	No. of flowers pollinated	No. of ripe fruit	Mean % fruit set and range for all genotypes	No. germinated	% germinated
S. sylvatica selfed	129(186)	219(445)	42(60):59-4	181	83
S. sylvatica x S. sylvatica	122	127	26:37-4	95	75
S. palustris selfed	125(80)	85(134)	17(42):31-2	44	52
S. palustris x S. palustris	110	209	47.5:63-21	189	90
S. sylvatica x S. palustris	144(81)	140(110)	24(33.5):44-0	10*	7
S. palustris x S. sylvatica	135(45)	0(49)	0(26)	0	0
S. palustris x S. ambigua	115	12	3 12-0	0	0
S. ambigua x S. palustris	31	0	0	0	0
S. sylvatica x S. ambigua	45	0	0	0	0
S. ambigua x S. sylvatica	27	0	0	0	0

*All died at the seedling stage.

(1969) with several other labiate species. Under what
conditions the mericarps will germinate in nature is not known.
The fruits studied were collected in August 1970 and germinated
in January 1971. Seedling establishment was extensive in the
competition experiment at the beginning of the second season in
early April. It may well be that either 6 months maturation or
vernalization at or below $0^{\circ}C$ is required to break dormancy.
In this investigation the fruits were treated at $4^{\circ}C$ and this
provided the only other treatment where any germination
occurred.

The pollen inviability scores, table 6, show a range for the
field populations from 10-90%. Generally, high pollen
inviability scores are associated with intermediate chromosome
number, both features indicating hybridization. They occur
together in the following populations:- H1A, H2, H4, H6, H7, H8,
H9, H10, H11, H12 and H13.

In all the populations investigated only two exhibit
character combinations inconsistent with one of the three
species categories. They are P6 and P13 both with high
inviability scores and high chromosome number. The two numbers
(2n = 102 and 2n = 103) are entirely consistent with the
reported number for S. palustris, so there is no indication of
the aneuploidy that would be expected in backcross hybrids with
S. palustris. An explanation of the inconsistencies exhibited
by these two populations is dependent on an examination of their
morphology (see General Discussion p. 136)

The results of the pollen germination test are shown in
table 7. S. palustris and S. sylvatica show no difference in
percentage germination, but have significantly different mean

Table 5. Percentage germination for P7 and S15, one month
from the start of the experiment (12 January –
12 February 1971). Sample size, n = 80.

		P7	S15
1.	Control	0	0
2.	Scoured	0	0
3.	High temperature	0	0
4.	Gibberellic acid	76.9($\pm$9.1)	72.5($\pm$10.5)
5.	Dark	0	0
6.	Vernalization	1.0	0.5
7.	Low temperature	0	0

Table 6. Mean pollen inviability scores for 29 field populations obtained during August 1970. Where the population range falls outside one category, the complete range is shown in brackets.

%categories	<10	10-30	30-50	50-70	70-90	>90
	P5	H12(10-50)	H8(30-90)	P6	P13	
	P7			H1A	H6	
	P9			H2	H9(50-70)	
	P10			H4	H10(50-90)	
	P11			H5	H11	
	P14			H7(50-90)	H13(50-90)	
Populations	P20				H14(50-90)	
	S10				H15(50-90)	
	S12					
	S13					
	S14					
	S15					
	S16					
	S18					
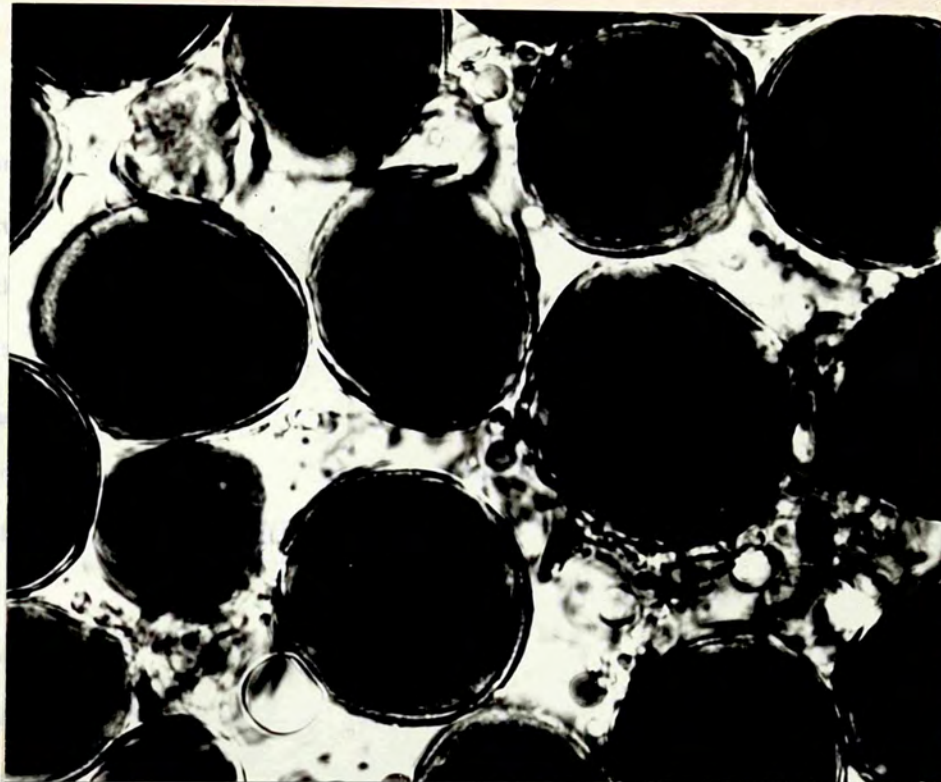
Fig. 9. S 15 with 90% round and stainable pollen grains.
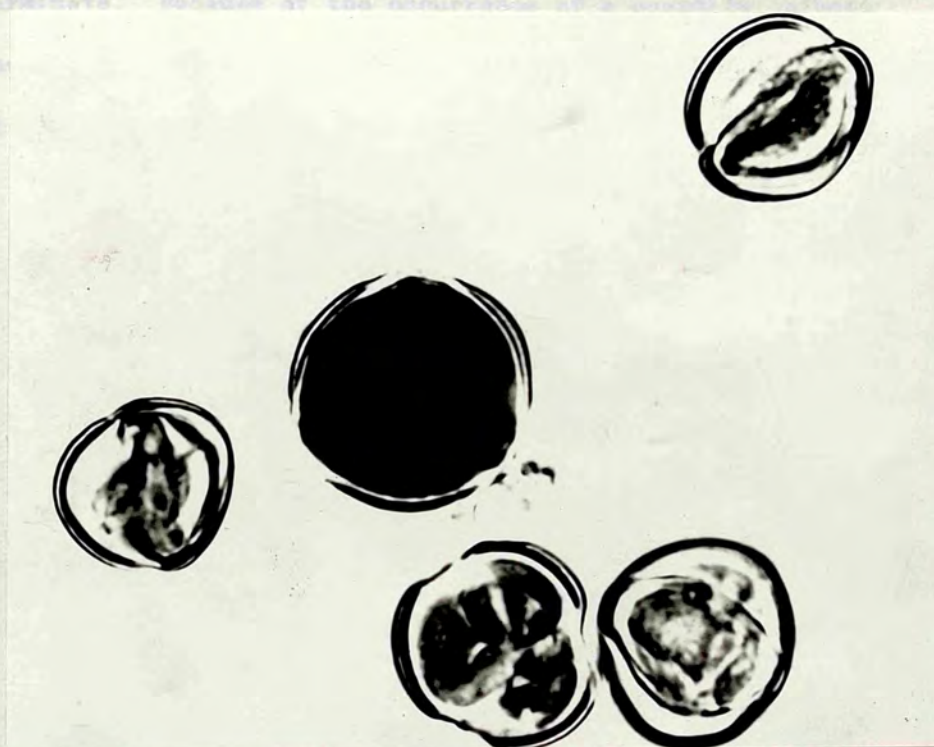
Fig. 10. H11 with 60-80% distorted and unstained pollen grains.

Figs. 9 and 10. Pollen of Stachys sylvatica (S15) and
Stachys ambigua (H11) mounted in acetocarmine.

pollen tube lengths. S. palustris pollen tubes are almost twice as long as those of S. sylvatica. This may account for the observed difference in fruit set in the experimental crosses. Significantly greater fruit set is obtained when S. sylvatica is pollinated by S. palustris (although embryo formation and development appears proportionately greater in the reciprocal cross).

Less than 5% of the pollen produced by S. ambigua germinates on sucrose agar. This is an important feature since, although a high proportion of round stainable grains are present in the pollen of S. ambigua, the majority will not germinate. Because of the occurrence of a quantity, albeit small, of fertile pollen in the anthers of S. ambigua backcrossing of the hybrid to either parent is a possibility.

Table 7. Percentage pollen germination and mean pollen
length with confidence limits at p = 0,05 in
Stachys spp.

		% germination	Mean pollen tube length and confidence limits at p = 0.05
S. ambigua	H4		
	H6		
	H13	< 5	ND
	H23		
	H24		
S. palustris	P5		
	P9	91 ± 8	0.613mms ± 0.06
	P19		
	P23		
S. sylvatica	S10		
	S12		
	S19	93 ± 5	0.340mms ± 0,023
	S20		
	S21		

3. NUMERICAL CHEMOTAXONOMY

INTRODUCTION

Biochemical investigations of plants, particularly in the last decade, have shown the intrinsic value of such studies for taxonomists, in the work cited by Alston and Turner (1963) and Hawkes (1968), for example. While chemotaxonomic procedures have diversified, there have been very few attempts to group the data obtained by numerical analysis. Most workers have relied on simple totals of shared pigments in assessing taxonomic relationships. However, as pointed out by Sokal and Sneath (1963), biochemical data are particularly suitable for numerical taxonomic procedures as some problems experienced with morphological data, such as defining unit characters, do not arise.

There are several coefficients of association that may be employed in taxonomic work and these are listed by Sokal and Sneath (1963: 129-130). Three coefficients seem particularly appropriate for chemotaxonomic work. One - the simple matching coefficient - is based on both negative and positive matches as an assessment of similarity. It is derived as follows,

$$SI_{SM} = \frac{\text{similarities } (+/+ - -/-)}{\text{similarities} + \text{dissimilarities}}$$

With Jaccard's coefficient, similarity is based only on positive matches, as follows,

$$SI_J = \frac{\text{similarities } (+/+)}{\text{similarities} + \text{dissimilarities}}$$

In the Dice modification, positive matches are weighted twice that of unmatched pairs.

$$SI_D = \frac{2 \times \text{similarities } (+/+)}{2 \times \text{similarities} + \text{dissimilarities}}$$

The simple matching coefficient of Sokal and Michener (1958) has already been employed in spot associations by a number of authors, for example, Jaworska and Nyborn (1967), Olsson (1967), Grant and Zandstra (1968), Dedio et al (1969) and Moore et al (1970). Not all of these workers found the procedure satisfactory and Moore et al considered that the coefficient actually obscurred trends in the data. They studied the geographical variation of leaf flavonoids in Empetrum rubrum and considered the affinities between samples based on the numbers of flavonoids shared by each pair more informative than the simple matching coefficient. Moore et al state that this is 'probably because the absence of a flavonoid, used in calculating each similarity index has much less biological significance than its presence'. On this basis the coefficient of Jaccard (1908) where negative matches (-/-) are excluded from assessment of the similarity index should be more satisfactory.

Jaccard's coefficient, which is sometimes referred to as the paired affinity index or similarity coefficient, has been employed by a number of workers including Harmey and Grant (1965), Matthews (1966), Jaworska and Nyborn (1967), Olsson (1967), Dedio et al (1969) and Taylor (1971). Again, there is no agreement on the general effectiveness of the procedure. Jaworska and Nyborn conclude from their study of the putative hybrid between Saxifraga caesia and S. aizoides using both coefficients that the matching coefficient is to be preferred as it takes into account negative matches which are not included in Jaccard's coefficient. Taylor reports that 'the cluster analysis failed to show any systematic grouping of taxa based on pigment similarities' even though the three species of Tiarella under consideration appear separable on features of

leaf morphology (Hitchcock et al 1961). This, of course, does not necessarily mean that the program is ineffective in discovering groupings if, on the basis of pigment similarity, none exist.

The third coefficient employed in this study, that of Dice (1945) is a modification of Jaccard's coefficient where positive pairs are given twice the weight of unmatched pairs, and negative matches are excluded. In practice, where chromatograms all have a similar number of spots the dendrograms produced from this coefficient of association will not be markedly different from those using the Jaccard coefficient on the same input data. If more than two chromatograms have the same number of spots in common but different totals then with Jaccard's coefficient their degrees of similarity will vary depending on the number of dissimilar spots. The larger the number of dissimilar spots, the lower the percentage similarity between each two chromatograms. With the Dice coefficient, the number of dissimilar spots will have less significance as the positive matches are doubled in both the numerator and denominator and the percentage similarities between these chromatograms will be closer.

The use of correlation coefficients and taxonomic distance procedures is not attempted in this study as both are more complex in programming for the computer than coefficients of association and are not really required when dealing with binary state data. The same reasoning has been applied to the selection of the weighted variable group procedure used to cluster the chromatograms rather than the commonly employed principal components or factor analysis. Gower (1966) has pointed out

that these procedures 'were primarily intended for use with
continuous (quantitative) variables'

<div align="center">MATERIALS AND METHODS</div>

Flower pigments and other compounds were extracted from 23
corolla samples collected in the field and sent by post directly
to the laboratories for processing. On receipt, the samples were
partially homogenized with methanol:hydrochloric acid (99:1 v/v)
in a Waring blender. The resulting solution was concentrated
using a rotary evaporator and stored at $4^{o}C$ in a refrigerator
for approximately one month before being spotted onto Whatman
no. 1 paper.

Two-dimensional paper chromatography was employed for
pigment separation using 15% acetic acid as the first solvent
and butanol:acetic acid:water in the proportions 5:1:3 v/v/v as
the second. Rf values were obtained for all spots visible in
daylight, ultra-violet light or in ultra-violet light after
treatment with ammonia. Identification of individual pigments
was not attempted.

The spots on the chromatograms were associated using
computer-programmed procedures. The first program was designed
by I.A. Stevenson to compare the Rf values of the spots on
separate chromatograms. By running the same sample with every
set of chromatograms when they were developed, the variation in
spot position produced by the running conditions was assessed.
The range of variation of spot position was 12% and this was
taken as the error level for the production of a series of
standards. The Rf values for the spots on chromatogram 1 were
all taken as standards. The Rf values of the spots on the next

chromatogram were compared with chromatogram 1. A spot with both Rf values falling within a 12% range of the Rf values of one standard was taken as identical and given the same standard number. Those spots not overlapping with the range of the standards on chromatogram 1 were given new standard numbers. The spots on chromatogram 3 were compared with the standards from 1 and 2; etc.

Once a standard number had been allocated to each spot on every chromatogram, the data were arranged in an nxt matrix, where n represents the standards with two states, present or absent. Column t contains the entities to be grouped by their association over all the standards (the so-called Q-technique). The programs involved in this procedure were written in Fortran IV by Dr. L. Morgan of the Computing and Statistics Department of Royal Holloway College.

Three different coefficients of association given in Sokal and Sneath (1963) were employed:-

1. Coefficient of Sokal and Michener (1958).

2. Coefficient of Jaccard (1908); Sneath (1957).

3. Coefficient of Dice (1945).

The similarity indices produced from all three coefficients range from 0-1, where 1 represents complete identity.

The chromatograms were then grouped by means of the weighted variable group clustering procedure given in Sokal and Sneath (1963), using Spearman's sums of variables for recomputing similarities. The criterion for admitting new members to clusters was the same as that used by Sokal and Michener (1958), i.e. 0.03. The results of the clustering procedure lead to the production of a dendrogram showing the relationships between chromatograms derived from samples of different populations.

The effectiveness of the three programs produced was first evaluated by their application to the results obtained by Moore, Harborne and Williams (1970) of flavonoid variation within Empetrum rubrum samples from S. America.

EVALUATION OF THE COEFFICIENTS OF ASSOCIATION

In their paper on the chemotaxonomy, variation and geographical distribution of the Empetraceae, Moore, Harborne and Williams (1970) discuss flavonoid variation within Empetrum rubrum samples from S. America (pp. 287-290, fig. 3 and tables 4 and 5). This work lends itself to the evaluation of the coefficients of association under consideration as Moore et al, provide two similarity matrices of the 18 samples, one based on shared flavonoid pairs and another on similarity index values (= simple matching coefficient).

The origin and habitat of the Empetrum rubrum samples are shown in table 8a. The two similarity matrices produced by Moore et al are shown in table 8b.

Moore et al conclude that the matrix based on shared flavonoids between samples is more informative than the 'statistically more appropriate similarity matrix' which 'tended to obscure the trends'. They confine their interpretations to a consideration of the shared pairs of flavonoids.

Greatest affinity was shown between samples 6-12. These all come from Nothofagus forest habitats of central and southern Tierra del Fuego and southern Patagonia. Less affinity with this group is shown by samples 4, 5, 13 and 14. This probably reflects spatial separation of samples rather than habitat differences, although 4, 13 and 14 come from Steppe habitats, since 5 comes from a forested habitat. This view is supported by the lack of affinity with the 6-12 forest group samples

shown by 3, 15 and 16, which also came from forest.

The decreasing chemical affinity shown by samples with the 6-12 forest group appears to be related to increasing distance of origin of the samples from the forest group sample area. While habitat differences seem less important than spatial separation, sample 6 which possesses high affinity with the forest group is geographically fairly close to 4 from the south Patagonian steppe, with which it has much lower affinities. There is, therefore, an ecological component involved in the relationships.

Samples 2 and 18 show decreasing affinity with geographical separation. Sample 1 does not completely agree with this.

Sample 17 does not accord with the general pattern. It should, on the basis of geography, show greatest chemical affinity with the NE Tierra del Fuego samples (13 and 14), but is closer to the central and southern Tierra del Fuego samples 8, 9, 10, 11 and 12.

These main conclusions of Moore et al were compared with the dendrograms (fig. 10 - 12) derived from the associations produced by each of the three coefficients. There is clearly little difference between the relationships obtained employing the Jaccard coefficient and the modification of Dice (see figs. 10 and 11 respectively). Both these coefficients ignore negative matches, a condition satisfying the objection of Moore et al that their similarity index tends to obscure the trends probably 'because the absence of a flavonoid...has much less biochemical significance than its presence'.

The dendrogram produced by the simple matching coefficient of Sokal and Michener (fig. 12) shows considerable differences

Table 8a.   Geographical origin and habitat of the 18
            Empetrum rubrum samples studied by Moore et al
            (1970).

1.   C. Chile: High Andes

2.   C. Chile;   High Andes c.2200m.

3.   S. Chile;   Nothofagus forest

4.   S. Chile;   Steppe

5.   W. Fuegia;   Nothofagus forest

6.   S. Chile;   Nothofagus forest

7.   W. Tierra del Fuego;   Nothofagus forest

8.   C. Tierra del Fuego;   Nothofagus forest

9.   "   "   "   "   "

10.   "   "   "   "   "

11.   "   "   "   "   "

12. S. Tierra del Fuego;   Nothofagus forest

13. N. Tierra del Fuego;   Steppe

14.   "   "   "   "

15. S.E. Fuegia;   Nothofagus forest

16.   "   "   "

17. Falkland Isles

18. Gough Island;   Cliffs

Table 8b. Numbers of flavonoids shared by pairs of Empetrum rubrum samples and similarity index values for flavonoid content between them. Data of Moore, Harborne and Williams (1970).

1. Shared flavonoids

Sample		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Chilean	1																		
Andes	2	6																	
W. Patagonia	3	6	4																
S. Patagonia steppe	4	7	5	7															
W. Fuegia	5	7	5	7	8														
S. Patagonia forest	6	9	6	7	8	8													
C. Fuegia forest	7	8	5	7	8	8	10												
	8	7	5	7	8	8	9	9											
	9	8	5	7	8	8	10	9	9										
	10	7	5	7	8	8	9	9	9	9									
	11	8	5	7	8	8	10	10	9	10	9								
S.C. Fuegia	12	7	5	7	8	8	9	9	9	9	9	9							
C. Fuegia steppe	13	7	5	7	8	8	8	8	8	8	8	8	8						
	14	6	5	7	7	7	8	8	8	8	8	8	8	8					
S.E. Fuegia	15	6	5	6	7	7	7	7	7	7	7	7	7	7	6				
	16	6	5	6	7	7	7	7	7	7	7	7	7	7	6	7			
Falkland Is.	17	8	5	7	8	8	9	9	8	9	8	9	8	8	7	7	7		
Gough I.	18	5	3	5	5	5	5	5	5	5	5	5	5	5	4	4	4	5	

Table 8b (cont.)

2. Similarity index values.

Sample		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Chilean Andes	1																		
W. Patagonia	2	67																	
S. Patagonia steppe	3	60	44																
W. Fuegia	4	78	56	88															
S. Patagonia forest	5	70	56	88	100														
C. Fuegia forest	6	82	55	64	73	73													
	7	73	45	70	80	80	91												
	8	64	50	78	89	89	82	90											
	9	73	45	70	80	80	91	100	90										
	10	64	50	78	89	89	82	90	100	90									
	11	73	45	70	80	80	91	100	90	100	90								
S.C. Fuegia	12	64	50	78	89	89	82	90	100	90	100	90							
C. Fuegia steppe	13	70	56	88	100	100	73	80	89	80	89	80	89						
	14	55	56	67	78	78	73	89	80	80	89	89	89	78					
S.E. Fuegia	15	55	63	75	88	88	64	70	70	70	78	70	78	88	67				
	16	55	56	67	78	78	58	64	70	64	70	70	70	78	60	88			
Falkland Is.	17	80	50	78	89	89	82	90	80	90	80	90	80	89	70	78	70		
Gough I.	18	50	34	63	56	56	42	45	50	45	50	45	50	56	40	44	40	50	

with the other two dendrograms. The relationships obtained by the three coefficients of association are compared with the conclusions of Moore et al.

The forest group of samples (6-12) considered by Moore et al to share the greatest affinity occur together in the dendrogram produced by simple matching (fig. 12) at 0.9 level of similarity, together with samples 14 and 17. This group only appears in the other two dendrograms at a low level of similarity and includes 3, 4, 5, 13, 14 and 15. In these two dendrograms samples 4, 5, 13 and 14 do not show less affinity with the forest group, but 14 is included in the group containing samples 6-12.

Samples 3, 15 and 16 in the dendrogram produced by simple matching certainly show as little affinity with the forest group 6-12 as do 4, 5 and 13 and these six samples form a group at the 0.91 level of similarity.

The lack of affinity between the geographically close samples 6 and 4 recorded by Moore et al is reflected in all three dendrograms. Samples 1, 2 and 18 in all three dendrograms show a marked lack of affinity with the other samples and no high degree of similarity between themselves. This illustrates the importance of spatial isolation as a factor in decreasing affinity between samples shown by Moore et al. In contrast to Moore et al's conclusion, sample 1 does appear to agree with this.

Sample 17 certainly shows a high level of affinity with the central and southern Tierra del Fuego samples 8, 9, 10, 11 and 12 (9 and 11, in particular) in the dendrogram produced by simple matching, in accordance with Moore et al's conclusion. This is not shown so clearly in the other two dendrograms.

All the conclusions reached by Moore et al are reflected in

the dendrogram derived by the simple matching coefficient with the exception of the position of sample 14. In this result it shows strong affinity with the forest group 6-12, a fact supported by its slightly higher similarity index values with that group than sample 13. Also, 13 shows much stronger associations with 3, 4 and 5 than does 14 (88, 100, 100 compared with 67, 78 and 78 respectively). The dendrograms produced by the Dice and Jaccard coefficients do not reflect Moore et al's conclusions as accurately as the third one.

In this study, the simple matching coefficient of Sokal and Michener, therefore, when employed with a weighted variable group clustering procedure, was the most effective method of the three coefficients of association investigated, in reflecting relationships between the chromatograms.

RESULTS

From the program devised by Ann Stevenson to produce standards, a total of 103 different ones were recognized from the 23 chromatograms at the 12% error level. 78% of these standards occurred in one or more of the putative S. ambigua samples, 64% in S. palustris and 39% in S. sylvatica. Putative samples of S. palustris and S.sylvatica each have 11 unique standards, and 24 occurred only in the S. ambigua samples. 37 standards occurred only once; 27 on 2 or 3 chromatograms; 25 on 4-7; 10 on 8-13; and 4 on 15 or more chromatograms. Of these last 4 standards, 2 are absent from only 4 chromatograms and one absent from only 5. They may represent spots occurring consistently, but in varying quantities, in all three taxa but undetected in 4 or 5 chromatograms.
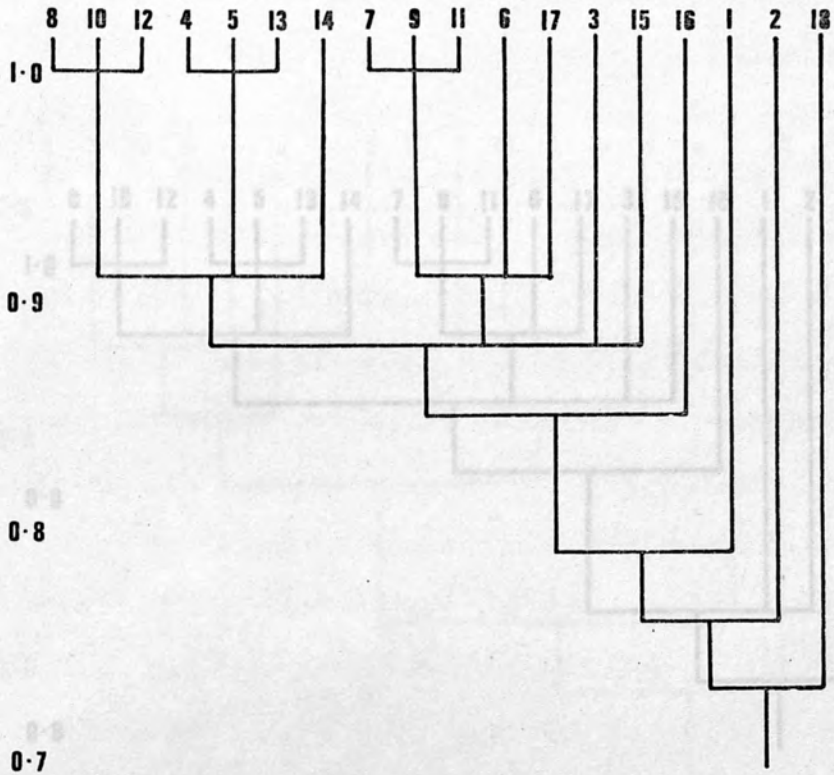
FIG. 10 DENDROGRAM OF THE RELATIONSHIPS BETWEEN 18 SAMPLES OF EMPETRUM RUBRUM, ASSOCIATED USING THE JACCARD COEFFICIENT. FROM THE DATA OF MOORE, HARBORNE AND WILLIAMS (1970).
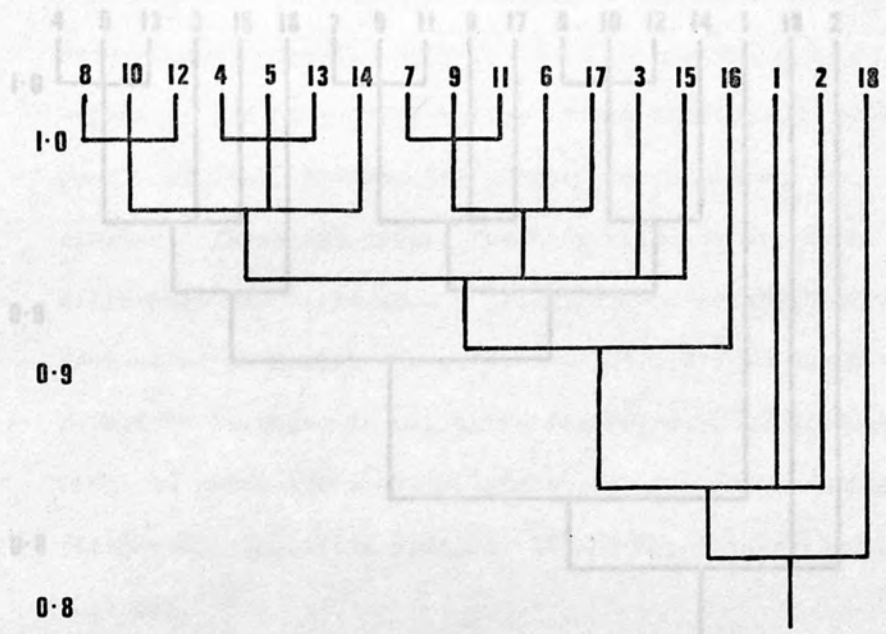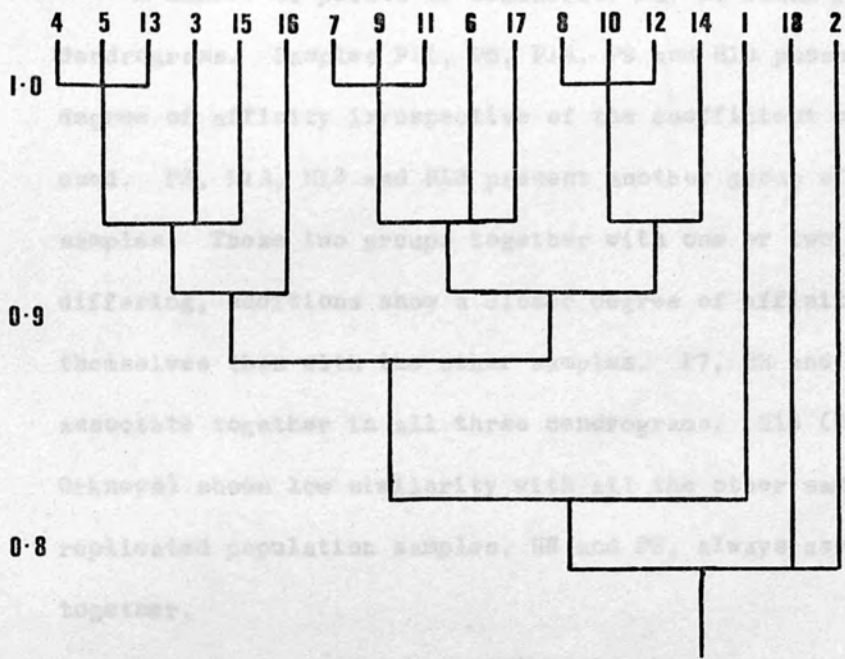
FIG. II DENDROGRAM OF THE RELATIONSHIPS BETWEEN 18 SAMPLES
OF EMPETRUM RUBRUM, ASSOCIATED USING THE DICE
COEFFICIENT. FROM THE DATA OF MOORE, HARBORNE AND
WILLIAMS (1970).

FIG. 12 DENDROGRAM OF THE RELATIONSHIPS BETWEEN 18 SAMPLES OF EMPETRUM RUBRUM, ASSOCIATED USING THE SIMPLE MATCHING COEFFICIENT. FROM THE DATA OF MOORE, HARBORNE AND WILLIAMS (1970).

Each of three coefficient of association programs written by Dr. Morgan was run with the results obtained for the Stachys samples. The dendrograms obtained are presented in figs 13, 14 and 15. Figs. 13 and 14 based on associations that ignore negative matches, show only minor differences from one another.

A number of points of similarity may be found in all three dendrograms. Samples P11, P5, P14, P9 and H10 possess a high degree of affinity irrespective of the coefficient of association used. P3, H1A, H12 and H13 present another group of similar samples. These two groups together with one or two minor, and differing, additions show a closer degree of affinity amongst themselves than with the other samples. P7, H8 and H1B associate together in all three dendrograms. H15 (Rousay, Orkneys) shows low similarity with all the other samples. The replicated population samples, H8 and P3, always associate together.

The simple matching coefficient, which proved most effective in representing the data of Moore, Harborne and Williams (1970), also appears to be the most effective one of the three in associating morphologically closely related Stachys populations (fig. 15). At the 0.75 level of similarity 3 groups are distinguished. The first group contains 12 samples in two sub-groups. The smaller sub-group has 5 samples, 4 putatively identified as S. palustris and 1 S. ambigua. They came from a wide area - Lake District, Perth, Argyll, Sutherland and Orkney. The larger sub-group contains 5 putative S. ambigua samples and 2 S. palustris (replicates of P3). They were derived mainly from two areas - Isle of Man and Orkney. The remaining sample came from Argyll.

In the second group are 3 putative S. ambigua and 1

S. palustris samples. They came from the Isle of Man, Dalmally
(replicated) and the Lake District.

The third group contains all the S. sylvatica samples, together
with 2 S. ambigua samples. The S. sylvatica populations came
from the Isle of Man and Orkney and the hybrid samples are
from the Isle of Man and Argyll.

H15 - a putative S. ambigua sample from Rousay, Orkneys -
is isolated from the other samples. The second and third
groups show a greater affinity with H15 and each other than with
the first group.

The dendrogram produced by the simple matching coefficient
is the only one that united the putative S. sylvatica samples.
In the other two dendrograms they show a low level of similarity
with each other generally associating with a number of different
sub-groups.

DISCUSSION

The strongest element in the association of samples of
Stachys by all three coefficients of association is their
taxonomic affinity. Geographical separation of samples is less
important. For example, the predominantly hybrid group occurring
in all three dendrograms comprising P3 replicates, H1A, H12, H13
and H14, with minor modifications, includes samples from Orkney
and the Isle of Man. Similarly the consistent group of
predominantly S. palustris samples - P11, P5, P14, P9 and H10 -
range from Orkney to the Lake District. The dendrogram reflecting
most accurately the taxonomic relationships of the samples
previously deduced from morphological and cytological evidence
was produced using the simple matching coefficient. This
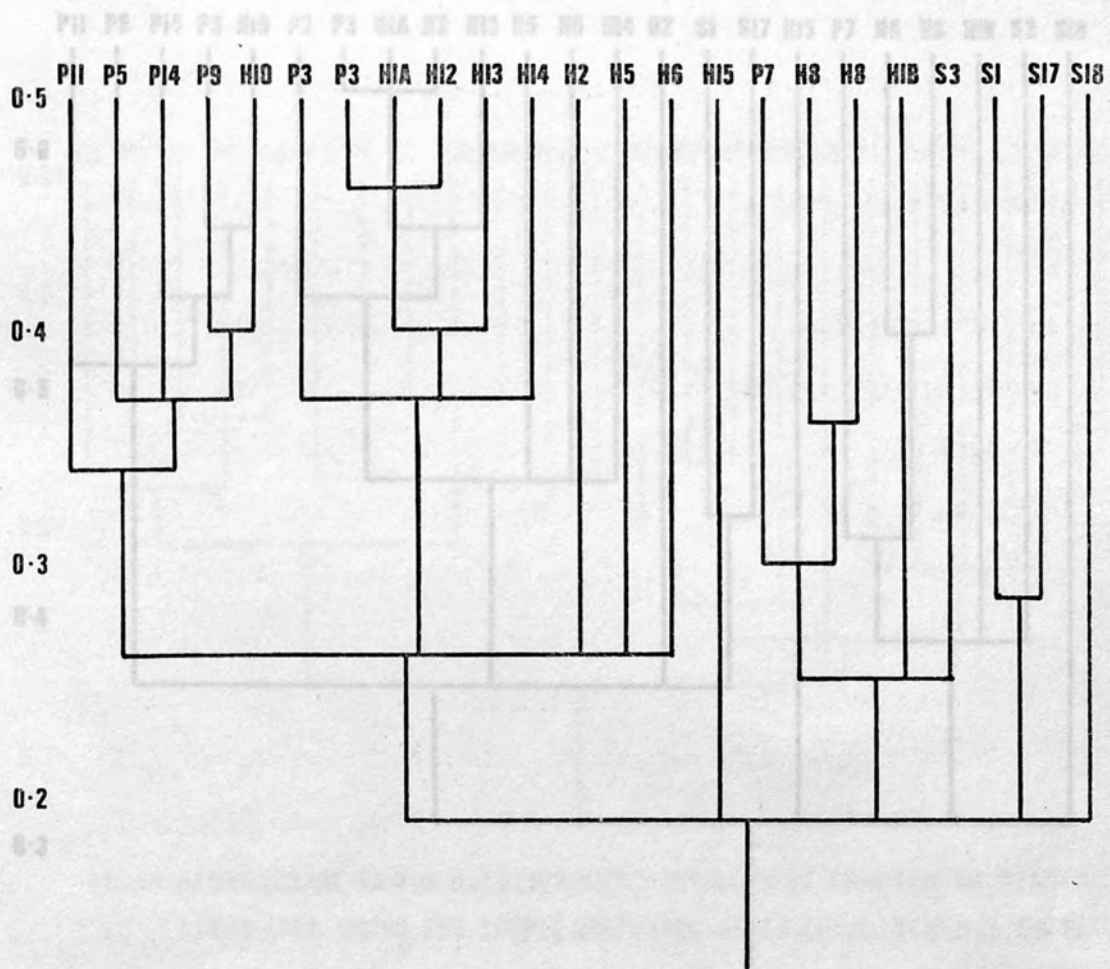
FIG. 13 DENDROGRAM OF THE RELATIONSHIPS BETWEEN 23 SAMPLES OF STACHYS, ASSOCIATED USING THE JACCARD COEFFICIENT. ORIGINAL DATA.

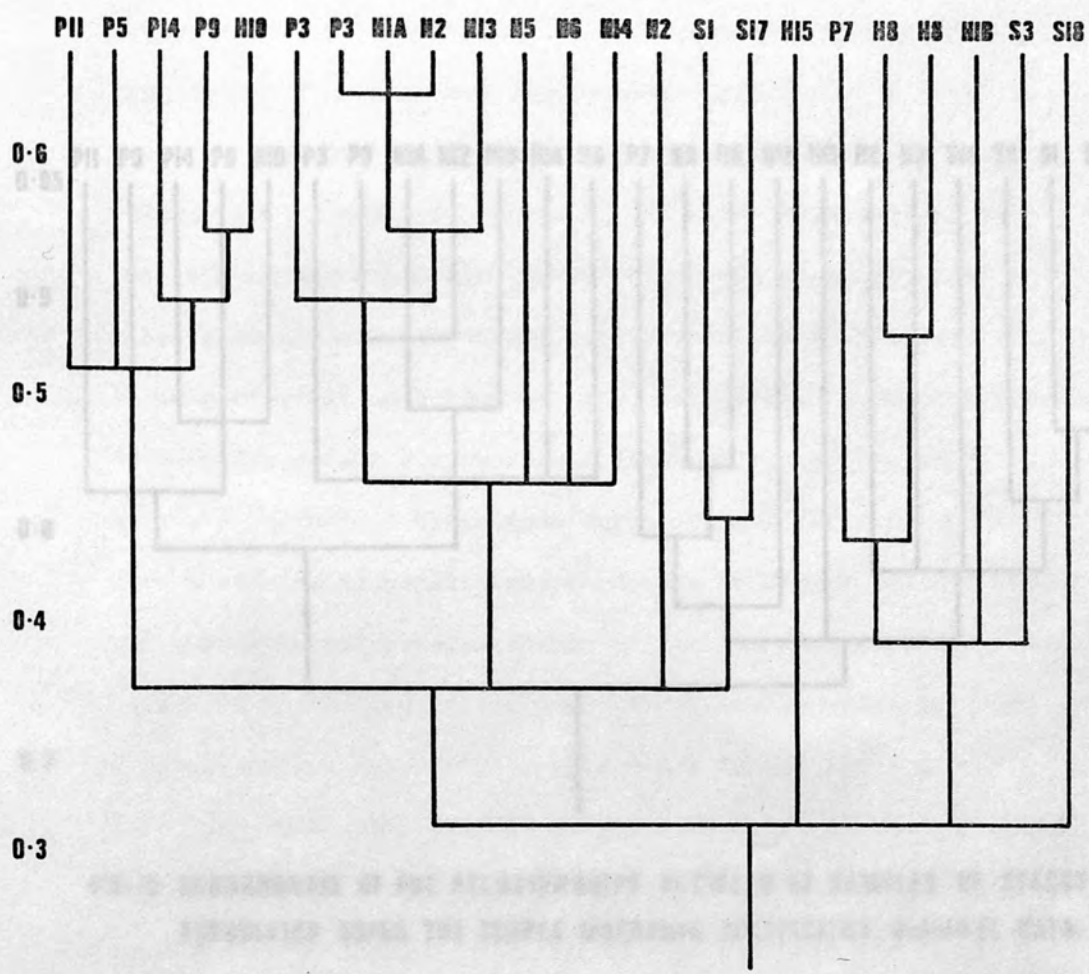FIG. 14 DENDROGRAM OF THE RELATIONSHIPS BETWEEN 23 SAMPLES OF STACHYS, ASSOCIATED USING THE DICE COEFFICIENT. ORIGINAL DATA.
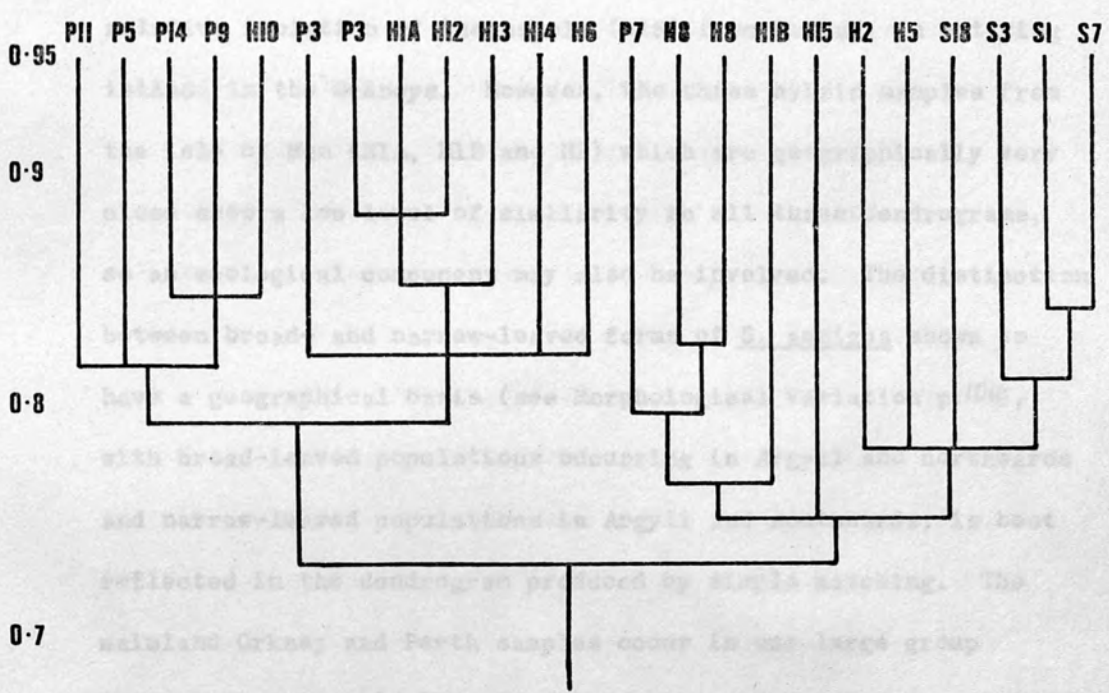
FIG. 15 DENDROGRAM OF THE RELATIONSHIPS BETWEEN 23 SAMPLES OF STACHYS, ASSOCIATED USING THE SIMPLE MATCHING COEFFICIENT. ORIGINAL DATA.

dendrogram is the only one in which the samples of <u>S. sylvatica</u> are associated together at a high level of similarity.

A geographical component, while less important than taxonomic affinity, is apparent from the associations produced. For example, the three hybrid samples from the mainland of Orkney - H12, H13 and H14 - always associate together with a high level of similarity. In agreement with this is the relative isolation of the sample (H15) from Rousay, an outlying island in the Orkneys. However, the three hybrid samples from the Isle of Man (H1A, H1B and H2) which are geographically very close show a low level of similarity in all three dendrograms, so an ecological component may also be involved. The distinction between broad- and narrow-leaved forms of <u>S. ambigua</u> shown to have a geographical basis (see Morphological Variation p.104), with broad-leaved populations occurring in Argyll and northwards and narrow-leaved populations in Argyll and southwards, is best reflected in the dendrogram produced by simple matching. The mainland Orkney and Perth samples occur in one large group (H10, H1A, H12, H13, H14 and H6) together with one from Argyll and one from the Isle of Man. The other group contains the hybrid samples H8, H1B, H15, H2 and H5, all from Argyll and the Isle of Man, except for the Rousay sample H15. Samples H1A and H15 are therefore misplaced for complete agreement with the leaf data.

The samples P3, H2 and S3, all from Billown Mooar, Isle of Man, show differences of affinity depending on the coefficient of association used. With the Jaccard and Dice coefficients H2 shows a low but greater affinity with P3 than S3. With the simple matching coefficient, H2 shows a greater affinity with S3

than P3. If H2 has arisen by hybridization between S3 and P3 it
is clear that it does not show the close affinity with P3 recorded
from leaf data. This may be taken as further evidence of a
separation between H2 and P3.

The taxonomic affinity of samples reflected in the
dendrogram produced by the simple matching coefficient shows
S. palustris to be very variable and S. sylvatica to be much
more uniform. This is in agreement with the evidence obtained
from a study of the morphological variation of the taxa.

The collection of corolla samples from 23 different
populations throughout Britain prevented concurrent sampling
and caused a delay in transferance of material to acid-methanol,
due to postage. It is unlikely that any important changes in
pigments occurred during postage as there appears to be general
agreement between the results obtained from fresh and air-dried
material, as shown, for example, by the work of Mabry et al
(1970) with Baptisia lecontei and Hymenoxys scaposa and Moore
et al (1970) with the Empetraceae. The absence of concurrent
sampling, however, may automatically induce differences between
populations associated on pigment comparisons. Taylor (1971),
for example, was able to show complete separation of Tiarella
plants collected in July from those collected in August, on the
basis of a comparison of leaf pigments. In the present investig-
ation, the 23 collections spanned a month and there is obviously
a possibility that some differences between chromatograms are
the result of variations in sampling times.

The work of Ball et al (1967) has shown that modification
can be induced in chromatographic spot patterns when plants are
grown under different environmental conditions. The case made

by Runemark (1968) for considering data obtained only from populations grown under the same environmental conditions cannot be justified solely on the grounds that spot patterns are environmentally modified. It is impracticable to ignore the variation observed in nature when considering morphological criteria in taxonomic and evolutionary studies. As pointed out by Sokal and Sneath (1963), the number of compounds in chromatograms will seldom 'be numerous enough and of a sufficiently wide genetic origin to give an adequate sample of the characters of the organism; therefore these methods should be used as an adjunct to others'. In the study of polyploidy and hybridization in particular, valuable taxonomic information may be obtained by a comparison of data obtained from field populations using the widest possible range of characters.

All extractable spots obtained on each chromatogram were employed in the computation of associations. This may therefore involve transitory metabolites in the computation, but their effect on the result will be minimized by the presence of pigments common to several samples. In any case, a metabolite present in a number of chromatograms represents a measure of similarity between them. The relative instability of such compounds is relatively unimportant in view of the variation in the pigment constitution of plants due to environmental conditions.

The chromatographic standard procedure was used with Stachys to minimize the variation in spot pattern on a chromatogram which may arise in development. Nevertheless, it is desirable to introduce a standard pigment of known Rf value into every spotted sample. Subsequent comparison of the Rf of the standard pigment under the developing conditions with its known Rf gives a

greater precision to the numerical values assigned to each spot.

The evidence presented in this study indicates that the simple matching coefficient of Sokal and Michener used in conjunction with the weighted variable group clustering procedure is the most effective of the three coefficients in analyzing the data obtained from the chemotaxonomic studies. Comparative biochemical studies lend themselves a numerical taxonomic treatment and when considering associations between large numbers of chromatograms computer analysis is the only efficient method available to fully utilize the data.

Important criticisms of the use of statistical treatment of the data obtained from chromatograms have been made by Hans Runemark (1968) and require some comment. His four main objections are summarized below:-

1. Scanty data. Analysis of quantitative and qualitative variation within and between populations on a large scale/are necessary to achieve a better understanding of the application of numerical techniques to the data.

2. Spot size varies with the solvent system used. Spot size comparisons between chromatograms must take account of this.

3. An unlimited number of coefficients of association can be used. Therefore almost any hypothesis held by the investigator can be supported, provided a suitable coefficient is selected.

4. Two-dimensional presentation in dendrograms of probable multidimensional relationships between samples cannot be recommended.

Runemark's first point that the data obtained from chemotaxonomic studies is quantitatively inadequate is no

justification for not developing statistical methods appropriate to their treatment. It is particularly important to develop and refine such techniques so that the data from the 'large-scale' investigations demanded by Runemark may be properly handled. The employment of procedures to compare the sizes of different spots certainly needs a more critical study than so far received. The use of correlation coefficients for estimating spot resemblance between chromatograms is clearly inappropriate and the biochemical distance assessment of similarity, as shown by Runemark, needs some refinement.

Different coefficients of association may produce dissimilar dendrograms as shown by this investigation. There is a danger that 'a posteriori' selection of coefficients on the grounds that they support the investigator's hypothesis may occur. However, all coefficients of association do not have the same intrinsic value when applied to biochemical data and should be properly evaluated by comparisons with previously worked data. For instance, in this investigation the selection of the two coefficients of association that ignore negative matches was based on the grounds that spot absences may have less biological significance than presences (Moore et al (1970). However, the simple matching coefficient, including negative matches, most accurately represented the data being evaluated. This also appeared to be the most effective coefficient in reflecting the relationships between the Stachys samples.

Runemark criticises the presentation of data in a dendrogram on the grounds that 'the relationships between the taxa compared may very well be multidimensional'. A dendrogram can simply and effectively illustrate the degree of affinity between

large numbers of individual OTUS and/or clusters, presenting their relationships in two dimensions. This obviously results in simplification of a multidimensional array of OTUS and must not be ignored. The intrinsic value of dendrograms in showing affinity between all investigated OTUS lies in presenting the relationships in an easily interpreted form. Inevitably it introduces the danger of oversimplification.

Runemark demonstrates that the number of spots on the chromatograms may sometimes be responsible for introducing differences in similarity coefficient values between chromatograms. The model he uses is the parent-hybrid relationship involving 9 chromatogram spots - 3 possessed by one parent and 6 by the other. The hybrid is presumed to possess all 9 and therefore using either the simple matching or Jaccard coefficients (negative matches do not occur in the comparisons) it shows similarity values of 0.33 and 0.67 with the two parents. The hybrid shows a greater degree of similarity with the parent possessing the greatest number of spots. This, however, is not a criticism of the methods of associating chromatograms but the interpretation of their resemblance. Even on the basis of shared spots, the hybrid exhibits a greater similarity with one parent - 6 shared pairs with one, and 3 with the other.

In practice, such a simple situation is unlikely to exist, especially when several population samples from each taxon are under investigation. Not all the parental spots will occur in the hybrids and with increasing numbers of samples negative matches correspondingly gain more prominence.

Spot number may be responsible for associating all the S. sylvatica samples with the simple matching coefficient. These

samples have 13 or 14 standards per chromatogram whereas the
remaining samples have an average of 18.5 standards ranging
from 16-20. The number of negative matches will therefore
be slightly higher in S. sylvatica - S. sylvatica comparisons than
in S. sylvatica - S. palustris/S. ambigua comparisons.

4. COMPETITION EXPERIMENTS

INTRODUCTION

Stachys ambigua has been noticed to frequently occur in the absence of one or even both of its putative parents. Perring (1962, 1968) has reported that where S. ambigua occurs in the absence of S. sylvatica, particularly in the Outer Hebrides, Orkney and Shetland, it often appears to be a relic of cultivation. Green (in preparation) writes, 'the hybrid occurs throughout much of the British Isles often in the absence of the parental species, especially S. sylvatica, and presumably distributed by man by fragmentation of the brittle rhizome.'

While distribution (accidental or desired) by fragments of rhizome may occur, it is also possible that the hybrid, once established at a site may enter into successful competition with the parental plants. This is particularly important as Stachys fruits are not equipped with any dispersal mechanism, so that the 'in situ' production of hybrid seed is likely to result in germination within the area of the maternal parent. Stachys ambigua may compete with its parents so successfully as to eliminate them from the immediate neighbourhood. As the F_1 hybrid is largely, if not completely, sterile effective competition will be dependent on extensive apomictic activity. Aggressive rhizome production by some plants will tend to result in their monopolization of soil space and nutrients and a sustained increase in the numbers of shoots produced annually.

A series of experiments was therefore set up to determine the effect the three taxa might have on one another in nature. Interpretations of the results of competition between the three

taxa in the experiments are based on aerial vegetative growth during two seasons and dry weight production of rhizome at the end of the second season. These two features are used in preference to studying amphimictic reproductive potential as all three taxa are predominately apomictic species. Harper (1961) has pointed out that competition 'between individuals acting at the seedling stage is less likely to be crucial in determining the distribution and abundance of vegetatively reproducing plants' than obligate amphimicts. Stachys ambigua is an almost total apomict since it rarely sets fruit.

Changes in aerial vegetative growth and rhizome production indicate successful or unsuccessful competition. This may eventually lead to total dominance or elimination of a taxon. Selective elimination of S. palustris or S. sylvatica by S. ambigua observed through the course of the experiment would lend support to the possible role of competition in producing isolated hybrid populations. However, large-scale eliminations during only two seasons growth are unlikely to occur. An increase over two years in aerial vegetative growth and/or rhizome production of one taxon at the expense of another indicates success that, if sustained, would eventually lead to total dominance.
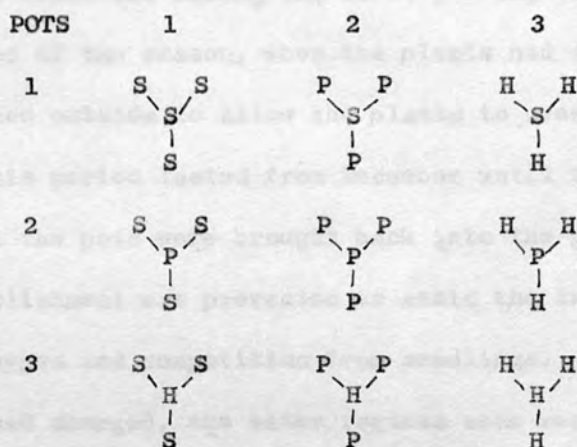
MATERIALS AND METHODS

The source of material used in the competition experiments was rhizome ramets from some clones which were used in the soil-water experiments. Four plants were grown together in single five-inch diameter plastic pots. A central plant of one taxon was surrounded by three from another taxon. In this way, the

interactions between two genotypes of different taxa were
investigated. Controls were set up employing four plants all of
one genotype in the same pot.

All possible combinations for three genotypes, one of each
taxon, were investigated making a total set of nine pots and a
schematic set is shown below using P, S and H to represent
S. palustris, S. sylvatica and S. ambigua respectively.

<u>Schematic set for the competition experiments</u>

```
POTS        1              2              3

  1      S     S        P     P        H     H
            S              S              S
            |              |              |
            S              P              H

  2      S     S        P     P        H     H
            P              P              P
            |              |              |
            S              P              H

  3      S     S        P     P        H     H
            H              H              H
            |              |              |
            S              P              H
```

Each set was replicated once so that the experiments could
be conducted under two water regimes - wet and dry. This was
designed to show any difference in competitive response related
to soil water level, as <u>S. palustris</u> and <u>S. sylvatica</u> have
marked ecological distinctions. Three different sets were studied
and the genotypes utilized in each were, as follows:-

    Set 1.   M4/L1/B8

    Set 2.   K1/R3/A7

    Set 3.   P3/Q8/S5

Set 2 and 3 had the basic two replicates, one for each
treatment, but with set 1, 4 replicates were employed, two in

each treatment.

In this way competition between two taxa could be studied at two different proportions (1:3 and 3:1) from plants grown in both cases at a combined density of 4 plants/pot.

The plants were grown in a greenhouse, the wet treatment obtained by placing pots in large plastic trays containing a constant water level of approximately two inches. The dry treatment was obtained by restricted watering until signs of wilting were observed. Measurements of vegetative height were taken on three occasions during the first growing season.

At the end of the season, when the plants had died back, the pots were placed outside to allow the plants to overwinter naturally. This period lasted from December until the end of March when all the pots were brought back into the greenhouse. Seedling establishment was prevented to avoid the introduction of alien genotypes and competition from seedlings. When the aerial parts had emerged, the water regimes were recommenced. The first new vegetative measurements were taken approximately one month after returning the plants to the greenhouse, and their subsequent growth was followed. At the termination of the experiment, rhizomes from each pot were separated and the dry weight production for each taxon per pot obtained. The dry weight production of aerial shoot mass was also determined.

RESULTS

The results of the experiments conducted are summarized in figs. 16-21 and tables 9 and 10. The diagram below illustrates the form of presentation of the data in tables 9 and 10.

Diagram to illustrate the form of presentation of the data in tables
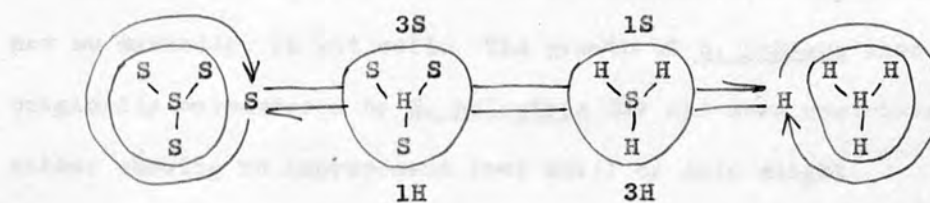
Performance (4 sets of data) of genotypes of <u>Stachys</u> in reciprocal competition at two original proportions. The letters in the circles represent the planting arrangement in the pot experiments; the letters outside the circles indicate how the data presented in the tables was obtained.

In figs. 16-21 the effect of interspecific competition on the growth of the combined genotypes of each taxon during two seasons is presented. As this is the result of the growth of plants in 4 pots, mean shoot height x mean number of shoots per pot is plotted against time. The number against the plot for each taxon refers to the original number of shoots in competition with the taxon being studied.

The effects of competition on the growth of <u>S. ambigua</u> can be seen in figs. 16 and 17. In wet soil, the second season's growth of <u>S. ambigua</u> was greater or (in one case) the same as the previous year's with both <u>S. sylvatica</u> and <u>S. palustris</u>. The growth of <u>S. ambigua</u> does not appear, therefore, to be inhibited in competition with <u>S. sylvatica</u> and <u>S. palustris</u> even when originally outnumbered by them.

In dry soils (fig. 17) again <u>S. ambigua</u> showed no inhibition in growth as a result of interspecific competition during the second season. In fact, in three conditions, with <u>S. sylvatica</u> at both proportions and when originally outnumbering <u>S. palustris</u>,

the growth performance was over twice the first year's figure. The improved growth in these three conditions occurred, although not so markedly, in wet soil. The growth of S. ambigua when originally outnumbered by S. palustris (⊝) was more restricted, either showing no improvement (wet soil) or only slight improvement (dry soil) in the second season.

The effects of competition on S. sylvatica in wet soil (fig. 18) and dry soil (fig. 19) was very obvious. In all conditions, the growth of S. sylvatica was markedly inhibited in the second season. In the wet treatment, when originally outnumbered by both S. palustris and S. ambigua, growth of S. sylvatica was zero or almost zero in the second year. However, effective growth was made by S. sylvatica during the first season.

The effects of competition on S. palustris (figs. 20 and 21) are more complex. In wet soil (fig. 20) when originally outnumbered by both S. ambigua and S. sylvatica, the growth of S. palustris in the second season reached the previous year's level. When in greater proportions, the growth of S. palustris was inhibited in competition with S. sylvatica (compared to the first year growth level) but the growth of S. palustris in the second season is enhanced when in competition with S. ambigua.

In dry soils a complete reversal of these growth responses was observed. Where S. palustris was originally outnumbered, its growth in the second season was inhibited in the presence of S. ambigua but enhanced in the presence of S. sylvatica. When outnumbering its competitors, the growth of S. palustris became inhibited in the presence of S. sylvatica but was enhanced in the presence of S. ambigua.

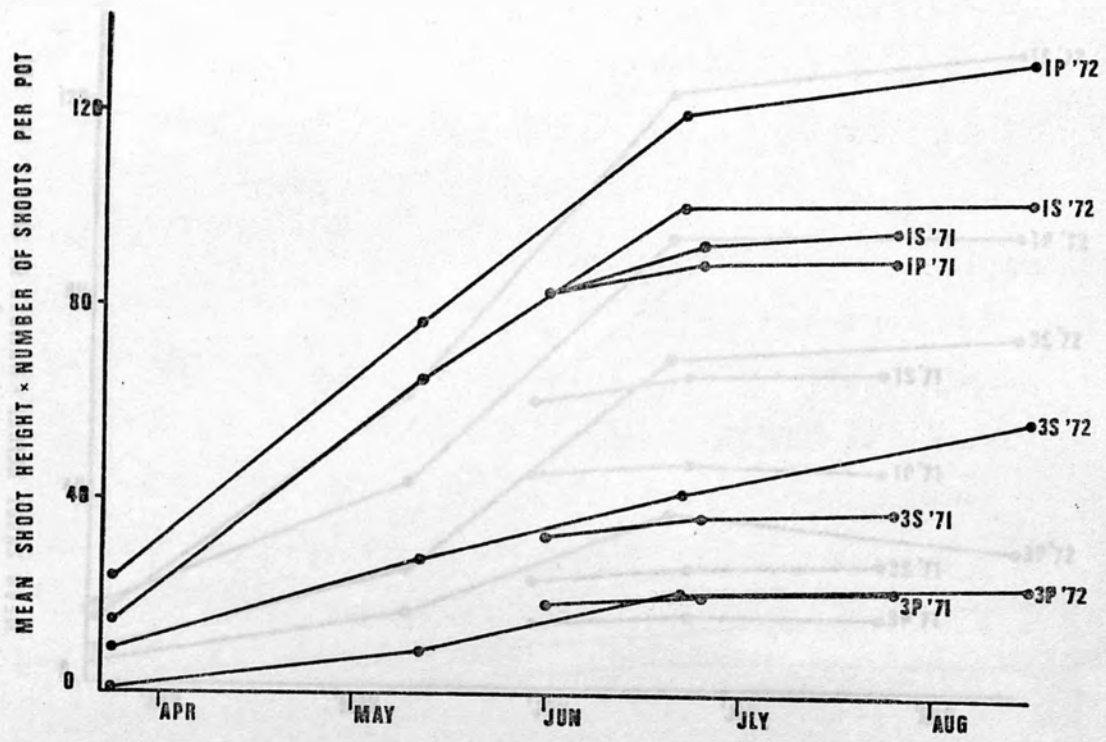At the termination of the experiments on 21st August, 1972
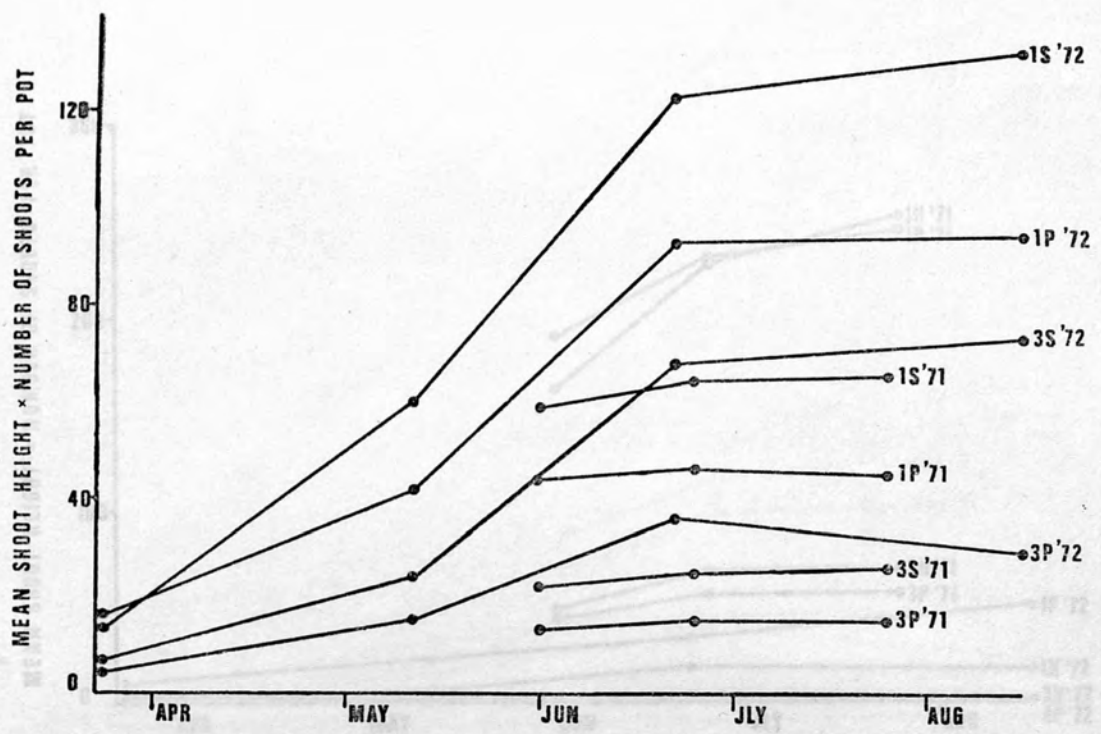
FIG.16 EFFECTS OF COMPETITION ON S.AMBIGUA L WET

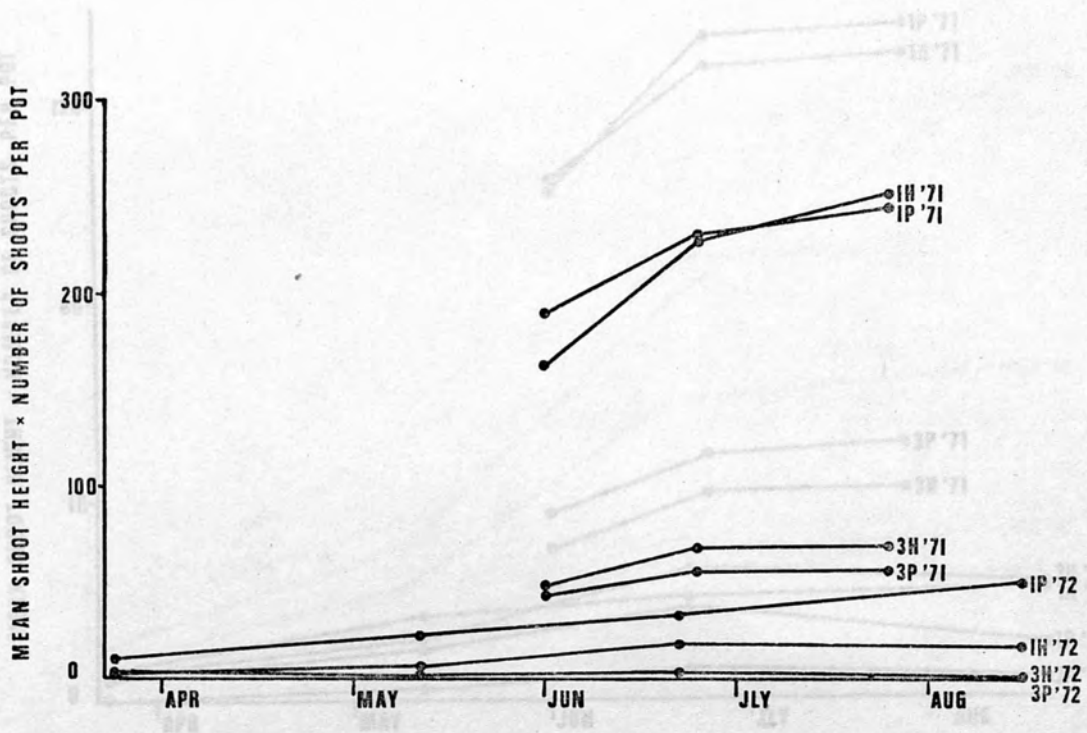FIG.17   EFFECTS OF COMPETITION ON S. AMBIGUA 2. DRY
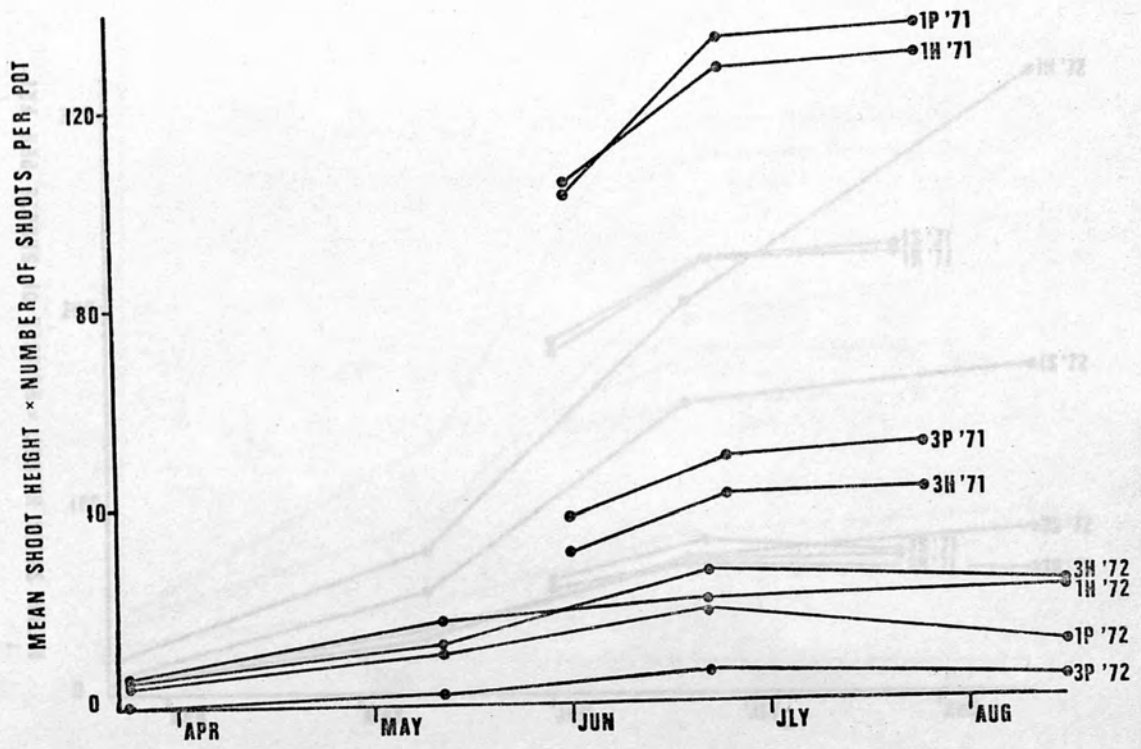
FIG.18 EFFECTS OF COMPETITION ON S. SYLVATICA L WET
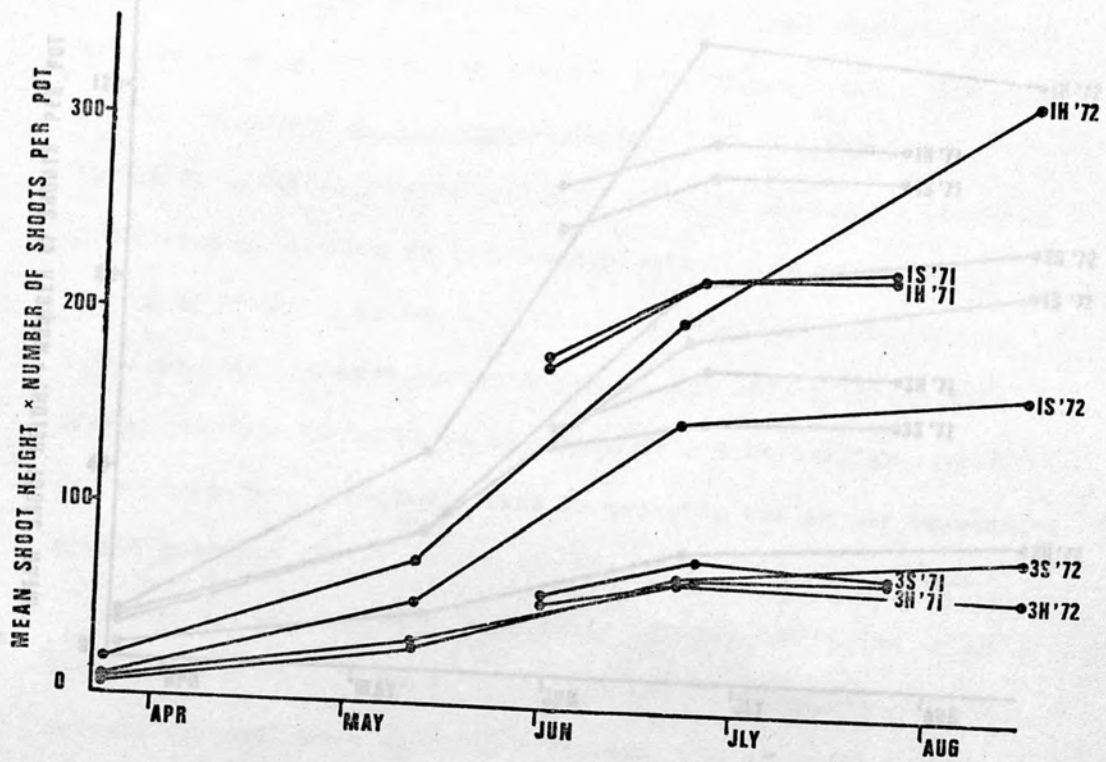
FIG.19 EFFECTS OF COMPETITION ON S. SYLVATICA 2. DRY
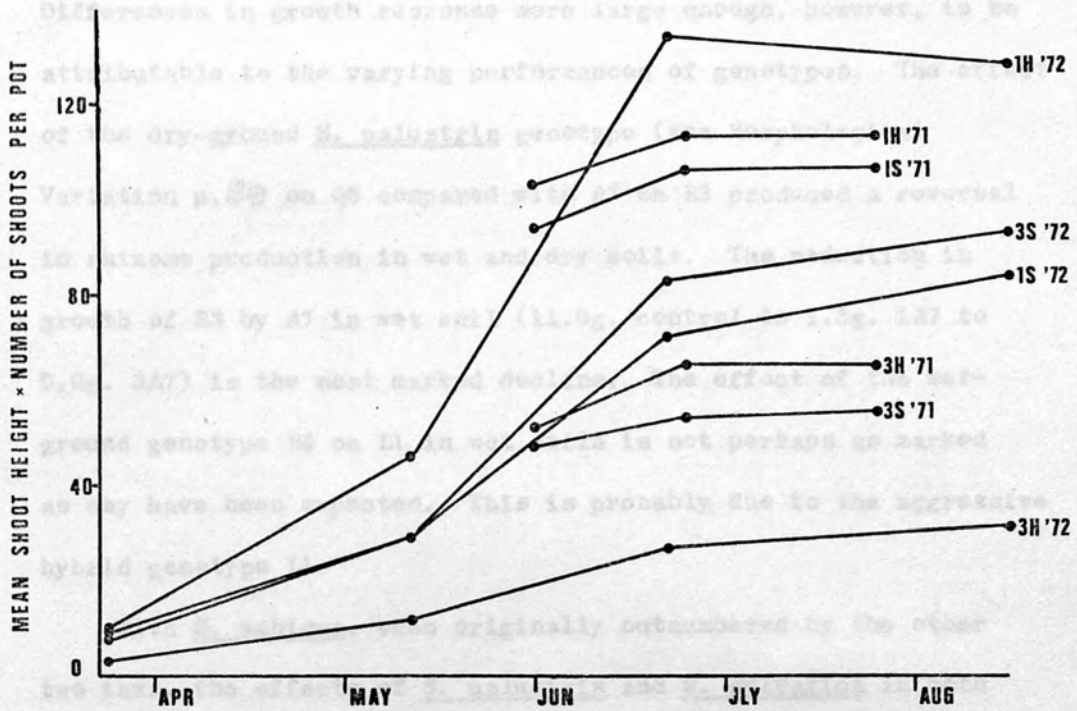
FIG.28 EFFECTS OF COMPETITION ON S.PALUSTRIS I.WET

FIG.21 EFFECTS OF COMPETITION ON S.PALUSTRIS 2.DRY

after 20 months, dry weight measurements were obtained of
separated aerial parts and rhizomes. These are presented in
tables 9 and 10 together with the results for all pots containing
single taxa.

When S. ambigua originally outnumbered the other two taxa,
no elimination of hybrid genotypes occurred even after 20 months.
Differences in growth response were large enough, however, to be
attributable to the varying performances of genotypes. The effect
of the dry-ground S. palustris genotype (see Morphological
Variation p. 134) on Q8 compared with A7 on R3 produced a reversal
in rhizome production in wet and dry soils. The reduction in
growth of R3 by A7 in wet soil (11.0g. control to 1.3g. 1A7 to
0.0g. 3A7) is the most marked decline. The effect of the wet-
ground genotype B8 on L1 in wet soils is not perhaps as marked
as may have been expected. This is probably due to the aggressive
hybrid genotype L1.

With S. ambigua, when originally outnumbered by the other
two taxa, the effects of S. palustris and S. sylvatica in both
wet and dry soil were markedly different. In wet soil S. ambigua
suffered a drastic reduction in rhizome production in the
presence of S. palustris, Q8 and R3 being eliminated. L1
produced small quantities of rhizome in spite of the fact that
its aerial production (0.2g. and 0.1g., table 10) is less than
that produced either by Q8 or R3. In the presence of S. sylvatica,
S. ambigua showed no elimination in wet or dry soils. With
S. palustris in dry soil, S. ambigua showed less rhizome,
production than with S. sylvatica (totals: 3.6g. compared to
18.9g.) but there was no elimination.

In dry soils, aerial growth of S. sylvatica when originally

outnumbering the other two taxa was recorded in 7 pots out of 8 (table 10). However, rhizomes were produced in only 3 out of the 8 pots. A similar reduction occurred in wet soils where only 2 pots contained rhizomes from 5 with aerial parts. The prospects for the third season would therefore have been a further substantial reduction in the aerial growth of S. sylvatica. Genotype P3 was less sensitive to wet soils than either K1 or M4, generally performing better than the others in each of the wet treatments.

When originally outnumbered by the other two taxa, the growth of S. sylvatica genotypes was even further diminished. In wet soil no pots contained rhizomes of S. sylvatica (table 9) and only 2 showed any in dry soil (from 5 pots with aerial shoots). Again, a third season would therefore have been expected to show a further substantial reduction in the aerial growth of S. sylvatica.

Taking the results from all the treatments together, the reduction in growth of S. sylvatica is slightly more marked in the wet compared with the dry soils.

The rhizome production of the S. palustris genotype S5, when originally outnumbering the other taxa was less in both wet and dry soils with S. sylvatica (P3) than with S. ambigua (Q8), which was not the case with the other two sets. This is probably the result of strong competition from P3. There was no elimination of S. palustris.

With S. palustris originally outnumbered, eliminations and reductions in rhizome production were recorded. In the presence of S. ambigua in dry soils A7 was eliminated and S5 produced only a small quantity of rhizome material. In the presence of

S. sylvatica 2-4 g. of rhizome material were produced by

S. palustris. Greater quantities of rhizome material were

generally produced in wet soil. S5 - the dry-ground genotype -

was eliminated by S. ambigua, while A7 and B8 showed no signs

of decline. The low value for S5 in the presence of S. sylvatica

(1.1 g. compared to 2.6 g. in dry soil) is probably due to the

growth of the least soil water sensitive of the three

S. sylvatica genotype (P3) and the poorly responsive S5 genotype.

DISCUSSION

From the two graphs illustrating the effect of interspecific

competition on the growth of Stachys sylvatica with S. palustris

and S. ambigua in both wet and dry soils, it is clear that this

species suffers considerable inhibition in growth during the

second season. At the same time, the growth of S. palustris in

competition with S. ambigua and S. sylvatica is almost always

greater with the latter than with S. ambigua under similar condi-

tions irrespective of the type of treatment. S. sylvatica is also

generally the poorer competitor when considering the growth of

S. ambigua in competition with S. palustris and S. sylvatica.

These results almost certainly reflect an unexpected

dependence on seedling establishment to maintain population

density. This may be deduced from two main reasons. First,

the greatest numbers of Stachys seedlings removed at the

beginning of the second season were always from pots originally

containing a predominance of S. sylvatica plants. Secondly,

the control pots originally containing 4 plants of S. sylvatica

show significantly lower dry weight production of both aerial

Table 8. Rhizome dry weight results (g.) recorded for individual genotypes of S. ambigua (H), S. palustris (P) and S. sylvatica (S) in competition at a density of 4 plants per pot. An arrow proceeds from the taxon under investigation to its competitor, the nearest set of data recording its growth at high original proportion (3:1) and the further set recording the growth at low original proportion (1:3). The following genotypes are in competition:- Q8/P3/S5   R3/K1/A7   L1/M4/B8

a. DRY SOIL

H
$$29.5 \begin{cases} 3.8 \ \text{Q8} \\ 4.9 \ \text{R3} \\ 10.5) \ \text{L1} \\ 10.3) \end{cases}$$

$$18.4 \begin{cases} 3.9 \ \text{Q8} \\ 6.0 \ \text{R3} \\ 5.6) \ \text{L1} \\ 2.9) \end{cases}$$

$$3.6 \begin{cases} 0.8 \ \text{Q8} \\ 1.0 \ \text{R3} \\ 0.2) \ \text{L1} \\ 1.6) \end{cases}$$

$$3.9 \begin{cases} 0.3 \ \text{S5} \\ 0.0 \ \text{A7} \\ 0.7) \ \text{B8} \\ 2.9) \end{cases}$$

$$13.2 \begin{cases} 2.2 \ \text{S5} \\ 5.9 \ \text{A7} \\ 3.2) \ \text{B8} \\ 1.9) \end{cases}$$

P
$$13.7 \begin{cases} 4.2 \ \text{S5} \\ 6.1 \ \text{A7} \\ 1.3) \ \text{B8} \\ 2.1) \end{cases}$$

$$0.5 \begin{cases} 0.0 \ \text{P3} \\ 0.0 \ \text{K1} \\ 0.5) \ \text{M4} \\ 0.0) \end{cases}$$

$$1.1 \begin{cases} 0.0 \ \text{P3} \\ 0.9 \ \text{K1} \\ 0.2) \ \text{M4} \\ 0.0) \end{cases}$$

$$16.2 \begin{cases} 1.6 \ \text{S5} \\ 8.3 \ \text{A7} \\ 3.0) \ \text{B8} \\ 3.3) \end{cases}$$

$$11.7 \begin{cases} 2.6 \ \text{S5} \\ 4.0 \ \text{A7} \\ 2.2) \ \text{B8} \\ 2.9) \end{cases}$$

$$36.2 \begin{cases} 4.4 \ \text{Q8} \\ 4.6 \ \text{R3} \\ 12.9) \ \text{L1} \\ 4.4) \end{cases}$$

$$18.9 \begin{cases} 0.6 \ \text{Q8} \\ 7.7 \ \text{R3} \\ 6.1) \ \text{L1} \\ 4.5) \end{cases}$$

$$1.5 \begin{cases} 1.5 \ \text{P3} \\ 0.0 \ \text{K1} \\ 0.0) \ \text{M4} \\ 0.0) \end{cases}$$

$$5.9 \begin{cases} 5.9 \ \text{P3} \\ 0.0 \ \text{K1} \\ 0.0) \ \text{M4} \\ 0.0) \end{cases}$$

S
$$8.1 \begin{cases} 1.9 \ \text{P3} \\ 2.1 \ \text{K1} \\ 2.7) \ \text{M4} \\ 1.4) \end{cases}$$

b. __WET SOIL__

P

5.0 S5
23.2 A7
7.4) B8
7.0)
42.6

H

8.3 Q8
11.0 R3
12.1) L1
5.0)
36.4

S

1.8 P3
0.7 K1
1.1) M4
0.0)
3.6

29.0 {8.8 Q8 / 11.3 R3 / 10.4) L1 / 8.5)

1.7 {0.0 Q8 / 0.0 R3 / 1.0) L1 / 0.7)

12.6 {0.0 S5 / 7.0 A7 / 3.3) B8 / 2.3)

31.1 {4.1 S5 / 8.2 A7 / 13.7) B8 / 5.1) 10.5 / 11.8

0.0 {0.0 P3 / 0.0 K1 / 0.0) M4 / 0.0)

34.1 {2.5 S5 / 12.8 A7 / 13.7) B8 / 5.1)

11.2 {1.1 S5 / 5.8 A7 / 2.1) B8 / 2.2)

1.7 {1.7 P3 / 0.0 K1 / 0.0) M4 / 0.0)

13.6 {2.9 Q8 / 3.2 R3 / 3.9) L1 / 3.6)

25.7 {7.2 Q8 / 4.3 R3 / 4.3) L1 / 9.9)

0.0 {0.0 P3 / 0.0 K1 / 0.0) M4 / 0.0)

0.9 {0.9 P3 / 0.0 K1 / 0.0) M4 / 0.0)
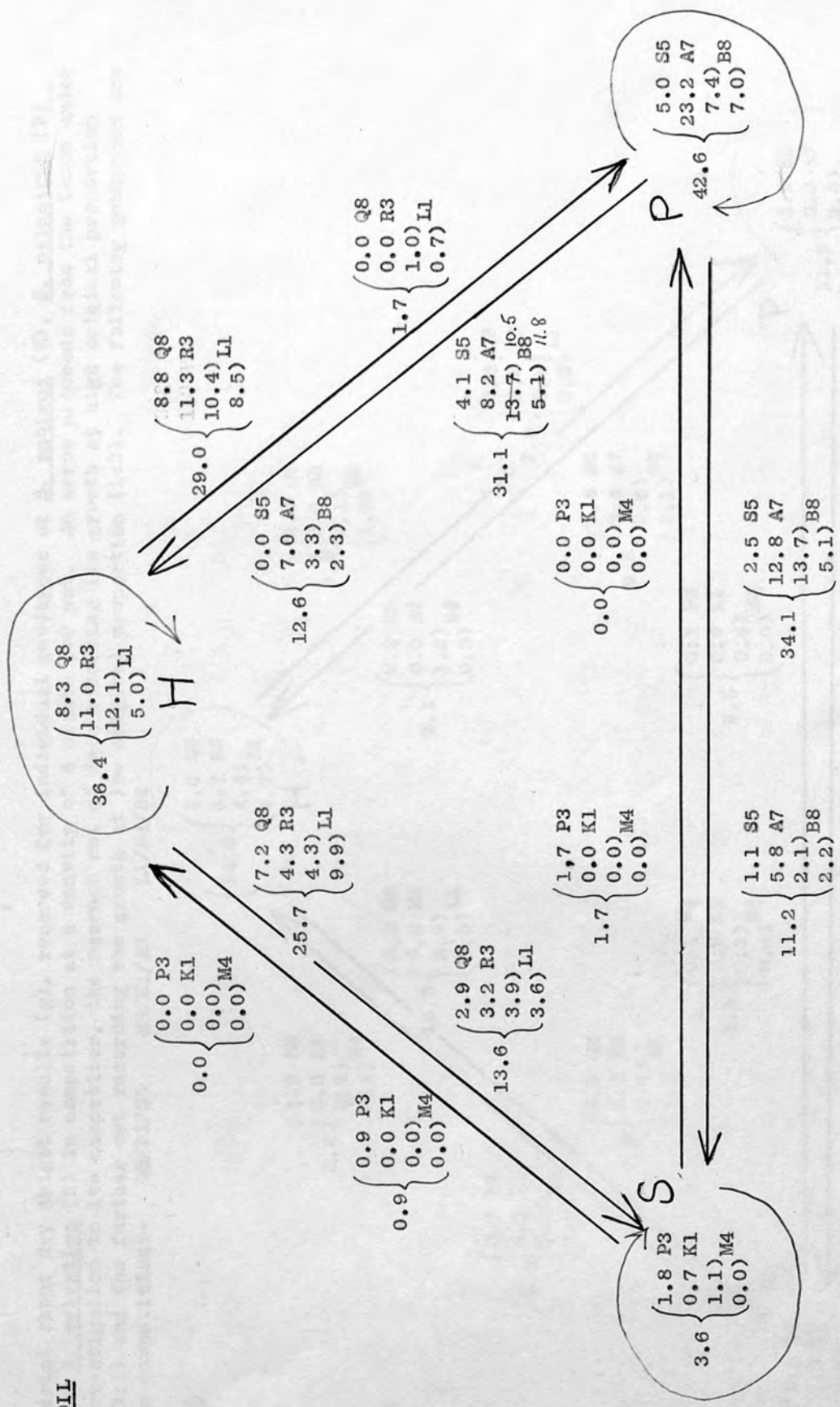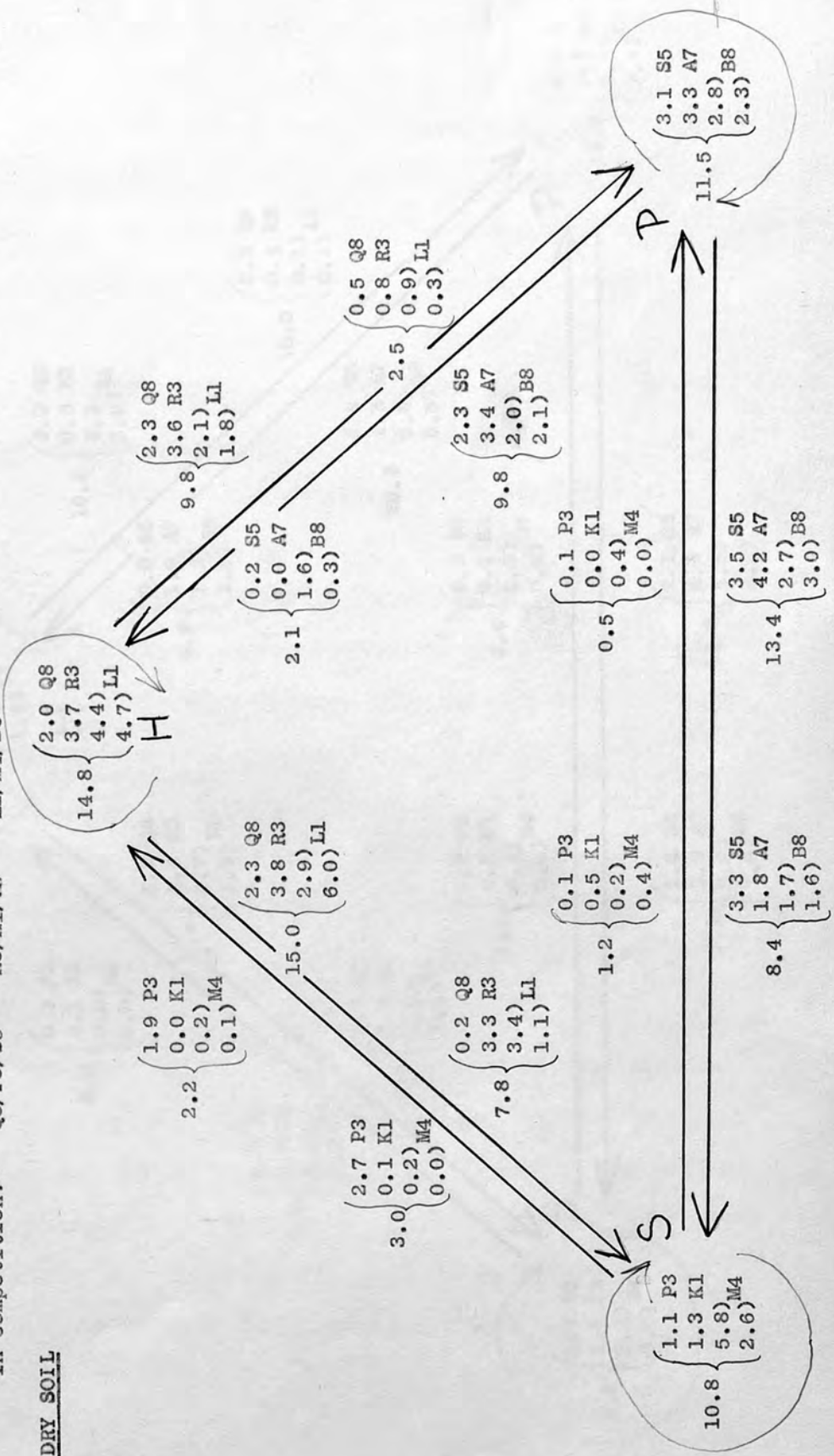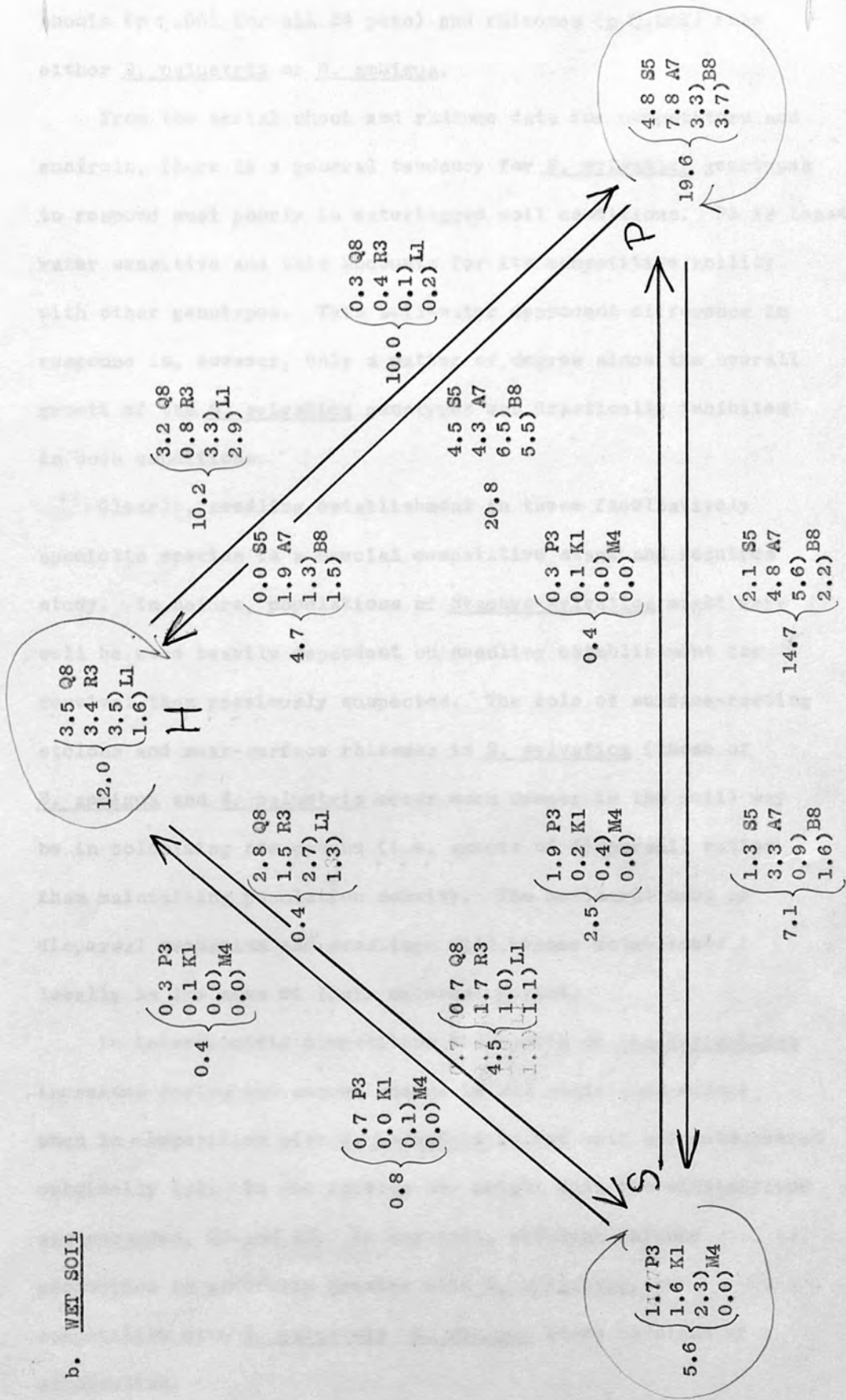
Table 10. Aerial shoot dry weight results (g). recorded for individual genotypes of S. ambigua (H), S. palustris (P) and S. sylvatica (S) in competition at a density of 4 plants per pot. An arrow proceeds from the taxon under investigation to its competitor, the nearest set of data recording its growth at high original proportion (3:1) and the further set recording the growth at low original proportion (1:3). The following genotypes are in competition:- Q8/P3/S5 R3/K1/A7 L1/M4/B8

a. DRY SOIL

H (self-loop):
14.8 { 2.0 Q8, 3.7 R3, 4.4) L1, 4.7) L1 }

P (self-loop):
11.5 { 3.1 S5, 3.3 A7, 2.8) B8, 2.3) B8 }

S (self-loop):
10.8 { 1.1 P3, 1.3 K1, 5.8) M4, 2.6) M4 }

H → P:
9.8 { 2.3 Q8, 3.6 R3, 2.1) L1, 1.8) L1 }
2.5 { 0.5 Q8, 0.8 R3, 0.9) L1, 0.3) L1 }

P → H:
2.1 { 0.2 S5, 0.0 A7, 1.6) B8, 0.3) B8 }
9.8 { 2.3 S5, 3.4 A7, 2.0) B8, 2.1) B8 }

H → S:
15.0 { 2.3 Q8, 3.8 R3, 2.9) L1, 6.0) L1 }
7.8 { 0.2 Q8, 3.3 R3, 3.4) L1, 1.1) L1 }

S → H:
2.2 { 1.9 P3, 0.0 K1, 0.2) M4, 0.1) M4 }
3.0 { 2.7 P3, 0.1 K1, 0.2) M4, 0.0) M4 }

P → S:
0.5 { 0.1 P3, 0.0 K1, 0.4) M4, 0.0) M4 }
1.2 { 0.1 P3, 0.5 K1, 0.2) M4, 0.4) M4 }

S → P:
13.4 { 3.5 S5, 4.2 A7, 2.7) B8, 3.0) B8 }
8.4 { 3.3 S5, 1.8 A7, 1.7) B8, 1.6) B8 }

(seeds (p < .001 for all 24 pots) and rhizomes (p < .001) from either *P. palustris* or *P. species*.

From the aerial shoot and rhizome data for wet soil and analysis, there is a general tendency for *P. palustris* seedlings to respond most poorly to waterlogged soil conditions. It is least water sensitive and this accounts for its competitive ability with other genotypes. This apparent difference in response is, however, only a matter of degree since the overall growth of *P. palustris* seedlings is drastically inhibited in both conditions.

Clearly seedling establishment in these facultatively speciating species ... special competitive ... any results study. ... populations of *Stachys* ... much ... well be ... heavily dependent on seedling establishment for these than previously suspected. The role of surface-rooting ... and near-surface rhizomes in *P. palustris* (none of *P. species* and *P. palustris* occur much deeper in the soil) may be in colonizing buried (i.e. growth below the soil surface) than maintaining root length density. ...

b. **WET SOIL**

4.8) S5
7.8) A7
3.3) B8
3.7)
19.6   P

0.3) Q8
0.4) R3
0.1) L1
0.2)
10.0

3.2) Q8
0.8) R3
3.3) L1
2.9)
10.2

4.5) S5
4.3) A7
6.5) B8
5.5)
20.8

0.0) S5
1.9) A7
1.3) B8
1.5)
4.7

3.5) Q8
3.4) R3
3.5) L1
1.6)
12.0   H

2.1) S5
4.8) A7
5.6) B8
2.2)
14.7

0.3) P3
0.1) K1
0.0) M4
0.0)
0.4

2.8) Q8
1.5) R3
2.7) L1
1.3)
0.4

1.9) P3
0.2) K1
0.4) M4
0.0)
2.5

1.3) S5
3.3) A7
0.9) B8
1.6)
7.1

0.3) P3
0.1) K1
0.0) M4
0.0)
0.4

0.7) Q8
1.7) R3
1.0) L1
1.1)
4.5

0.7) P3
0.0) K1
0.1) M4
0.0)
0.8

1.7) P3
1.6) K1
2.3) M4
0.0)
5.6   S

shoots (p < .001 for all 24 pots) and rhizomes (p < .001) than either S. palustris or S. ambigua.

From the aerial shoot and rhizome data for competitors and controls, there is a general tendency for S. sylvatica genotypes to respond most poorly in waterlogged soil conditions. P3 is least water sensitive and this accounts for its competitive ability with other genotypes. This soil-water dependent difference in response is, however, only a matter of degree since the overall growth of the S. sylvatica genotypes was drastically inhibited in both conditions.

Clearly, seedling establishment in these facultatively apomictic species is a crucial competitive stage and requires study. In nature, populations of Stachys sylvatica might very well be more heavily dependent on seedling establishment for survival than previously suspected. The role of surface-rooting stolons and near-surface rhizomes in S. sylvatica (those of S. ambigua and S. palustris occur much deeper in the soil) may be in colonizing new ground (i.e. agents of dispersal) rather than maintaining population density. The mericarps have no dispersal mechanism and seedlings will become established locally in the area of their maternal parent.

In interspecific competition the growth of Stachys ambigua increases during the second season in all conditions except when in competition with S. palustris in wet soil and outnumbered originally 1:3. In the rhizome dry weight data two eliminations are recorded, Q8 and R3. In dry soil, although rhizome production is generally greater with S. sylvatica, in competition with S. palustris, S. ambigua shows no signs of elimination.

The growth responses of S. palustris in the different

treatments are more variable than those of S. ambigua. The depression of growth during the second growing season caused by S. ambigua in the proportion 3:1 in dry soil has resulted in the elimination of A7 and near elimination of S5. A further elimination occurs, this time in wet soil conditions. S5 is eliminated by Q8 (S. ambigua) when outnumbered originally 1:3. The other two genotypes of S. palustris (A7 and B8) in the same treatment show no signs of elimination. This difference in response is probably due to the fact that S5 is a genotype from dry ground producing significantly fewer rhizomes in wet soil than the normal forms represented by A7 and B8 (see Morphological Variation p. 125), and hence it is a poorer competitor.

One anomalous result recorded is on the graph of the growth of S. palustris under wet soil conditions (fig. 19). A depression in growth is caused by S. sylvatica in proportion 1:3 during the second season. This is unexpected since the recorded growth of all three S. sylvatica genotypes under these conditions is zero. S. palustris would therefore have been expected to show an enhancement in growth during the second season. The reasons for this result are obscure but may be dependent on the root space occupied and nutrients utilized by S. sylvatica during the first growing season. Enhanced growth of S. palustris may be dependent on the increase in the available root space by breakdown of roots and renewal of minerals. If this has been the cause, an equal or greater depression of the growth of S. palustris by S. sylvatica (in proportion 1:3) in dry soil may also be expected. This is the case (see fig. 20), and 2 out of 4 pots show some aerial growth of S. sylvatica, a slightly better performance than in wet soil.

As already noted, the response of different genotypes of the same taxon to their growing conditions is not always the same, and this results in differences in interspecific competition between a number of genotypes from two taxa. Thus, S5 is eliminated by the S. ambigua genotype Q8 (proportion 3:1) in wet soil, when the other two genotypes of S. palustris (A7 and B8) respond and compete well producing a high dry weight of rhizome. Because of the responses of S5, Q8 produces greater dry weight of rhizome in wet soil at the original proportion 3Q8:1S5 than in dry soil (8.8g compared with 3.9g in dry soil). Since A7 responds well in wet conditions, R3 at the original proportion 3R3:1A7 produces greater weight of rhizome in dry soil than wet (6.0g:1.3g). Amongst S. sylvatica genotypes, P3 is the least soil water sensitive, the other two growing noticeably better in the drier soil conditions.

Two seasons growth only have evidently not been a long enough period of time to observe many definitive effects of interspecific competition between the three taxa. However four conclusions emerge:-

1. S. ambigua is an effective competitor when in high concentration in both wet and dry soils. In low concentration it is susceptible to elimination by S. palustris - particularly when in competition in wet soil conditions.

2. S. sylvatica is dependent on seedling establishment for maintaining population density. S. palustris shows no such population dependence on seedlings. S. ambigua is, of course, almost entirely, if not totally, dependent on clonal production to maintain populations.

3. The results of interspecific competition between two taxa

vary depending on the responses of the genotypes selected, to
the conditions employed in the experiment.

4. No evidence is presented to show conclusively that
S. ambigua may eliminate either of the parental species from
its habitat. The sensitivity to competition of outnumbered
plants, as an F_1 hybrid in the presence of its maternal parent
would be, must represent a significant barrier to the establish-
ment of the hybrid in undisturbed habitats. Once established,
plants produced from rhizomes reaching areas less densely
colonized by the parents are likely to be most successful. For
this reason disturbed habitats (as pointed out by Anderson,
1948 and reviewed by Baker, 1951) may present the best areas
for establishment and survival of hybrids such as S. ambigua.

5. MORPHOLOGICAL VARIATION

INTRODUCTION

The range of variation of Stachys palustris, and S. ambigua in Britain and Europe appears to have caused problems in separating them. Wilcock (1969) has shown from herbarium studies that S. palustris and S. ambigua overlap in leaf morphology, but show a marked discontinuity separating them from S. sylvatica (see fig. 22).

A more detailed investigation of the variation of leaf and other characters in field populations was undertaken in an attempt to discover and define the phenotypic range of potentially discriminating characters. Areas of Britain particularly investigated were those where S. ambigua appears to occur most commonly. This was determined on the basis of the field and herbarium records of S. ambigua making up the distribution map published in the Critical Supplement to the Atlas of the British Flora (Perring and Sell, 1968). These regions are: Orkney, Argyll, Isle of Man and the Lake District.

Populations of S. ambigua and S. palustris were studied most intensively because of the reported overlap in morphology of these taxa. Investigations of inter-and intra-population variability of these two taxa at the same and different sites provides information on the origin of the observed overlap. Perring and Sell (1968) and Green (in preparation) have reported that this is likely to be the result of the backcrossing of S. ambigua with S. palustris. The phenotypic plasticity of leaf characters was assessed by growing selected genotypes under controlled environmental conditions. Comparisons of the growth
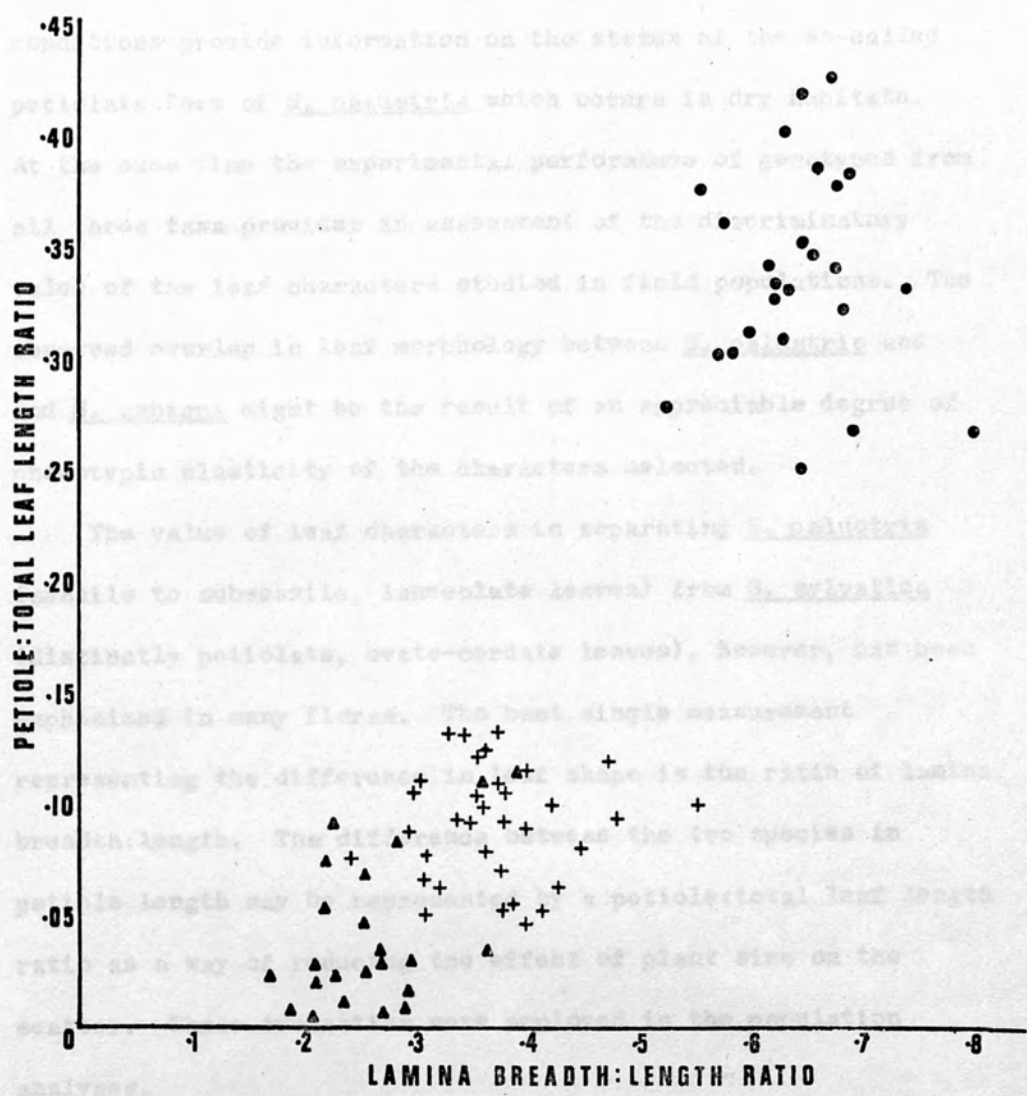
FIG.22 SCATTER DIAGRAM OF S.PALUSTRIS▲,S.AMBIGUA+,AND S.SYLVATICA● , PREPARED FROM HERBARIUM MATERIAL.

of selected genotypes of S. palustris under wet and dry soil
conditions provide information on the status of the so-called
petiolate form of S. palustris which occurs in dry habitats.
At the same time the experimental performance of genotypes from
all three taxa provides an assessment of the discriminatory
value of the leaf characters studied in field populations. The
observed overlap in leaf morphology between S. palustris and
and S. ambigua might be the result of an appreciable degree of
phenotypic plasticity of the characters selected.

The value of leaf characters in separating S. palustris
(sessile to subsessile, lanceolate leaves) from S. sylvatica
(distinctly petiolate, ovate-cordate leaves), however, has been
emphasized in many floras. The best single measurement
representing the difference in leaf shape is the ratio of lamina
breadth:length. The difference between the two species in
petiole length may be represented by a petiole:total leaf length
ratio as a way of reducing the effect of plant size on the
scatter. These two ratios were employed in the population
analyses.

The evaluation of characters that reputedly distinguish
S. ambigua from S. palustris over their total range in Britain
alone, seems long overdue. The variation of S. sylvatica was
included to complete the picture of variability and provide data
on introgression.

MATERIALS AND METHODS

The variation of leaf, corolla and verticil characters
were studied in the field. The variation of leaf characters were
further investigated, together with rhizome production, by

experimental cultivation of specimens grown **under different**
environmental conditions. The field data were obtained by 35
population samples at sites in Britain during 1970 and 1971.
Plants were collected and grown at Royal Holloway College to
provide experimental material.

A site was defined as a geographical area of variable size
and shape (but not exceeding 200 m. at its widest point) within
which free gene exchange between plants was a likely possibility.
All the plants within one site were treated as a single
population. At each sample site every plant used as a source of
data was referred to one of the three taxa and allocated either
the letter P (S. palustris), H (S. ambigua) or S (S. sylvatica).
These are referred to as population samples. Numbers were
allocated to population samples for the following reasons:-

1. To distinguish localized and isolated groups of plants
of the same species within the population.

2. To distinguish groups of plants of the same species
from different populations.

3. To distinguish overlapping groups of plants of the same
species within the population but recognizable by the presence
of one or more discriminating characters. For example, the light
and dark-flowered forms of S. sylvatica at Dalmally, S13 and
S14.

Analysis of populations of one taxon only was undertaken
by sampling all the flowering shoots within a quadrat of variable
size but containing approximately 20 suitable specimens. Where
taxa grew together the quadrat was widened to obtain data from
approximately 40 flowering shoots. Most populations either
contained only a single taxon or the taxa were localized in

different areas within the site. At sites where <u>S. ambigua</u> and one or both of its putative parents grew together, all flowering shoots within the enlarged quadrat were sampled to ensure that intermediates between the taxa were detected. The number of stems examined in a population sample ranged from 12-23. The population at Kilchrenan is an exception where 60 plants were sampled. Here, the population appeared to consist of only one taxon with highly variable verticil characters. Groups of uniform plants occurred within the population. In order to sample the population adequately a transect, 135m. long x 1.5m. wide, was taken through the population and the individual plants were numbered in approximately sequential order.

The data for each population are presented either in the form of a scatter diagram of all measurements or as plots of the mean and its confidence limits at p = 0.05 level of confidence.

LEAF data were obtained from the third vegetative node on the plant, taking the first vegetative node as the one immediately below the first whorl of the inflorescence and counting downwards. The aim of selecting one vegetative node was to compare leaves of closely similar physiological ages. Measurements from both leaves at the node were taken and the petiole:total leaf length and lamina breadth:length ratios subsequently averaged. The lamina breadth was measured in its widest part. The two ratios were plotted together to produce a scatter diagram based on leaf data for the populations sampled. Specimens morphologically referable to <u>S. palustris</u> on the basis of leaf features on the scatter diagrams will have low values for each ratio and so appear nearest the origin.*

*<u>Footnote</u>: See next page.

Although not mentioned in any flora, in the field, VERTICILS of S. palustris and S. ambigua often gave the impression of being denser and more prominent than those of S. sylvatica. This feature appeared to depend on a larger number of open flowers in the inflorescence at one time and, quite frequently, larger numbers of flowers at any one verticil. As an estimate of the number of flowers at one whorl of the inflorescence, all the flowers were counted at the second whorl, taking the first whorl as the lowest one in the inflorescence. The number of open flowers in the inflorescence were counted in quadrats where the majority of plants were neither just beginning nor just finishing their period of flowering.

Four COROLLA characters were measured from one flower of each plant examined in a quadrat. The characters, illustrated in figure 23, are:-

1. Length of corolla
2. Length of corolla tube
3. Lower lip measurement A
4. Lower lip measurement B

*Footnote: re previous page.

The axes, and in particular the y axis, tend slightly to exaggerate the same difference between values at points progressively farther away from the origin. LOG_{10} scale plots of both ratios prevent this trend but replace the scatter with the reverse effect - a gradual exaggeration progressively nearer the origin. As LOG_{10} ratio plots were no more satisfactory, simple ratios were plotted, but it should be remembered that populations of S. sylvatica and S. palustris, both with the same degree of variability in nature, will be represented by slightly unequal scatters and, consequently unequal confidence limits.
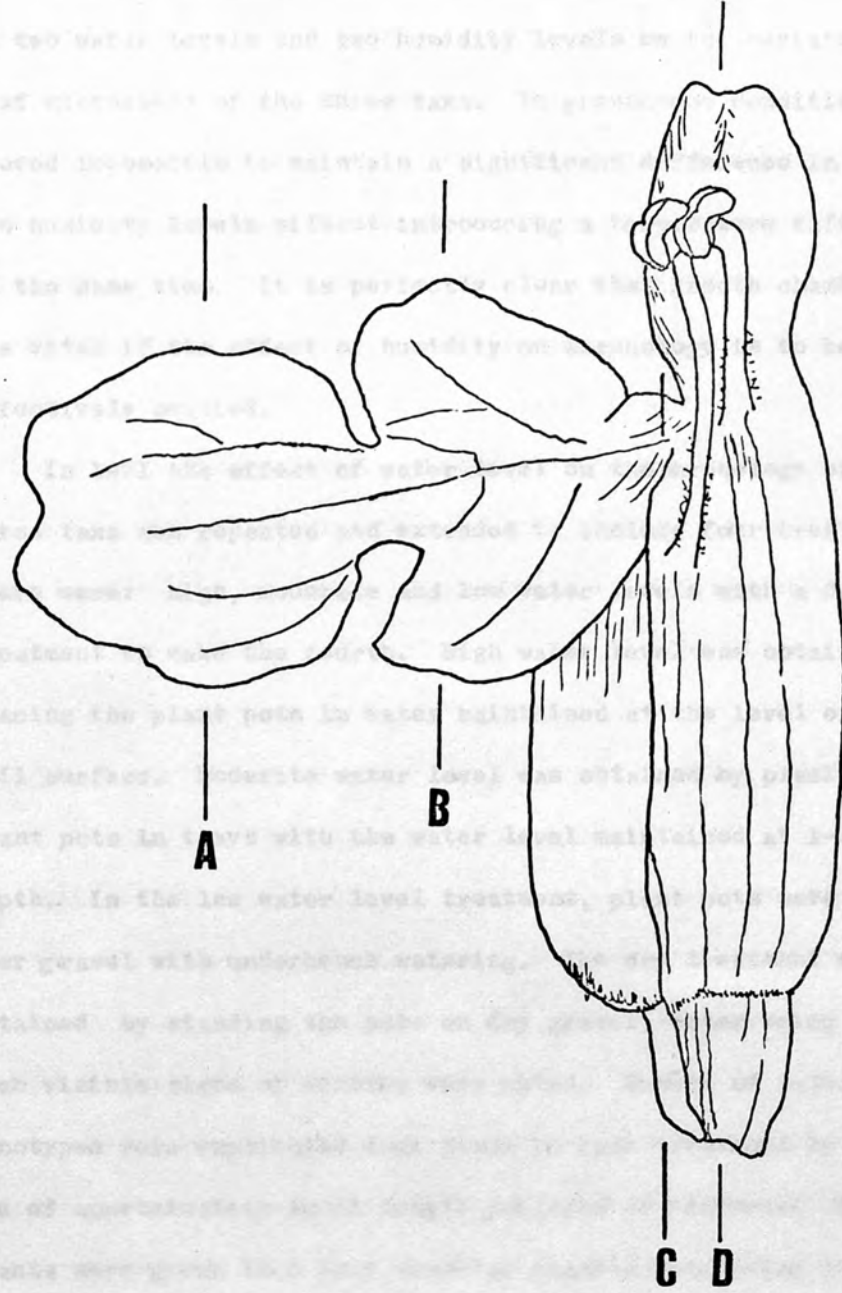
FIG. 23 COROLLA OF S. SYLVATICA SPREAD OUT AND CUT AWAY TO ILLUSTRATE
THE PARAMETERS FOR MEASUREMENT.
    A. LOWER LIP MEASUREMENT A
    B. LOWER LIP MEASUREMENT B
    C. LENGTH OF COROLLA TUBE
    D. LENGTH OF COROLLA

A pilot experiment was set up in 1970 to test the effect of two water levels and two humidity levels on the variation of leaf characters of the three taxa. In greenhouse conditions it proved impossible to maintain a significant difference in the two humidity levels without introducing a temperature difference at the same time. It is perfectly clear that growth chambers are vital if the effect of humidity on morphology is to be effectively studied.

In 1971 the effect of water level on the morphology of the three taxa was repeated and extended to include four treatments. There were: high, moderate and low water levels with a dry treatment to make the fourth. High water level was obtained by placing the plant pots in water maintained at the level of the soil surface. Moderate water level was obtained by placing the plant pots in trays with the water level maintained at 1-2" in depth. In the low water level treatment, plant pots were placed over gravel with underbench watering. The dry treatment was obtained by standing the pots on dry gravel, water being supplied when visible signs of wilting were noted. Ramets of selected genotypes were replicated four times in each treatment by the use of approximately equal length portions of rhizomes. All plants were grown in 5 inch diameter plastic pots using potting compost prepared by the Botanical Supply Unit. All pots were fitted with an identical mixture of potting compost.

Data were obtained from the plants when all the specimens had reached flowering. Both leaves at each of the four nodes below the lowest whorl of the inflorescence were measured, giving 8 values for each character from each plant. As every genotype was replicated 4 times in each treatment a total of 32

measurements was obtained for each treatment. Means and confidence limits at p = 0.05 level of probability for the lamina breadth:length ratio are plotted on the graphs.

The rhizomes from the plants of S. palustris and S. ambigua grown in the controlled environments were harvested at the termination of the experiment. Rhizome production in each treatment was evaluated by the determination of fresh and dry weight of rhizomes produced per plant.

The controlled environment experiments were set up again in 1972 using different genotypes. On this occasion only two soil water levels were studied - high water level and dry treatments.

RESULTS

1. Variation in leaf characters

1:1. Variation in field populations

All possible combinations of taxa can be found at different sites in Britain. S. ambigua occurs most commonly in the absence of one or both putative parents. The data presented illustrate a wide range of field situations.

At Glendaruel, Argyll all three taxa occur together (see fig. 24) and are readily separable on leaf morphology since no overlap occurs in the sample ranges of the three taxa.

At the Dalmally site (fig. 25) a similar situation exists. Here, no intermediates are shown between the three taxa although in one part they all grew together (S13 + 14, H7 and P10). The population means of S13 and S14 for both characters do not differ significantly. These two forms were distinguished by a flower colour difference - pale pink and dark red corollas; a difference maintained in cultivation. H8 and H9 represent localized
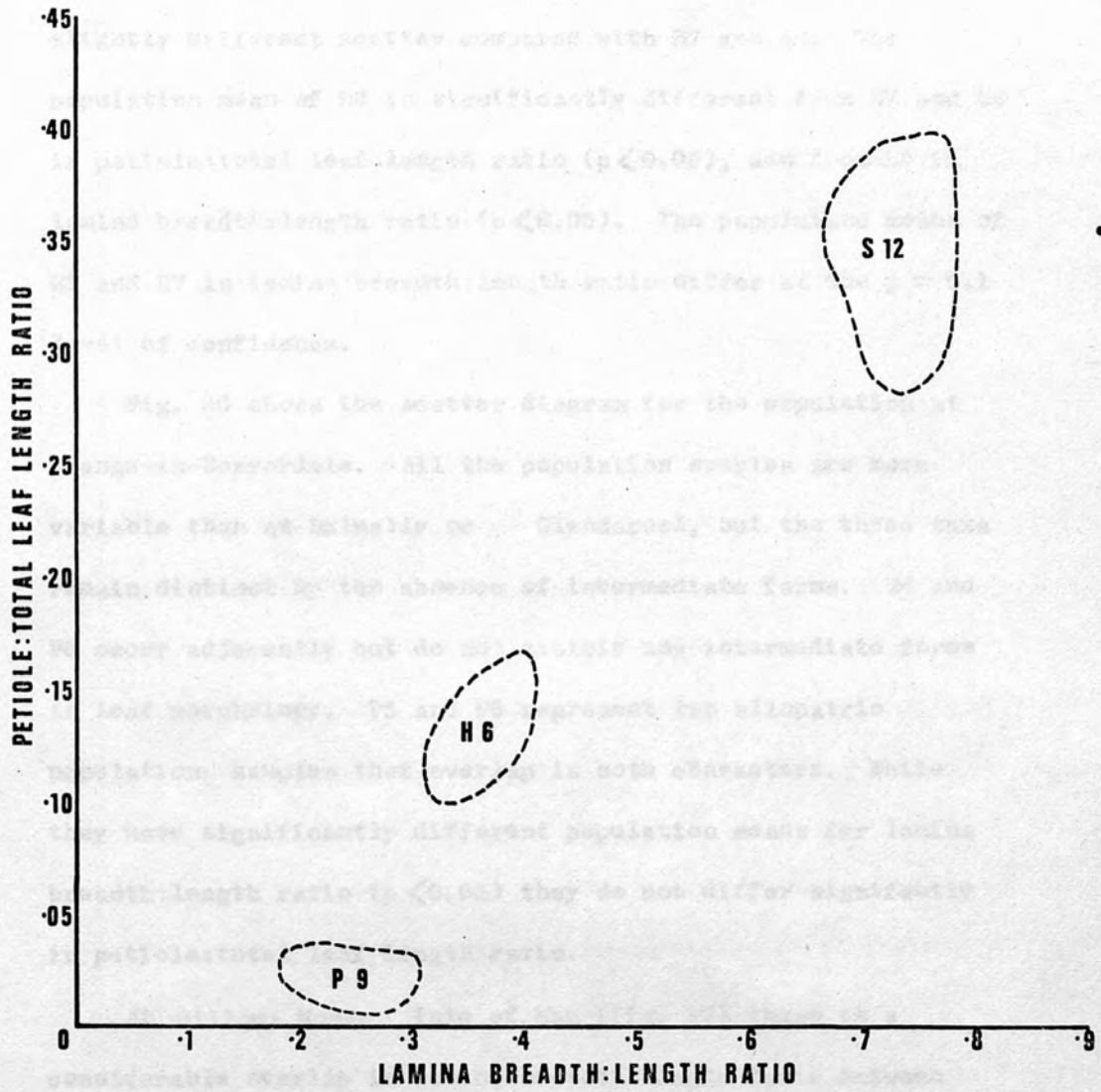
FIG.24 SCATTER DIAGRAM OF STACHYS POPULATIONS AT GLENDARUEL, ARGYLL.

clusters of plants occurring within the population. H8 has a slightly different scatter compared with H7 and H9. The population mean of H8 is significantly different from H7 and H9 in petiole:total leaf length ratio ($p < 0.05$), and from H9 in lamina breadth:length ratio ($p < 0.05$). The population means of H8 and H7 in lamina breadth:length ratio differ at the $p = 0.1$ level of confidence.

Fig. 26 shows the scatter diagram for the population at Grange-in-Borrowdale. All the population samples are more variable than at Dalmally or Glendaruel, but the three taxa remain distinct by the absence of intermediate forms. H4 and P6 occur adjacently but do not exhibit any intermediate forms in leaf morphology. P5 and P6 represent two allopatric population samples that overlap in both characters. While they have significantly different population means for lamina breadth:length ratio ($p < 0.05$) they do not differ signifantly in petiole:total leaf length ratio.

At Billowa Mooar Isle of Man (fig. 27) there is a considerable overlap in lamina breadth:length ratio between S. ambigua and S. palustris and the two taxa appear to be connected by a chain of intermediates. However, the population means for both characters are significantly different ($p < 0.05$). At present all three taxa are localized in their distribution without any overlap. H2 occurs amongst marshland plants such as Iris pseudacorus and Epilobium hirsutum growing around the edge of a pond, and in the alder wood surrounding the inlet stream to the north. S3 occurs on the periphery of the pond and wood, by the farm track. P3 occurs by the track following the stream northwards from the A5, and is a very small group of approximately
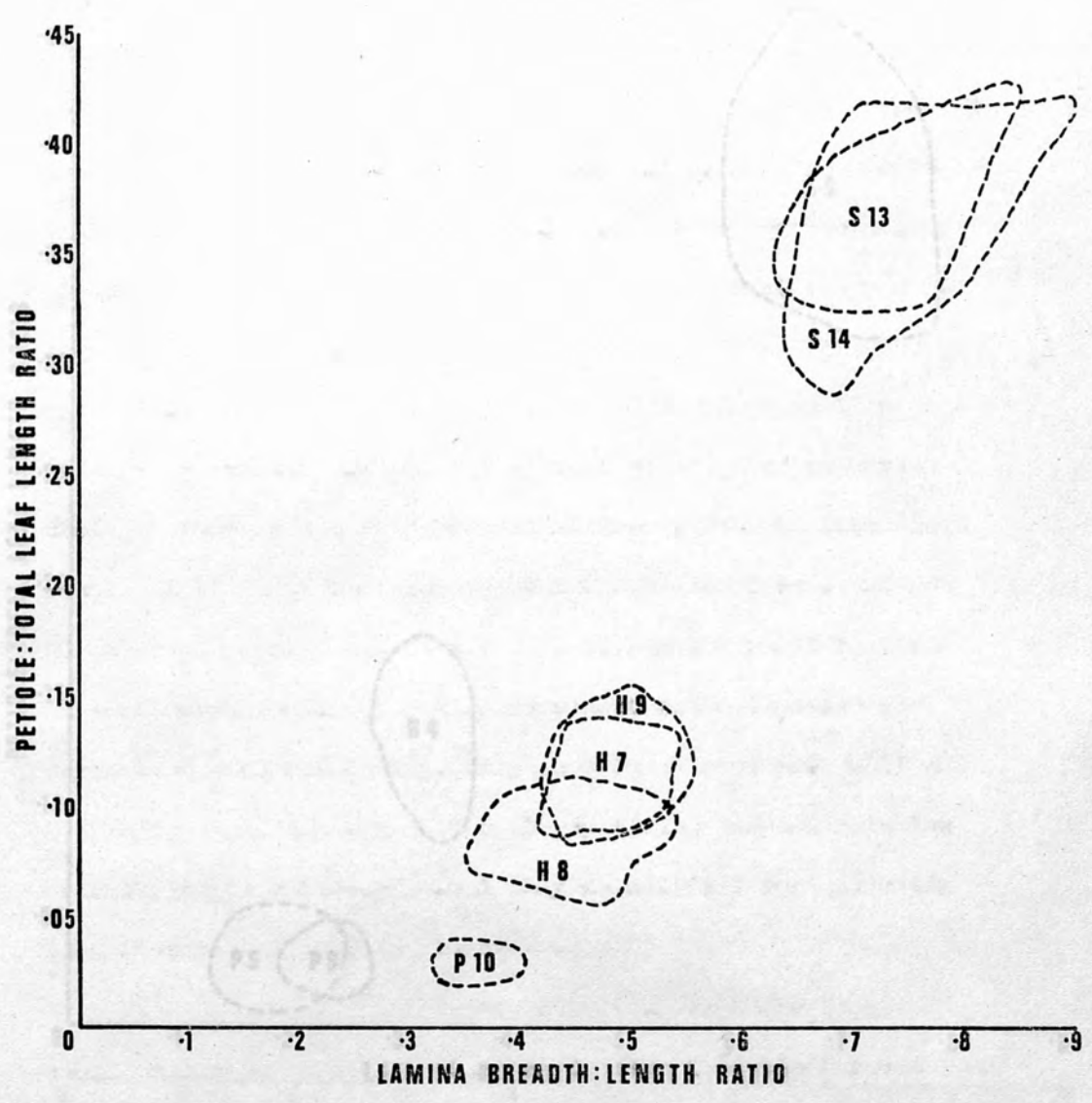
FIG.25 SCATTER DIAGRAM OF STACHYS POPULATIONS AT DALMALLY, ARGYLL.
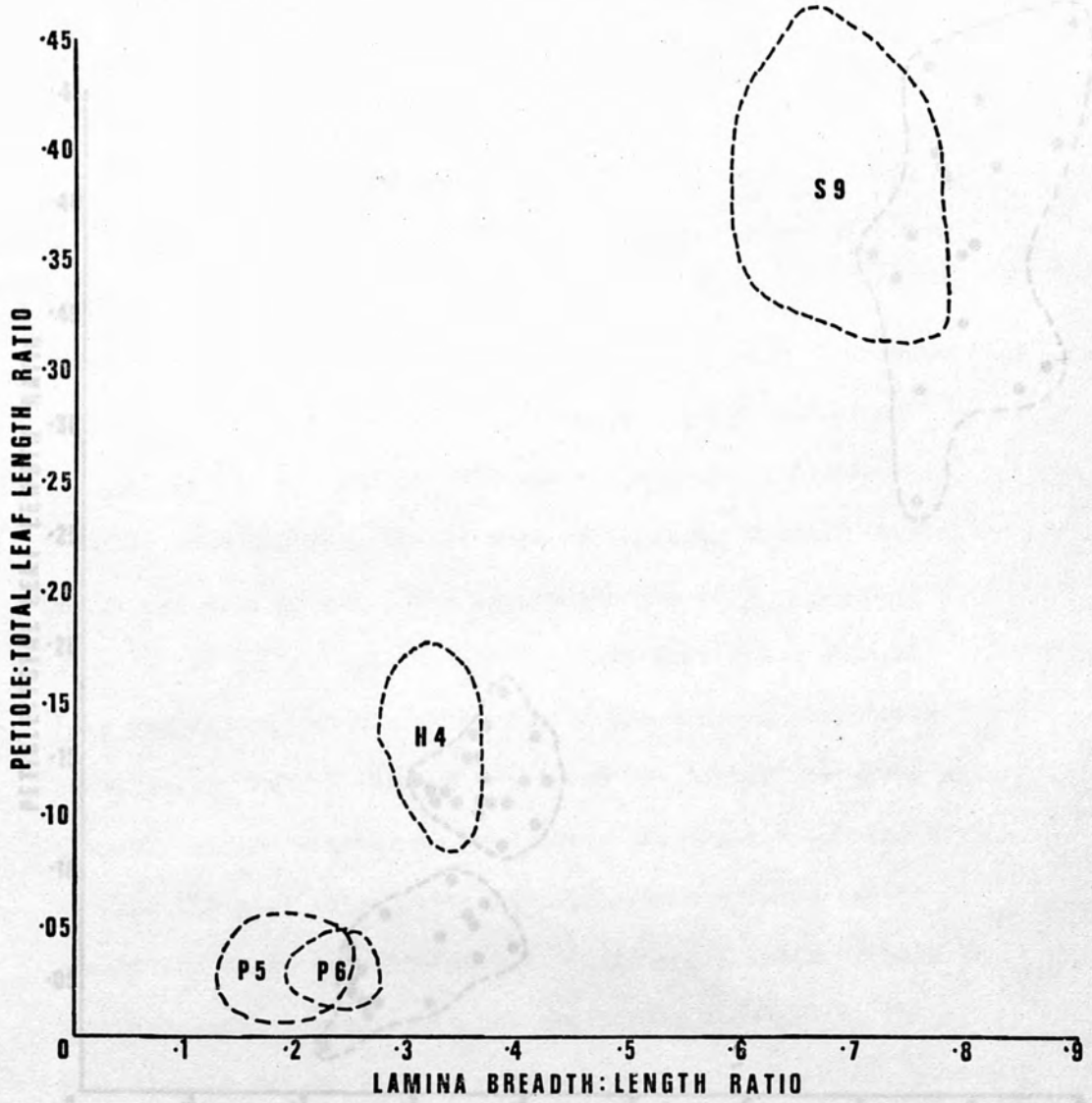
FIG. 26 SCATTER DIAGRAM OF STACHYS POPULATIONS AT GRANGE-IN-
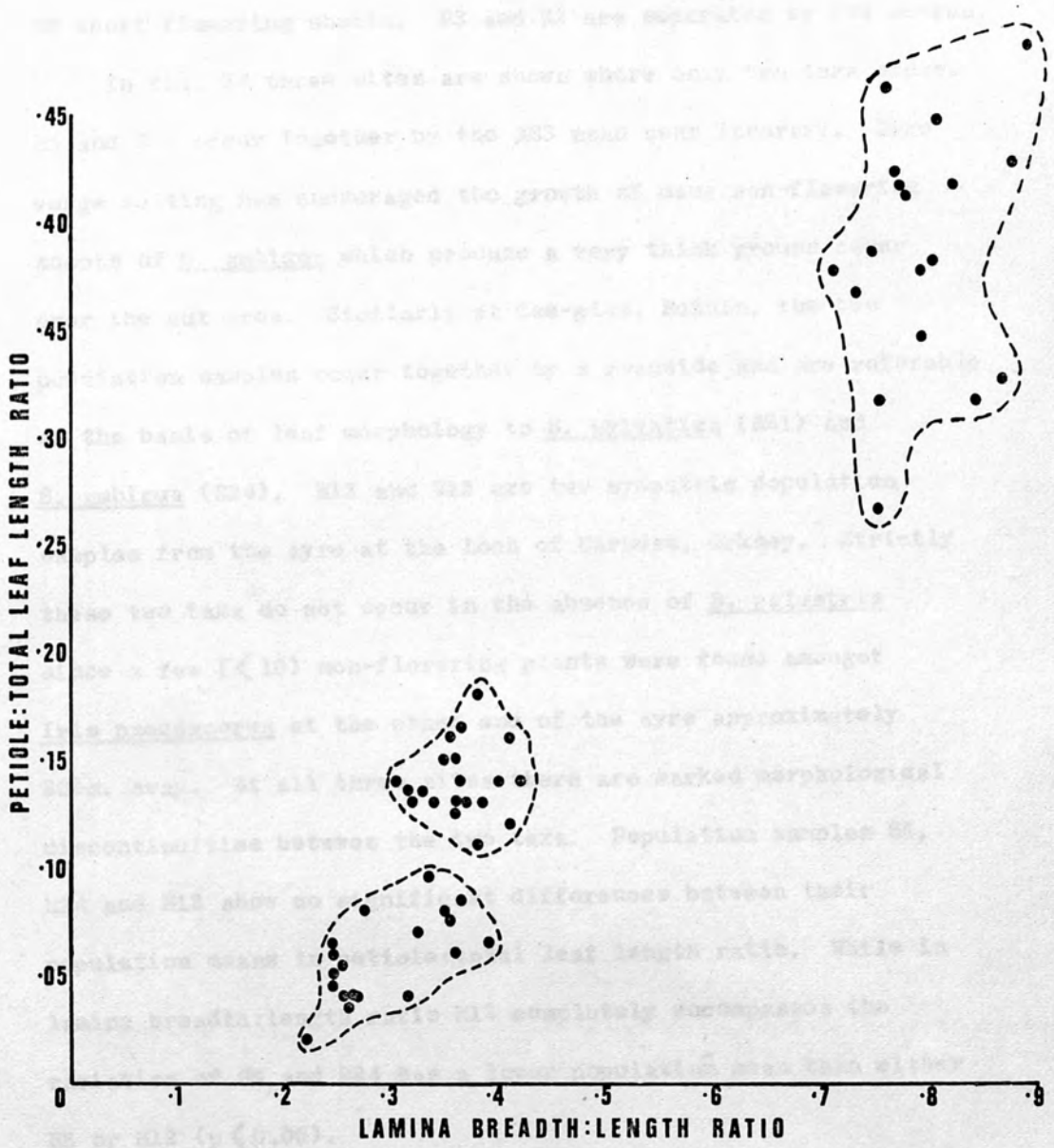BORROWDALE, LAKE DISTRICT.

FIG.27 SCATTER DIAGRAM OF STACHYS POPULATIONS AT BILLOWN MOOAR, ISLE OF MAN.

30 short flowering shoots. P3 and H2 are separated by 100 metres.

In fig. 28 three sites are shown where only two taxa occur. H5 and S10 occur together by the A83 road near Inverary. Here verge cutting has encouraged the growth of many non-flowering shoots of S. ambigua which produce a very thick ground cover over the cut area. Similarly at Cae-glas, Ruthin, the two population samples occur together by a roadside and are referable on the basis of leaf morphology to S. sylvatica (S21) and S. ambigua (H24). H12 and S15 are two sympatric population samples from the ayre at the Loch of Carness, Orkney. Strictly these two taxa do not occur in the absence of S. palustris since a few ($<$10) non-flowering plants were found amongst Iris pseudacorus at the other end of the ayre approximately 200m. away. At all three sites there are marked morphological discontinuities between the two taxa. Population samples H5, H24 and H12 show no significant differences between their population means in petiole:total leaf length ratio. While in lamina breadth:length ratio H12 completely encompasses the variation of H5, and H24 has a lower population mean than either H5 or H12 (p$<$0.05).

At Stonethwaite in the Lake District two taxa occur together by a ditch in a wet meadow (fig. 29). H23 is more variable in petiole:total leaf length ratio than lamina breadth:length ratio and has one of the widest confidence limits of any hybrid population sample for that character. P19 is more variable in both characters than many population samples referable on leaf morphology to S. palustris. In spite of the variability of both taxa, a discontinuity in the scatter exists between them.
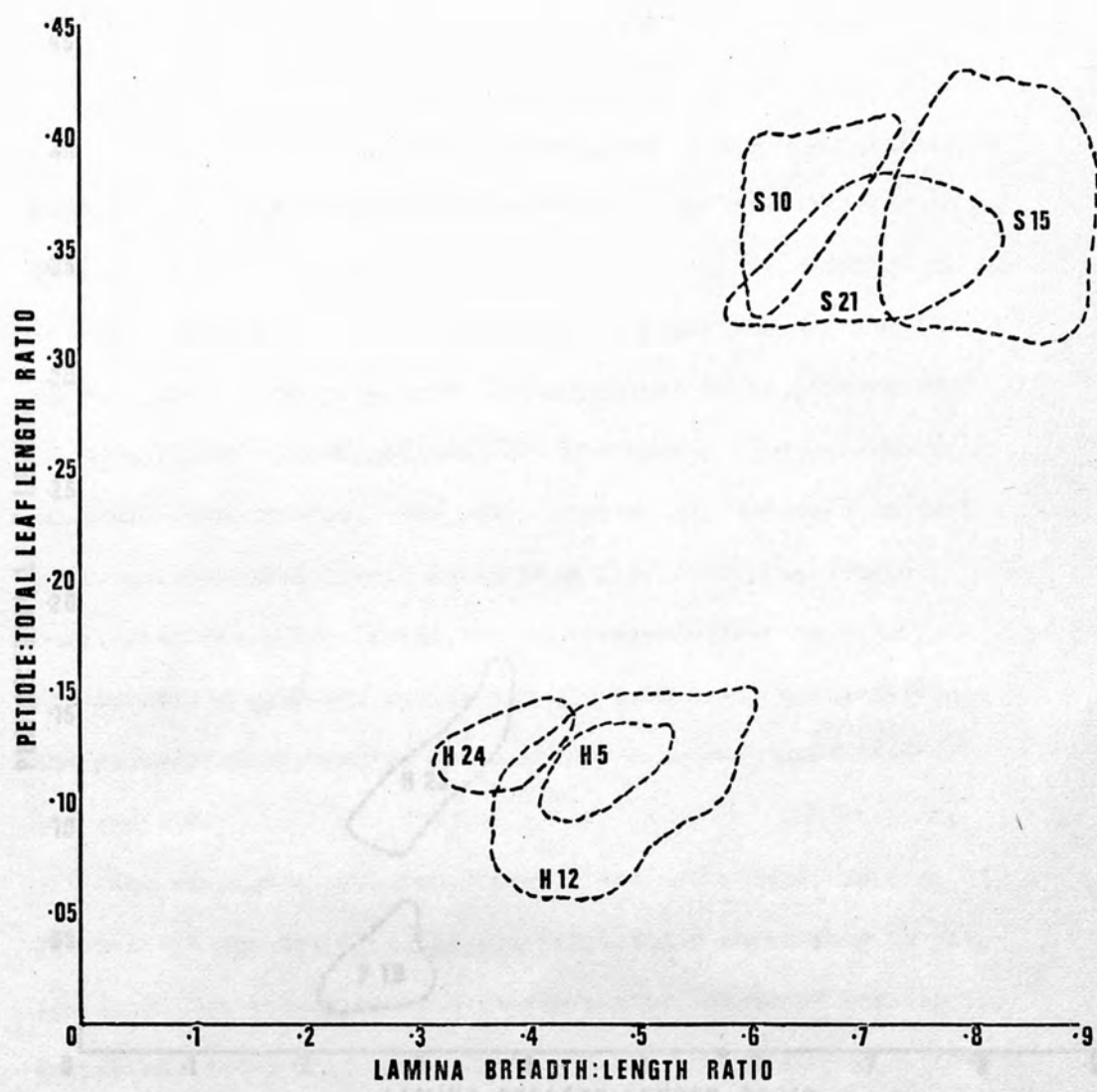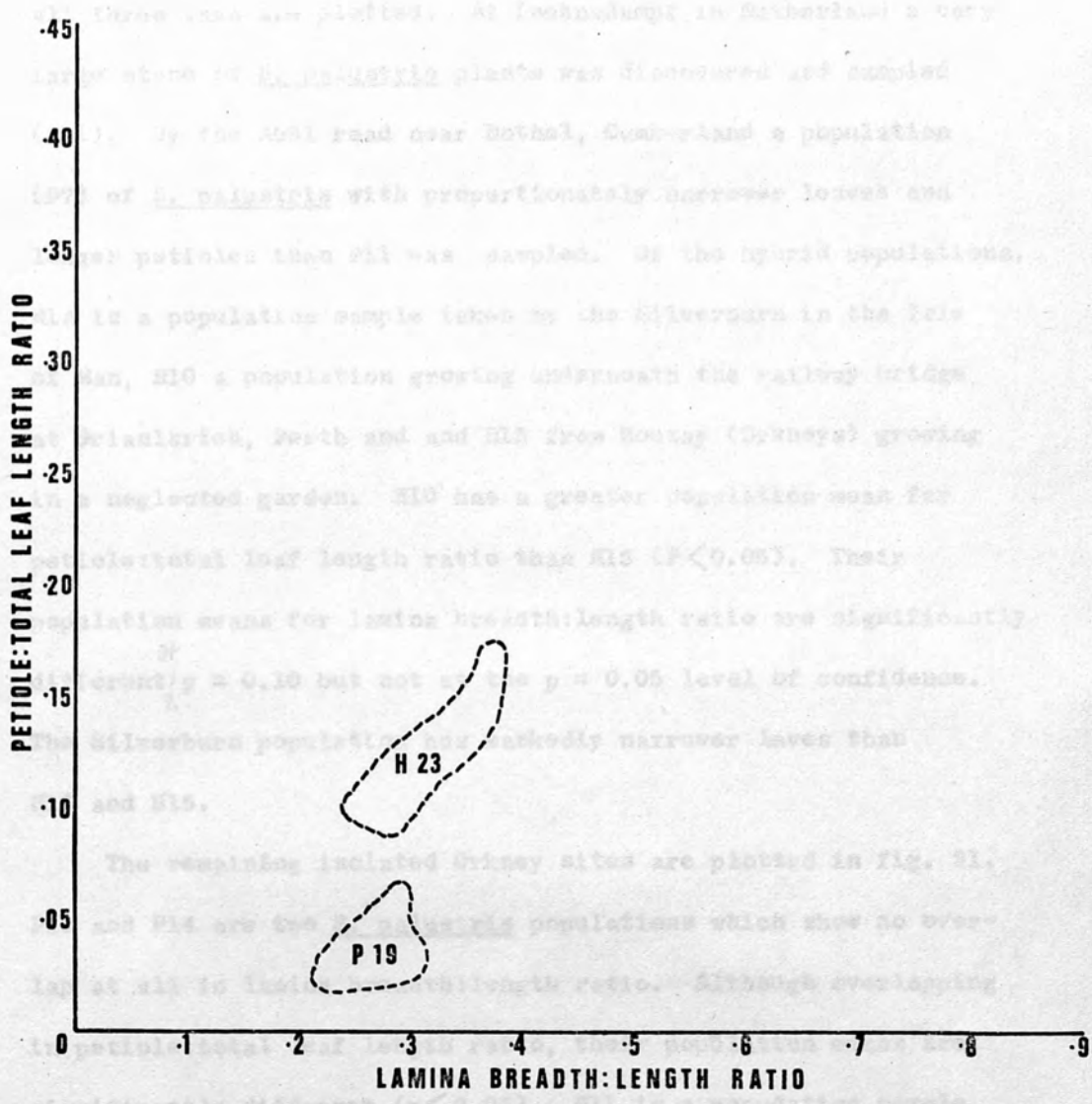
FIG.28 SCATTER DIAGRAM OF STACHYS POPULATIONS AT 3 SITES WHERE
ONLY 2 TAXA OCCUR.

INVERARY, ARGYLL   H5;S10
LOCH OF CARNESS, ORKNEY   H12; S15
RUTHIN, DENBIGH   H24; S21

FIG. 29 SCATTER DIAGRAM OF STACHYS POPULATIONS AT STONETHWAITE, LAKE DISTRICT.

In figs. 30 and 31, a number of isolated populations of all three taxa are plotted. At Inchnadampf in Sutherland a very large stand of S. palustris plants was discovered and sampled (P11). By the A591 road near Bothel, Cumberland a population (P7) of S. palustris with proportionately narrower leaves and longer petioles than P11 was sampled. Of the hybrid populations, H1A is a population sample taken by the Silverburn in the Isle of Man, H10 a population growing underneath the railway bridge at Crianlarich, Perth and and H15 from Rousay (Orkneys) growing in a neglected garden. H10 has a greater population mean for petiole:total leaf length ratio than H15 ($P < 0.05$). Their population means for lamina breadth:length ratio are significantly different at $p = 0.10$ but not at the $p = 0.05$ level of confidence. The Silverburn population has markedly narrower laves than H10 and H15.

The remaining isolated Orkney sites are plotted in fig. 31. P13 and P14 are two S. palustris populations which show no overlap at all in lamina breadth:length ratio. Although overlapping in petiole:total leaf length ratio, their population means are significantly different ($p < 0.05$). H11 is a popudation sample taken from a neglected garden on Hoy. H13 and H14 are population samples taken at Netherhouse farm garden (H13) and at Netherhouse farm dump (H14), an area of mire used as a waste tip. Here, the tip has been covered over with loose soil which has become colonized by S. ambigua (see fig. 32). Population means for both characters are not significantly different. When compared with H13 and H14 for both characters the population mean of H11 is significantly lower ($p < 0.05$).
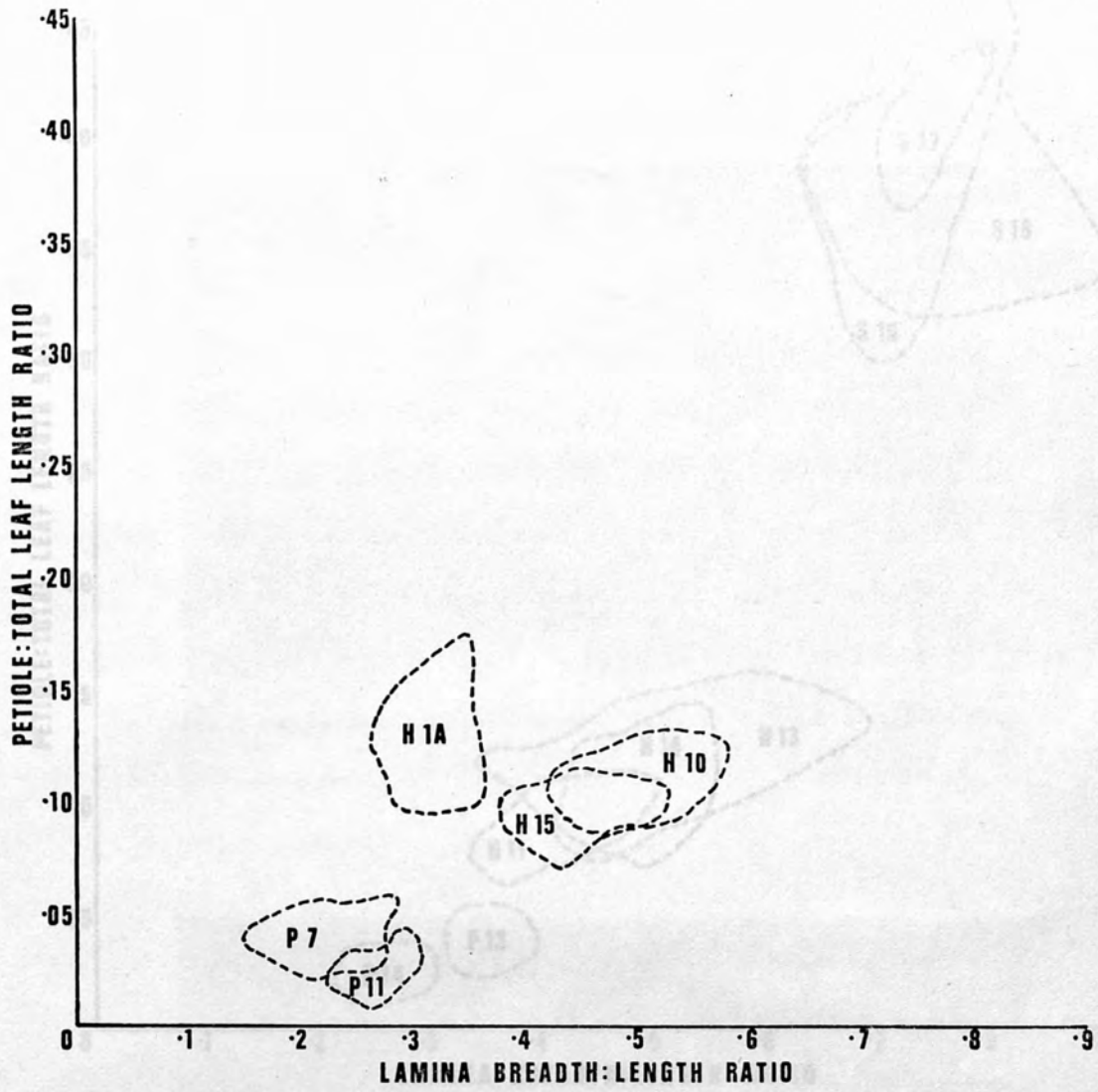
S16 and S17 are two populations of S. sylvatica growing

FIG. 30   SCATTER DIAGRAM OF STACHYS POPULATIONS AT FIVE SITES
WHERE ONLY ONE TAXON OCCURS.
INCHNADAMPF, SUTHERLAND PII
CARLISLE P7
SILVERBURN, ISLE OF MAN   HIA
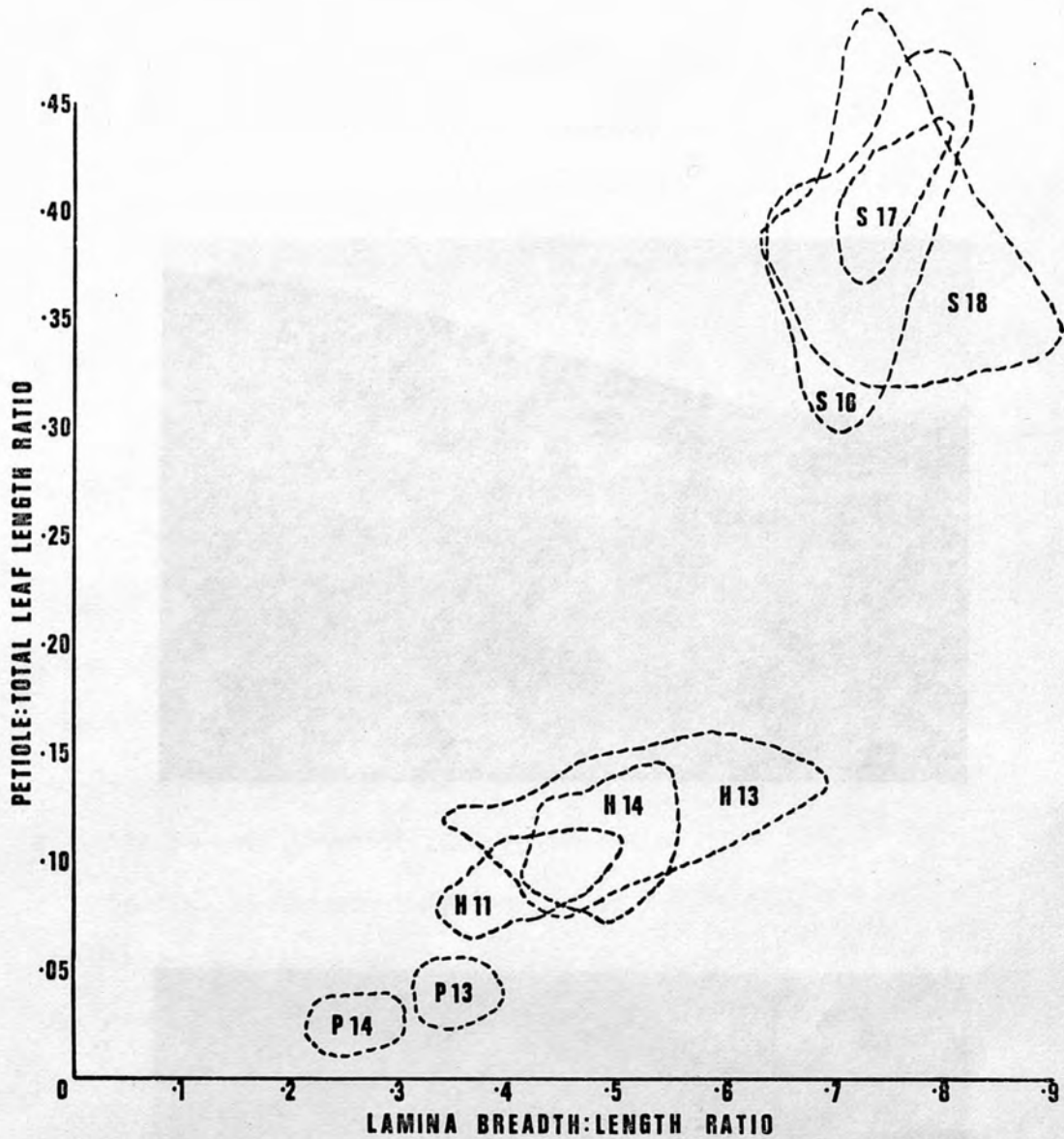CRIANLARICH, ARGYLL   HIO
ROUSAY, ORKNEYS   H I5

FIG.31 SCATTER DIAGRAM OF STACHYS POPULATIONS AT SEVERAL
SITES IN ORKNEY WHERE ONLY ONE TAXON OCCURS.

WAULKMILL BAY P 13

WIDEFORD HILL P 14

WHITE GLEN, HOY H 11

NETHERHOUSE FARM H 13

NETHERHOUSE DUMP H 14

KIRK BURN BU, HOY S 16

KIRK BURN BU, HOY S 17

ROUSAY S 18

Fig. 32. Two site photographs of Netherhouse Farm and dump on
Orkney. _Stachys ambigua_ plants, probably originating
from the farm's garden, are rapidly colonizing the
covering soil.

near one another at Kirk Burn Bu', Hoy. S18 occurs by the ayre
at the Loch of Scockness, Rousay. There are no significantly
differences between the means of any of the three populations
for either character.

In fig. 33 a population of S. palustris growing in a sugar
beet field at Aylmerton, Norfolk has been sampled (P20). It is
highly variable in both characters. Using confidence limits as
an assessment of the degree of variability; when compared with
all the 9 other populations of S. palustris sampled, P20 is 2.4
times more variable in lamina breadth:length ratio and 2.2
times more variable in petiole:total leaf length ratio. When
compared with the least variable populations, P20 is 3.6 times
more variable in lamina breadth:length ratio and 3.2 times more
variable in petiole:total leaf length ratio.

In fig. 34 the means and confidence limits at p = 0,05 for
all the populations sampled for leaf characters are plotted.
The data from which the scatter diagram has been obtained are
presented in table 11. The S. sylvatica populations form a
separate and easily recognizable taxon with broad leaves and
long petioles. However, it is clear that no such discontinuity
exists in leaf characters between S. palustris and S. ambigua.
The groups, labelled B and C. are distinguished. In group C
occur all the population samples considered as S. palustris:
P3, P5, P6, P9, P10, P11, P13, P14, P19 and P20. The highly
variable population P20 has the greatest lamina breadth:length
ratio and petiole:total leaf length ratio, and therefore occurs
nearest to groups A and B. In the absence of P20 there would
be a minor discontinuity between groups A/B and C.

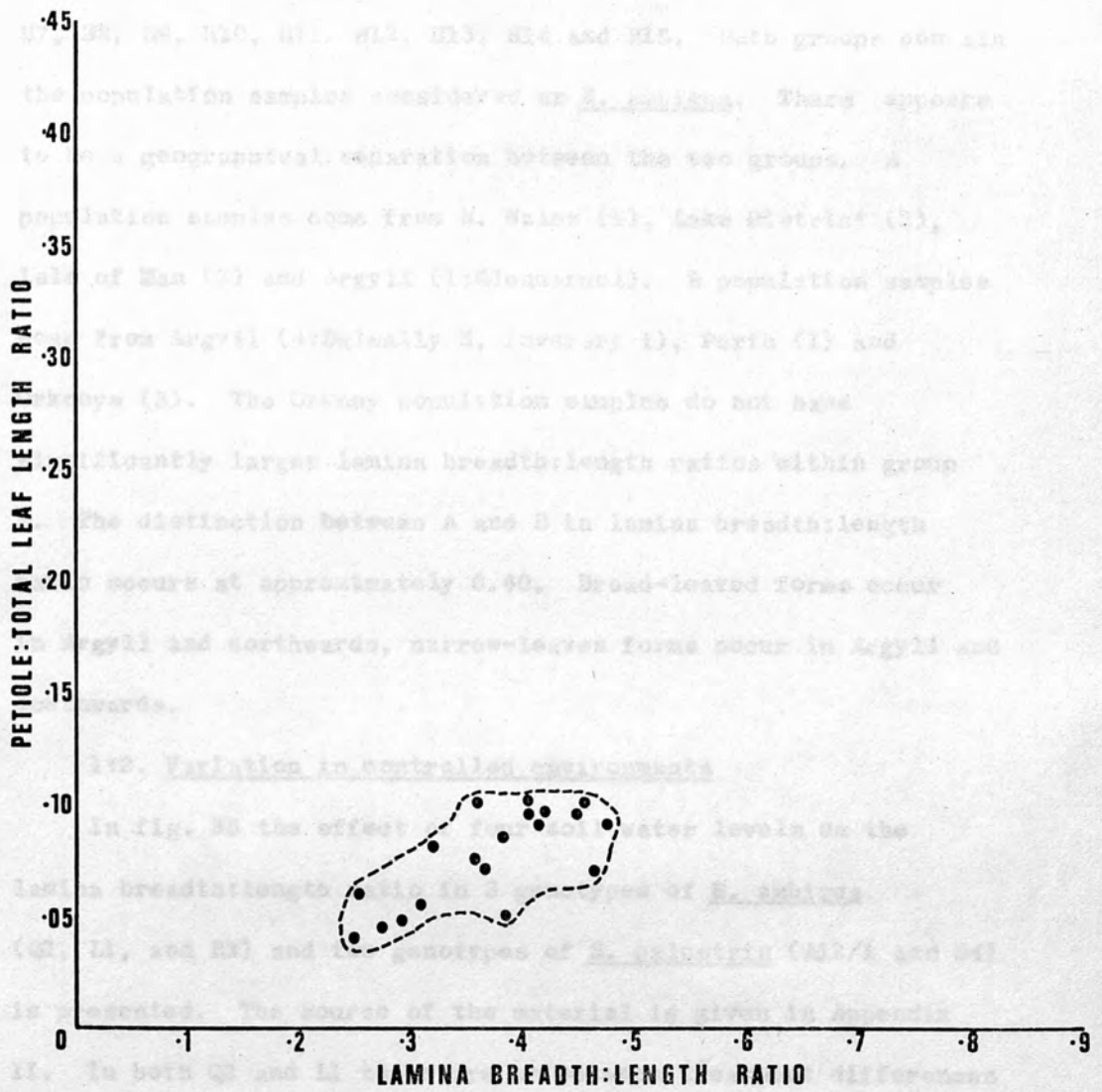Group A contains H1A, H2, H4, H6, H23 and H24 and is

FIG.33   SCATTER DIAGRAM OF STACHYS PALUSTRIS POPULATION AT AYLMERTON, NORFOLK.

Axis labels:
- Y-axis: PETIOLE:TOTAL LEAF LENGTH RATIO
- X-axis: LAMINA BREADTH:LENGTH RATIO

distinct from B by having narrower leaves. In group B are H5, H7, H8, H9, H20, H11, H12, H13, H14 and H15. Both groups contain the population samples considered as S. ambigua. There appears to be a geographical separation between the two groups. A population samples come from N. Wales (1), Lake District (2), Isle of Man (2) and Argyll (1:Glendaruel). B population samples come from Argyll (4:Dalmally 3, Inverary 1), Perth (1) and Orkneys (5). The Orkney population samples do not have significantly larger lamina breadth:length ratios within group B. The distinction between A and B in lamina breadth:length ratio occurs at approximately 0.40. Broad-leaved forms occur in Argyll and northwards, narrow-leaves forms occur in Argyll and southwards.

1:2. Variation in controlled environments

In fig. 35 the effect of four soil water levels on the lamina breadth:length ratio in 3 genotypes of S. ambigua (Q2, L1, and R3) and two genotypes of S. palustris (A12/1 and S4) is presented. The source of the material is given in Appendix II. In both Q2 and L1 there are no between treatment differences at $p = 0.05$. In R3, the two driest treatments exhibit higher lamina breadth:length ratios compared with the high water level treatment ($p < 0.05$).

In S14, the lamina breadth:length ratios are lower in the dry treatment than moderate and low water levels ($p < 0.05$) and in the high water level ($p = 0.10$).

In A12/1, there is no significant difference in lamina breadth:length ratio between treatments, but the within-treatment variation increases consistently with lower water levels. A12/1 is 3 times more variable in the dry treatment
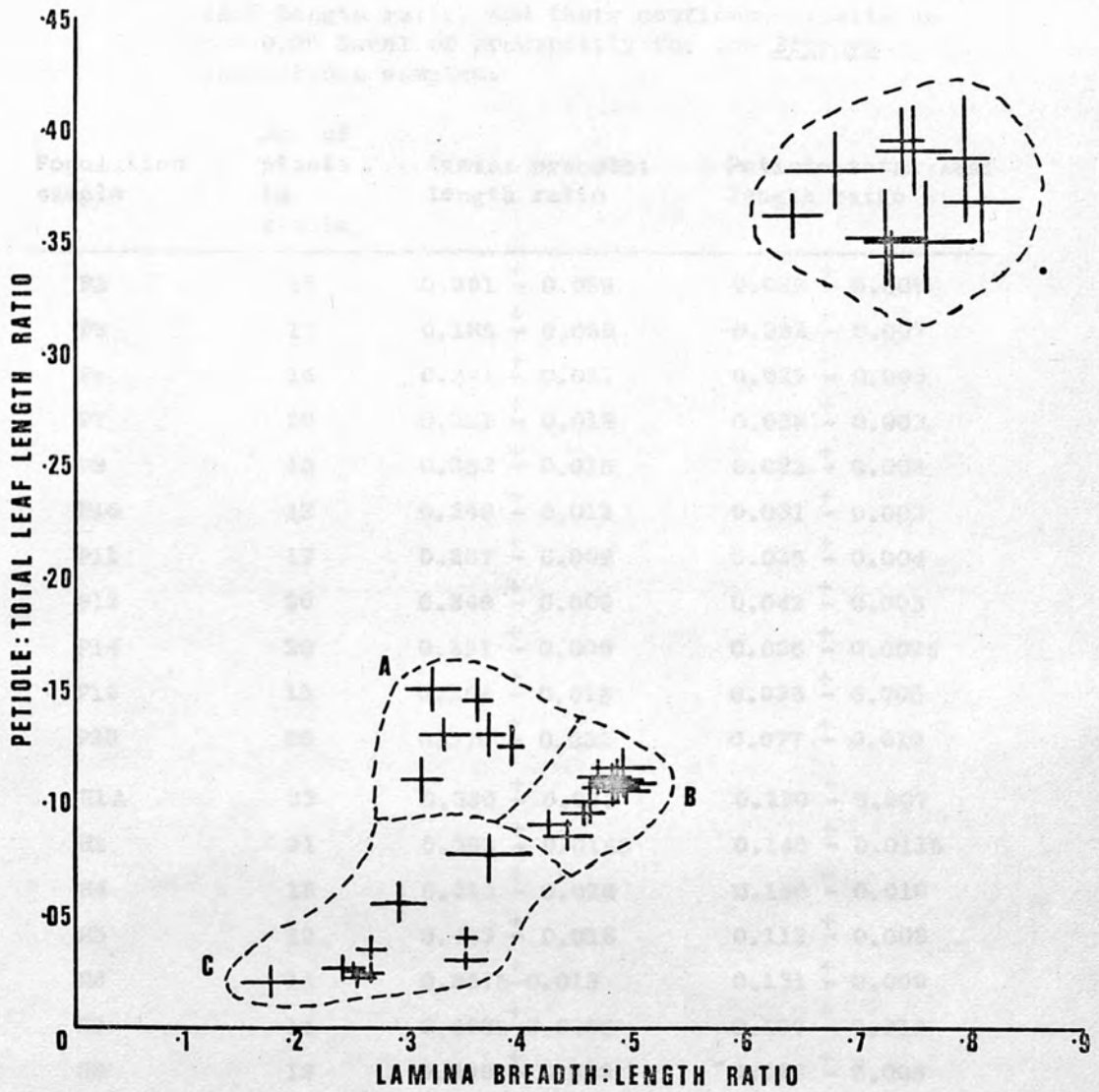
FIG. 34 SCATTER DIAGRAM OF ALL STACHYS POPULATIONS.
Means and confidence limits at p=0·05 are plotted.

Table 11. Mean lamina breadth:length ratio and petiole:total leaf length ratio, and their confidence limits at $p = 0.05$ level of probability for the <u>Stachys</u> populations sampled.

Population sample	No. of plants in sample	Lamina breadth: length ratio	Petiole:total leaf length ratio
P3	18	0.291 ± 0.026	0.055 ± 0.009
P5	17	0.185 ± 0.029	0.022 ± 0.007
P6	15	0.241 ± 0.021	0.027 ± 0.006
P7	20	0.221 ± 0.018	0.039 ± 0.003
P9	15	0.253 ± 0.015	0.022 ± 0.003
P10	12	0.349 ± 0.012	0.031 ± 0.003
P11	17	0.267 ± 0.009	0.025 ± 0.004
P13	20	0.349 ± 0.009	0.042 ± 0.003
P14	20	0.251 ± 0.009	0.026 ± 0.0025
P19	15	0.264 ± 0.015	0.036 ± 0.006
P20	20	0.370 ± 0.032	0.077 ± 0.010
H1A	23	0.330 ± 0.023	0.130 ± 0.007
H2	21	0.363 ± 0.0145	0.146 ± 0.0115
H4	18	0.320 ± 0.026	0.150 ± 0.010
H5	12	0.467 ± 0.018	0.112 ± 0.008
H6	16	0.3675 ± 0.013	0.131 ± 0.009
H7	14	0.4805 ± 0.0285	0.107 ± 0.013
H8	19	0.440 ± 0.022	0.086 ± 0.006
H9	13	0.486 ± 0.022	0.110 ± 0.011
H10	15	0.493 ± 0.022	0.107 ± 0.006
H11	20	0.424 ± 0.019	0.091 ± 0.006
H12	20	0.462 ± 0.025	0.099 ± 0.010
H13	21	0.488 ± 0.032	0.112 ± 0.009
H14	22	0.492 ± 0.032	0.108 ± 0.007
H15	21	0.455 ± 0.019	0.096 ± 0.004
H23	15	0.304 ± 0.020	0.119 ± 0.011
H24	15	0.391 ± 0.008	0.125 ± 0.007

Population sample	No. of plants in sample	Lamina breadth: length ratio	Petiole:total leaf length ratio
S3	17	0.796 ± 0.025	0.384 ± 0.028
S9	18	0.680 ± 0.044	0.380 ± 0.035
S10	13	0.643 ± 0.027	0.3605 ± 0.020
S12	16	0.731 ± 0.014	0.3425 ± 0.020
S13/14	26	0.724 ± 0.037	0.350 ± 0.022
S15	15	0.810 ± 0.036	0.367 ± 0.017
S16/17	28	0.742 ± 0.018	0.393 ± 0.018
S18	16	0.748 ± 0.035	0.388 ± 0.020
S21	18	0.762 ± 0.062	0.349 ± 0.029
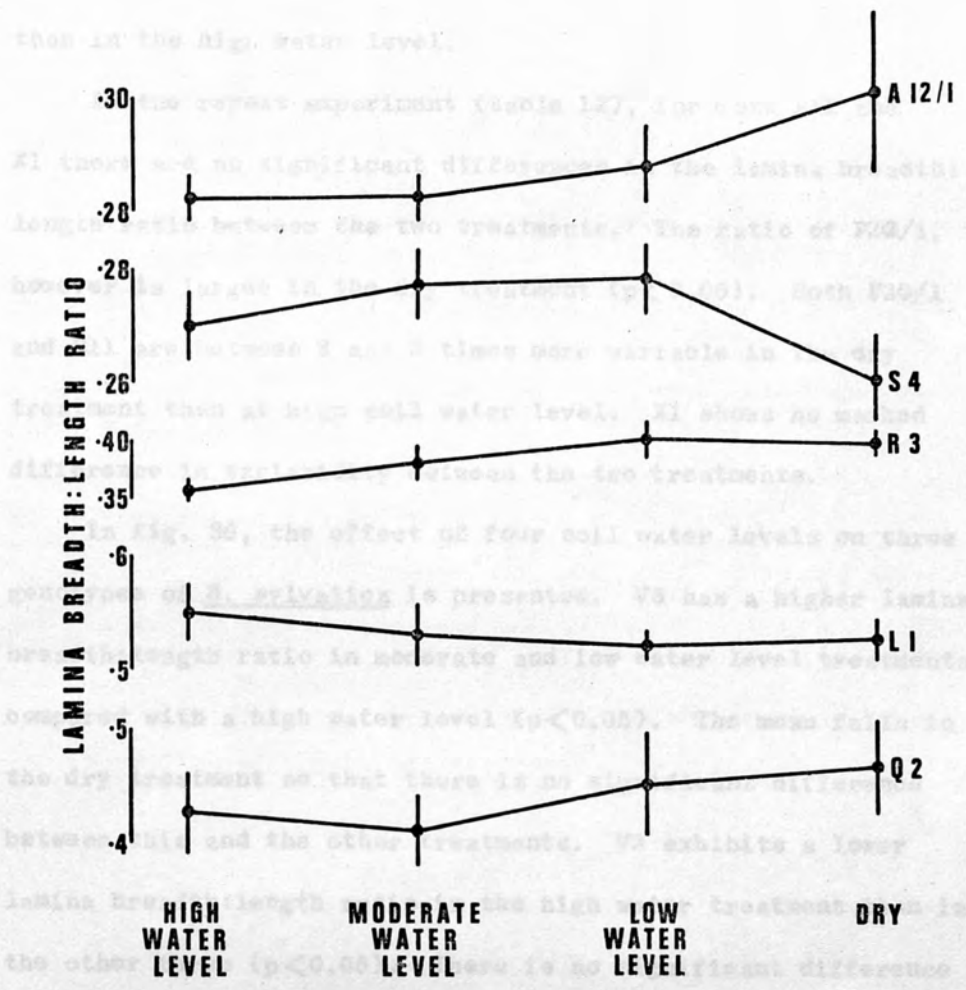
FIG. 35 EFFECT OF FOUR SOIL WATER LEVELS ON LAMINA BREADTH:LENGTH
RATIO IN CLONES OF S.AMBIGUA (R3, LI AND Q2) AND S.PALUSTRIS
(A 12/I AND S4).

than in the high water level.

In the repeat experiment (table 12), for both B11 and X1 there are no significant differences in the lamina breadth: length ratio between the two treatments. The ratio of P20/1, however is larger in the dry treatment ($p < 0.05$). Both P20/1 and B11 are between 2 and 3 times more variable in the dry treatment than at high soil water level. X1 shows no marked difference in variability between the two treatments.

In fig. 36, the effect of four soil water levels on three genotypes of S. sylvatica is presented. V3 has a higher lamina breadth:length ratio in moderate and low water level treatments compared with a high water level ($p < 0.05$). The mean falls in the dry treatment so that there is no significant difference between this and the other treatments. V2 exhibits a lower lamina breadth:length ratio in the high water treatment than in the other three ($p < 0.05$). There is no significant difference between treatments in the lamina breadth:length ratio of K5.

2. Variation in other characters

2:1. Flower production

Flower production was evaluated by determining two characters: the number of open flowers/inflorescence and the number of flowers occurring at the second whorl of the inflorescence. Populations just beginning or ending their period of flowering were not considered.

The result is plotted in fig. 37 and shows populations of S. sylvatica to have a low rate of flower production. Plants of S. sylvatica are extremely constant in the number of flowers produced at one whorl of the inflorescence. Only rarely does the number deviate from 6. In these cases the number is usually 1(-5), a feature commonly occurring at the first whorl of the

Table 12. Effect of two soil water levels on lamina breadth: length ratio and rhizome production in 3 genotypes of S. palustris.

| | HIGH WATER LEVEL | | DRY | |
	$\bar{x}$ Lamina breadth:length ratio	$\bar{x}$ Dry weight rhizomes Gms/pot	$\bar{x}$ Lamina breadth: length ratio	$\bar{x}$ Dry weight rhizomes Gms/pot
P20/1	$0.321**\pm.010$	10.4*	$0.360**\pm.026$	3.9
B11	$0.283*\pm.009$	9.6*	$0.291*\pm.025$	5.2
X1	$0.294*\pm.011$	4.8**	$0.310*\pm.009$	4.6

Means and within treatment confidence limits at p = 0.05 are given.

** Point differing significantly from the other two means.
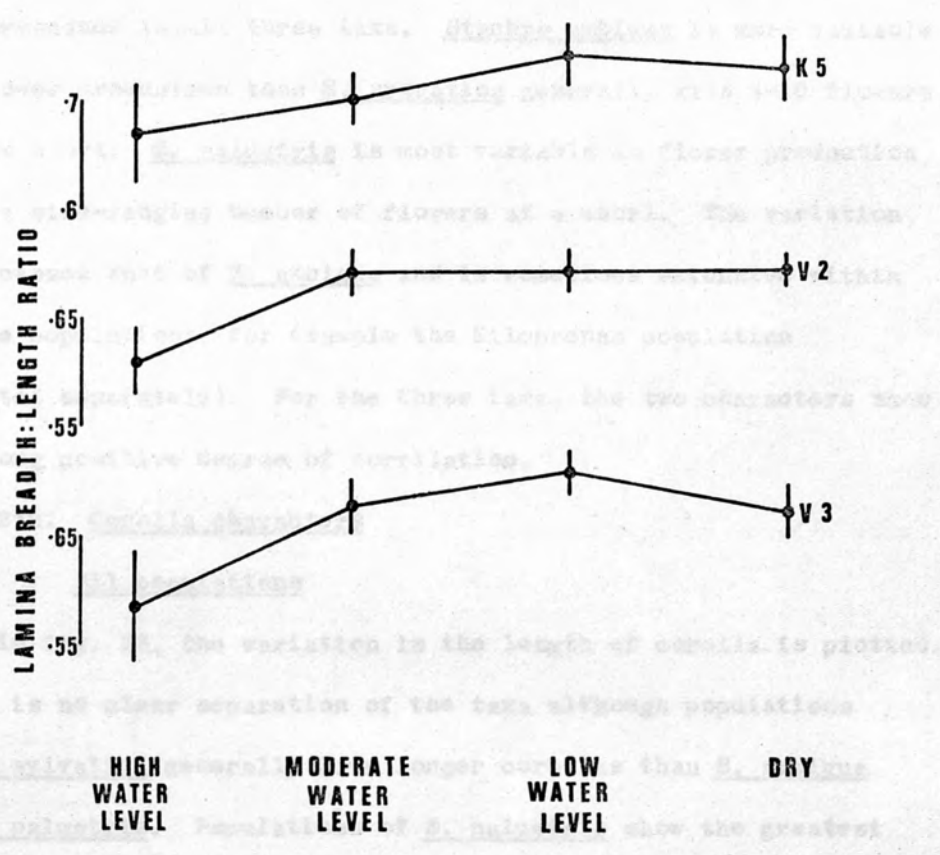
* Points differing significantly from one other mean.

LAMINA BREADTH:LENGTH RATIO

- K 5
- V 2
- V 3

·7
·6
·65
·55
·65
·55

HIGH WATER LEVEL   MODERATE WATER LEVEL   LOW WATER LEVEL   DRY

FIG. 36 EFFECT OF FOUR SOIL WATER LEVELS ON LAMINA BREADTH:LENGTH RATIO IN THREE CLONES OF S. SYLVATICA.

inflorescence in all three taxa. <u>Stachys ambigua</u> is more variable
in flower production than <u>S. sylvatica</u> generally with 6-10 flowers
at one whorl. <u>S. palustris</u> is most variable in flower production
with a wide-ranging number of flowers at a whorl. The variation
encompasses that of <u>S. ambigua</u> and is sometimes extensive within
single populations, for example the Kilchrenan population
(treated separately). For the three taxa, the two characters show
a strong positive degree of correlation.

2:2. <u>Corolla characters</u>

<u>All populations</u>

In fig. 38, the variation in the length of corolla is plotted.
There is no clear separation of the taxa although populations
of <u>S. sylvatica</u> generally have longer corollas than <u>S. ambigua</u>
or <u>S. palustris</u>. Populations of <u>S. palustris</u> show the greatest
degree of between-population-variability encompassing the range
of <u>S. ambigua</u>, and part of <u>S. sylvatica</u>. Population P13
(Waulkmill Bay, Orkney) has a distinctly small corolla length,
and P11 (Inchnadampf, Sutherland) a markedly long one.

In fig. 39, the variation of the length of the corolla tube
is plotted. The pattern of variation is the same as the corolla
length, indicating a positive correlation between the two
characters. H2, H12 and P7 have proportionately long tubes and
H15 has a proportionately short one.

The variation of the corolla lower lip measurement A
(shown in fig. 23) is plotted in fig. 40. This character appears
remarkably uniform throughout most of the populations sampled
and is not taxonomically useful. Between-population variability
varies considerably, for example S3 is 6 times more variable than
S18. P11 has a distinctly larger corolla lower lip measurement

FIG. 37   FLOWER PRODUCTION IN STACHYS POPULATIONS.

Means and confidence limits at p=0·05 are plotted.

S 17
P 11
S 15
S 18
S 3
S 16
S 13/14
H 13
P 3
H 23
H 1A
H 14
H 15
P 19
P 7
H 12
H 11
H 7
P 14
H 2
H 10
P 10
P 13

POPULATIONS

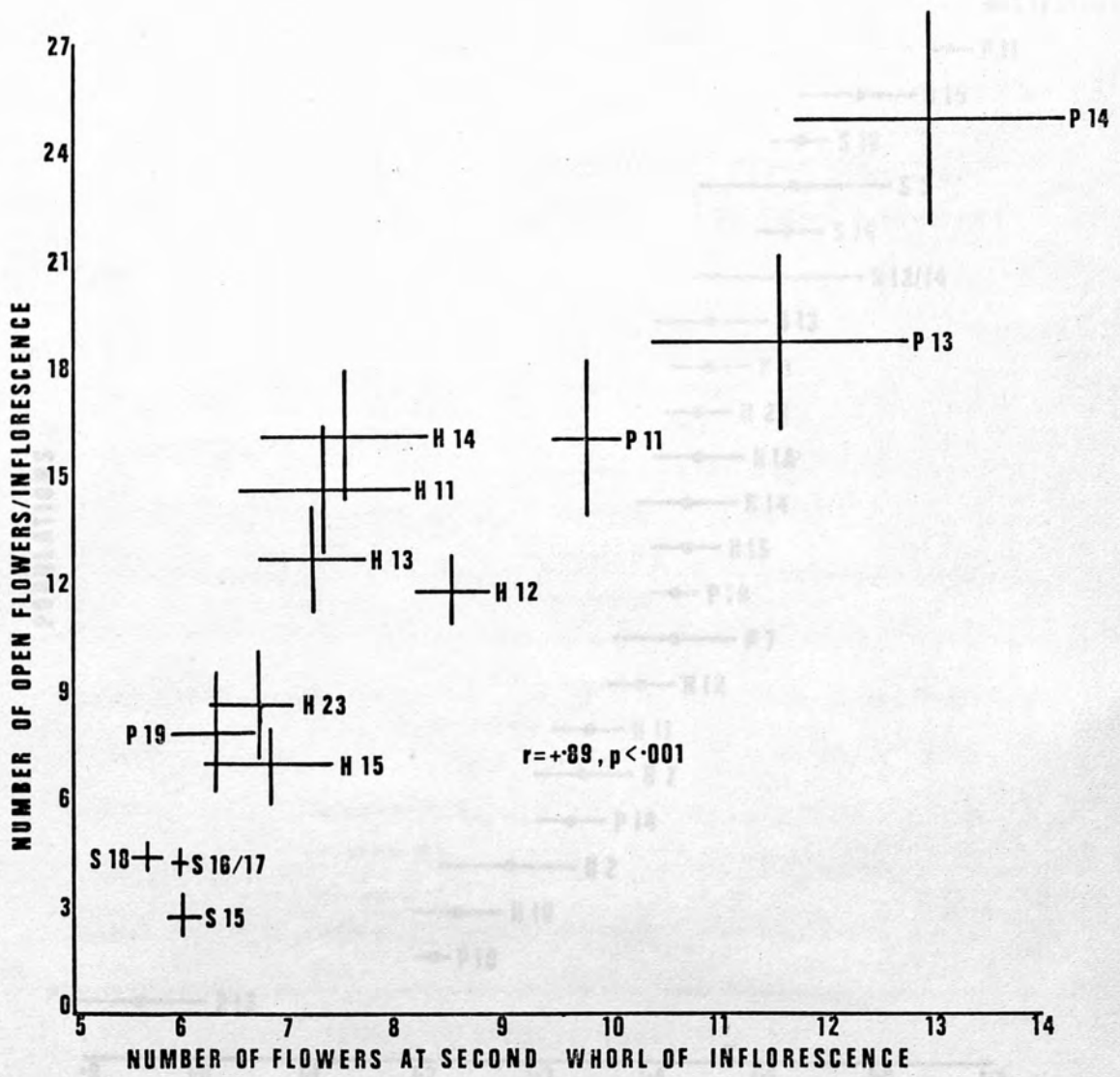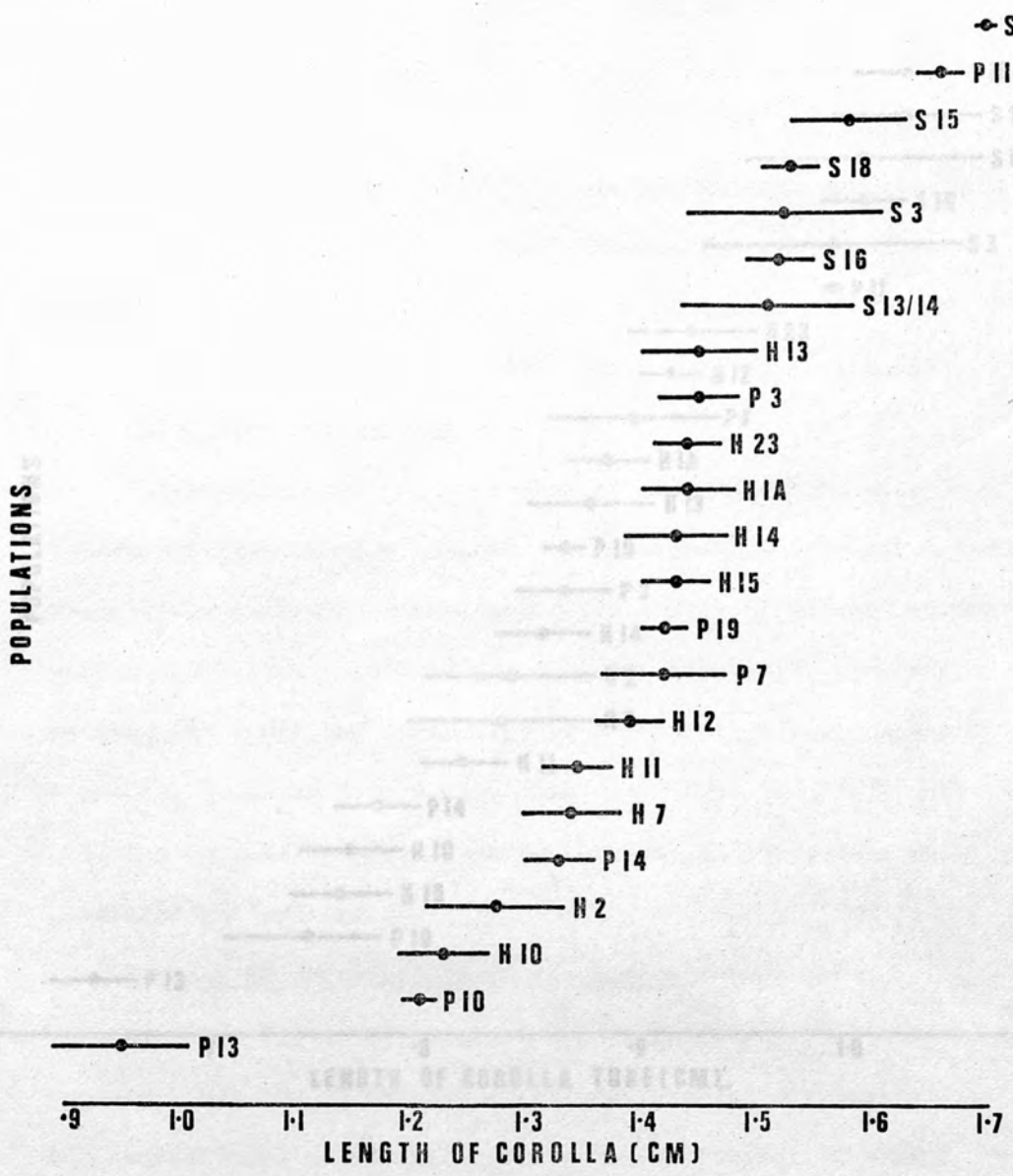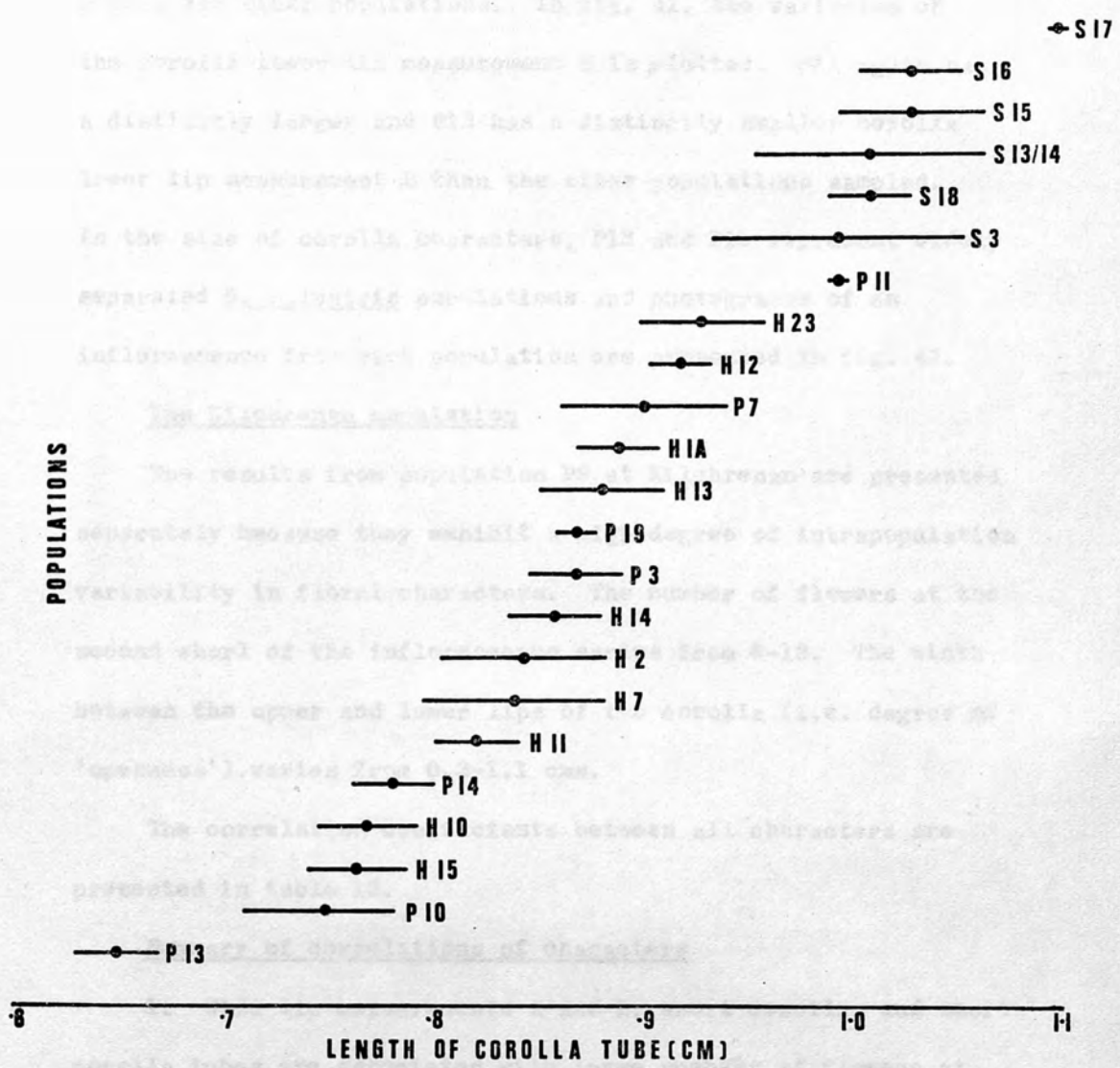| ·9 | 1·0 | 1·1 | 1·2 | 1·3 | 1·4 | 1·5 | 1·6 | 1·7 |

LENGTH OF COROLLA (CM)

FIG. 38 VARIATION OF COROLLA LENGTH IN STACHYS POPULATIONS.
Means and confidence limits at p=0·05 are plotted.

FIG. 39 VARIATION OF COROLLA TUBE LENGTH IN STACHYS POPULATIONS.
Means and confidence limits at p=0·05 are plotted.

A than the other populations. In fig. 41, the variation of the corolla lower lip measurement B is plotted. P11 again has a distinctly larger and P13 has a distinctly smaller corolla lower lip measurement B than the other populations sampled. In the size of corolla characters, P13 and P11 represent widely separated S. palustris populations and photographs of an inflorescence from each population are presented in fig. 42.

The Kilchrenan population

The results from population P8 at Kilchrenan are presented separately because they exhibit a high degree of intrapopulation variability in floral characters. The number of flowers at the second whorl of the inflorescence varies from 6-18. The width between the upper and lower lips of the corolla (i.e. degree of 'openness').varies from 0.3-1.1 cms.

The correlation coefficients between all characters are presented in table 13.

Summary of correlations of characters

1. Wide lip measurements A and B, short corollas and short corolla tubes are associated with large numbers of flowers at the second whorl of the inflorescence and 'closed' flowers (i.e. with a narrow opening between the upper and lower lips of the corolla).

2. The lip characters A and B are not strongly correlated with the other corolla characters.

Two independent variables are therefore associated with the same combination of characters - viz. large flower numbers at a whorl of the inflorescence and closed flowers.

The transect (fig. 43) passes through regions of variable and uniform plants. Plants 31-44 show little variation in the corolla characters studied. They have long corollas and corolla
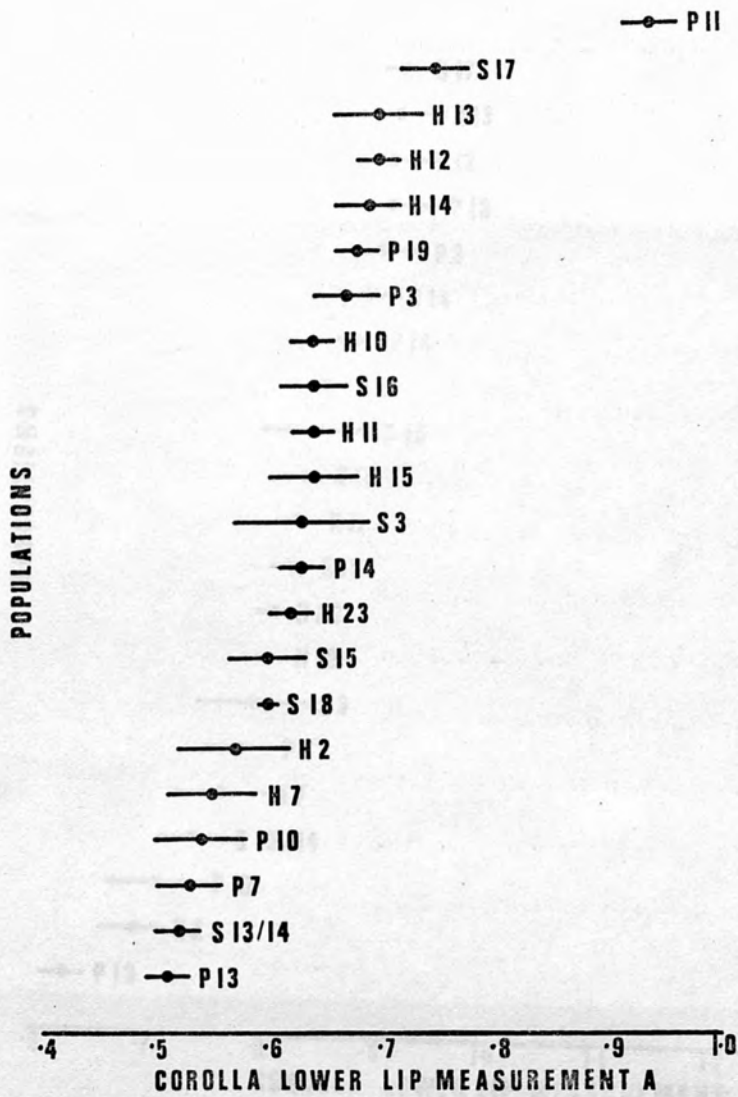
FIG. 40   VARIATION OF COROLLA LOWER LIP MEASUREMENT A IN STACHYS
POPULATIONS.

Means and confidence limits at p=0·05 are plotted.
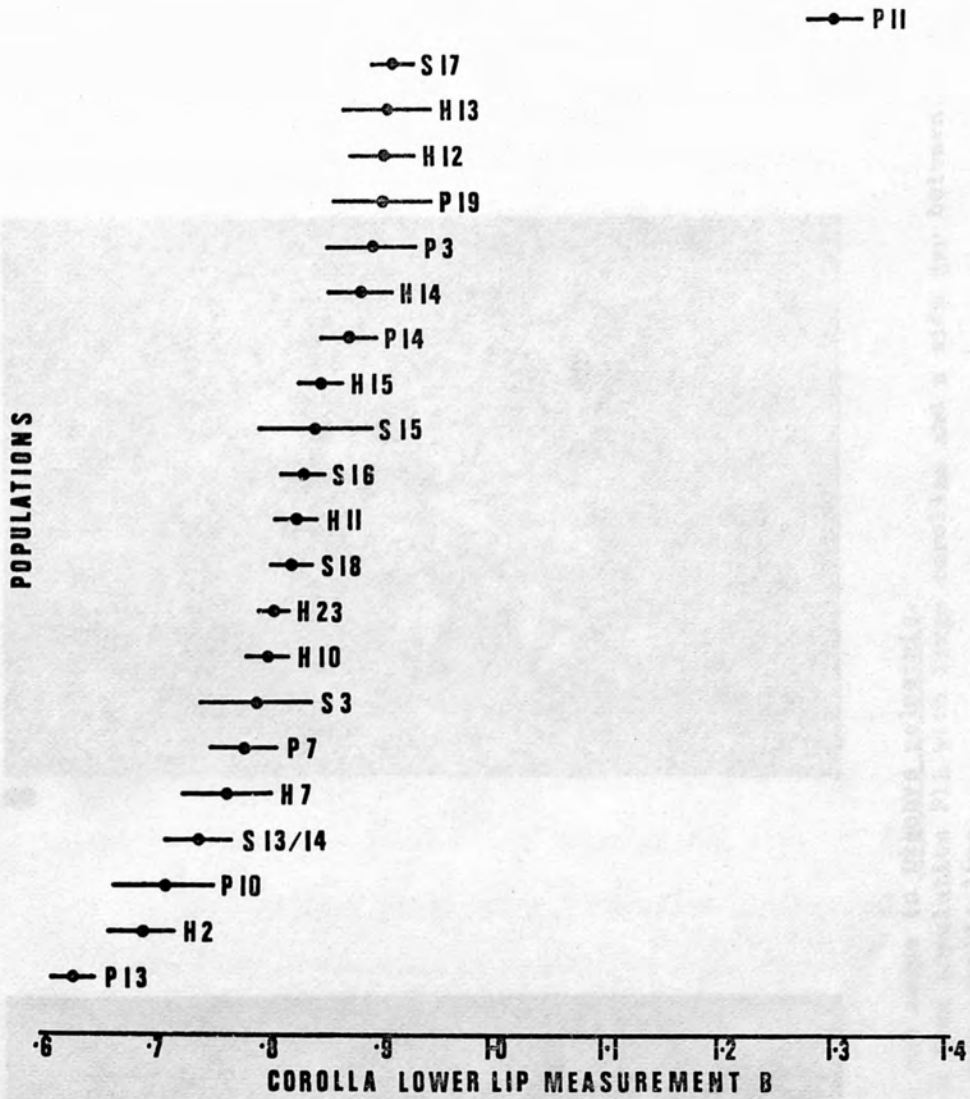
FIG. 41 VARIATION OF COROLLA LOWER LIP MEASUREMENT B IN STACHYS POPULATIONS.
Means and confidence limits at p=0·05 are plotted.

Fig. 42. Variation of corolla size and shape in *Stachys palustris*.

A x 1.8. An inflorescence from population Pl1 with large corollas and a wide gap between the upper and lower corolla lips.

B x 2.0. An inflorescence from the male-sterile population Pl3 with small corollas and a narrow gap between the corolla lips.

tubes, open flowers (c. 1.05 cms between the corolla lips) and
low flower numbers at the second whorl (6). From plants 44-60
there is a gradual gradation towards high flower numbers at a
whorl and closed flowers. Plants from 0-44 show more abrupt
intrapopulation variation.

In fig. 44, a scatter diagram illustrates the relationship
between the number of flowers at the second whorl of the
inflorescence and the width between the upper and lower lips of
the corolla for the plants sampled at Kilchrenan. While the
two characters are negatively correlated ($p < 0.001$) at least
two forms in the population may be recognized. One with open
flowers and low numbers at the second whorl of the inflorescence,
the other with large numbers of 'closed' flowers at a whorl.
Illustrations of the two forms are shown in fig. 45.

2:3. Rhizome production under controlled environments

In the results for rhizome production (fig. 46-49)
significances for plots have been obtained using the Mann-
Whitney (1947) ranking tests as confidence limits of the mean are
inappropriate for small samples.

In fig. 46, the fresh weight production of rhizomes/plant
for three genotypes of S. ambigua are plotted. Within treatment
responses are generally characteristic for each genotype, as are
between treatment responses. For L1, rhizome production is
significantly greater in the moderate water level treatment
than in the other three, but this genotype does not perform
significantly better in high soil water than in dry conditions.
On the other hand, R3 performs progressively better with
increasing soil water level. Q2 performs slightly better in
the two wet treatments than in the two drier ones. The results

Table 13. Correlation coefficients and probability values for the Kilchrenan population.

	C.L.	C.T.L.	L.L.A.	L.L.B.	COROLLA WIDTH	NO. AT 2ND WHORL
Corolla length						
Corolla tube length	+.85 $p<.001$					
Lower lip measurement A	-.22 $p>.05<.10$	-.16 $p>.10$				
Lower measurement B	-.28 $p=.05$	-.22 $p>.05<.10$	+.76 $p<.001$			
Width between upper and lower lips of corolla	+.78 $p<.001$	+.80 $p<.001$	-.42 $p<.001$	-.54 $p<.001$		
Number of flowers at 2nd whorl of inflorescence	-.65 $p<.001$	-.57 $p<.001$	+.52 $p<.001$	+.53 $p<.001$	-.75 $p<.001$	

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COROLLA LENGTH

COROLLA TUBE LENGTH

LOWER LIP MEASUREMENT A

LOWER LIP MEASUREMENT B

WIDTH BETWEEN UPPER AND LOWER LIPS OF COROLLA

NUMBER OF FLOWERS AT SECOND WHORL OF INFLORESCENCE

·5 CMS

·5 CMS

·5 CMS

·5 CMS

PLANT NUMBERS ALONG 135·1·5 METRE TRANSECT
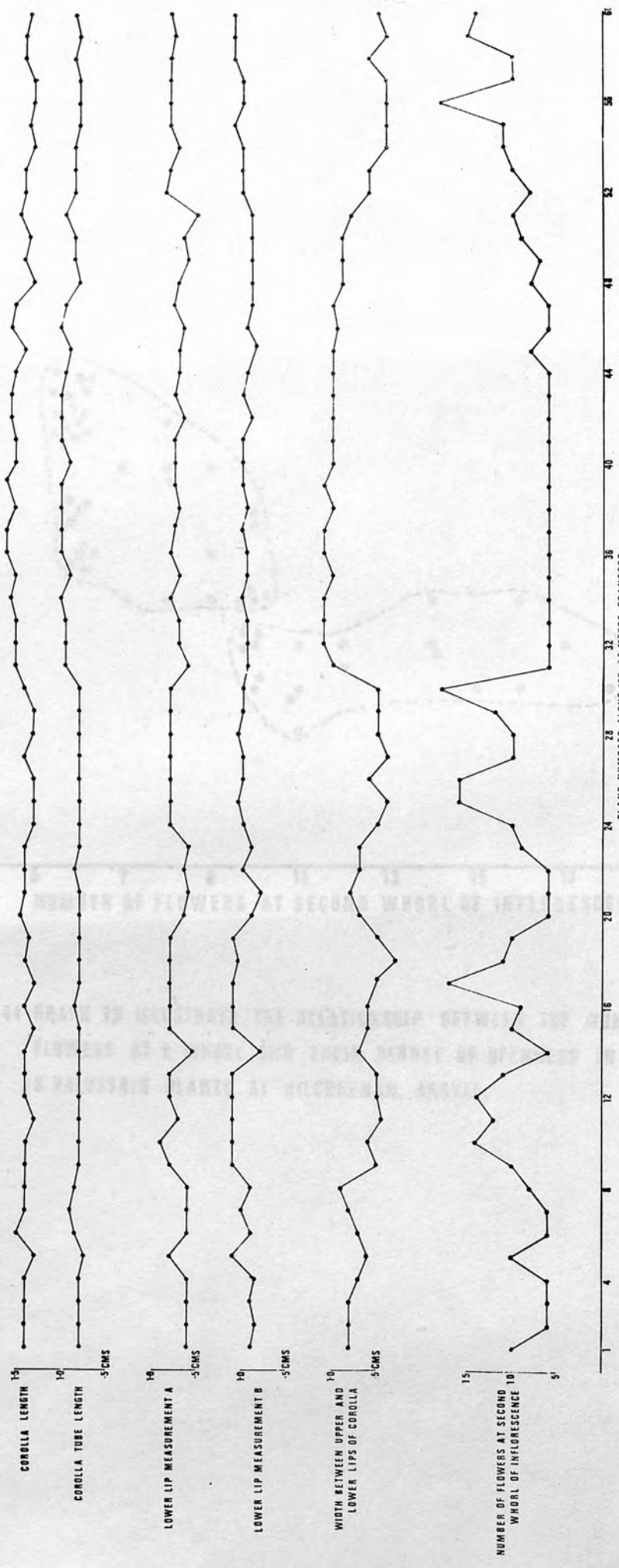
FIG.43 FLORAL VARIATION OF S.PALUSTRIS AT KILCHRENAN, ARGYLL
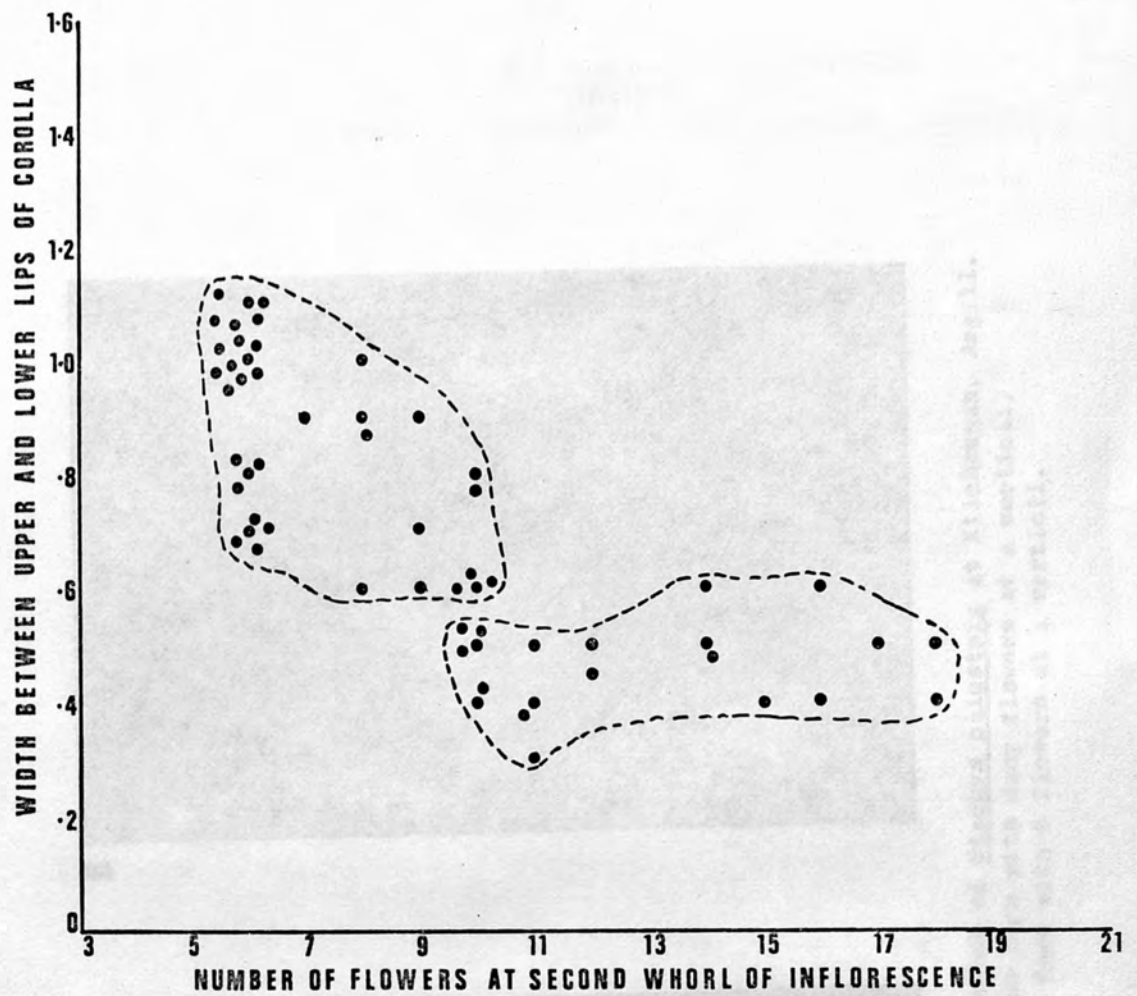
FIG. 44 GRAPH TO ILLUSTRATE THE RELATIONSHIP BETWEEN THE NUMBER OF FLOWERS AT A WHORL AND THEIR DEGREE OF OPENNESS IN S. PALUSTRIS PLANTS AT KILCHRENAN, ARGYLL.

Fig. 45. Variation of the inflorescence of <u>Stachys palustris</u> at Kilchrenan, Argyll.
A x 0.8. 'Closed'-flowered form with many flowers at a verticil.
B x 1.0. 'Open'-flowered form with 6 flowers at a verticil.

are essentially similar for the dry weight analysis (fig. 47).

The fresh and dry weight production of rhizomes/plant for three genotypes of S. palustris are presented in figs. 48 and 49 respectively. S4 shows no significant change in rhizome production over the four soil water treatments. Both A12/1 and B11 produce significantly greater quantities of rhizome material in the two wettest treatments than in the two driest (p < 0.01). They also produce significantly greater quantities of rhizome (d.w.) than S4 in the two wettest treatments.

In the repeat experiment using two different genotypes (table 12) both B11 and P20/1 produce significantly greater quantities of rhizome material than X1 at high water level. There are no significant differences between the performances of the three genotypes in the dry treatment. P20/1 and X1 are genotypes collected from dry ground conditions, B11 from typical marshland.

DISCUSSION

The overall picture obtained from a study of the variation of leaf characters in nature (illustrated in fig. 34) corresponds very closely with the results obtained from earlier herbarium studies (fig. 22). The overlap between S. palustris and S. ambigua observed in leaf characters has undoubtedly led authors (for example, Perring and Sell, 1968) to the conclusion that backcrossing of the hybrid to S. palustris occurs in nature. The presence of a marked discontinuity in the variation of leaf characters between S. ambigua and S. sylvatica, has been taken to indicate that introgression involves one parent only.

Close examination of the field sites where all three taxa

FIG. 46 EFFECT OF FOUR SOIL WATER LEVELS ON RHIZOME PRODUCTION (F.W.)
IN THREE CLONES OF S. AMBIGUA.

Means and within treatment significant differences at $p = 0.05$ are
plotted.

xx Points differing significantly from the other two means.

x Points differing significantly from one other mean.

FIG. 47 EFFECT OF FOUR SOIL WATER LEVELS ON RHIZOME PRODUCTION (D.W.)
IN THREE CLONES OF S. AMBIGUA.

Means and within treatment significant differences at p=0·05 are
plotted.

××Points differing significantly from the other two means.
×Points differing significantly from one other mean.

FIG. 48 EFFECT OF FOUR SOIL WATER LEVELS ON RHIZOME PRODUCTION (F.W.) IN THREE CLONES OF S. PALUSTRIS.

Means and within treatment significant differences at p=0·05 are plotted.

ᵡᵡ Points differing significantly from the other two means.

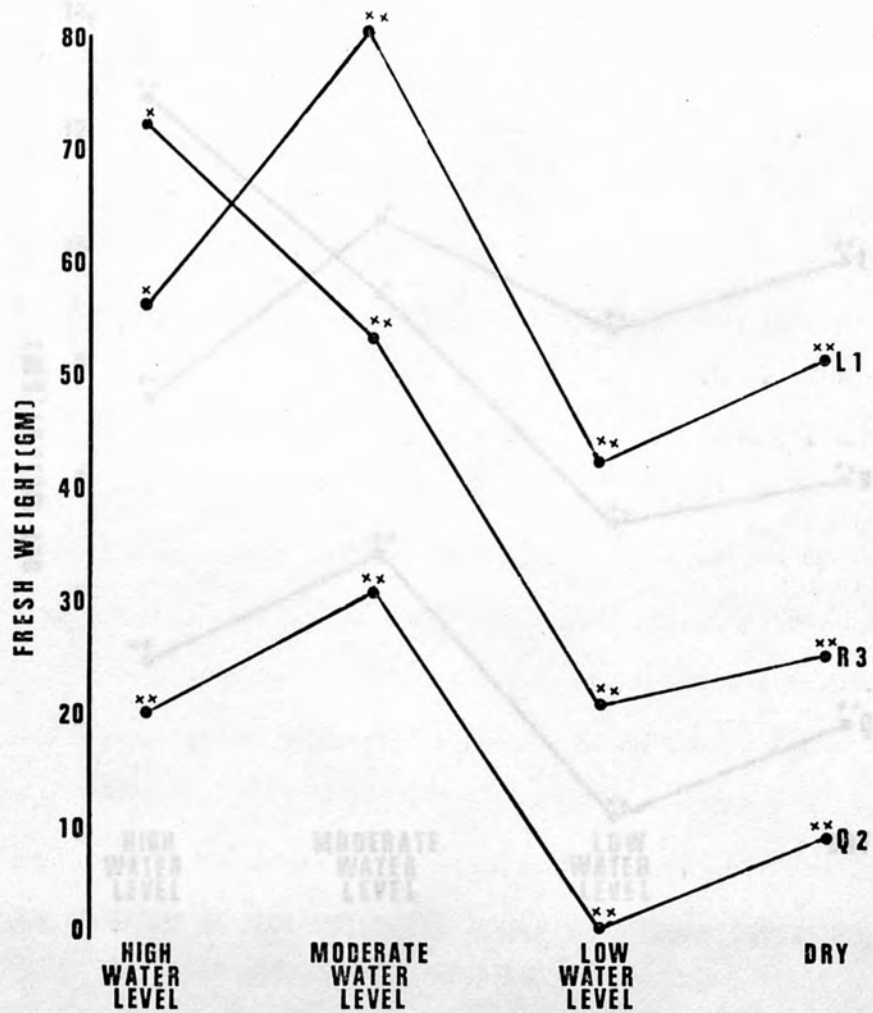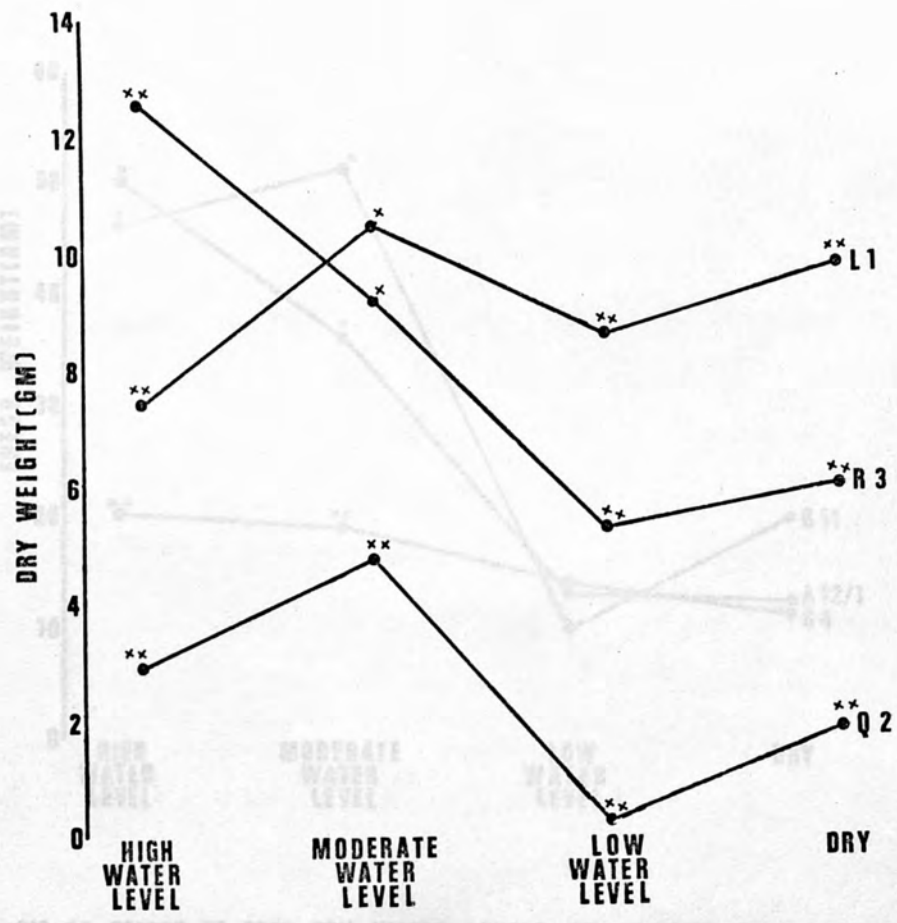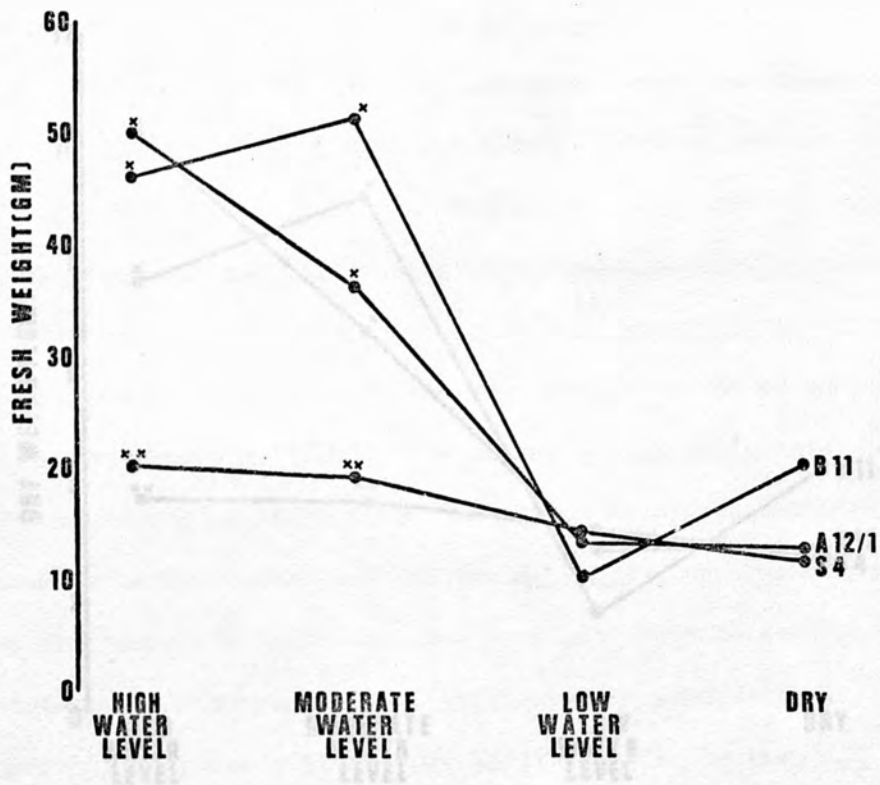ᵡ Points differing significantly from one other mean.
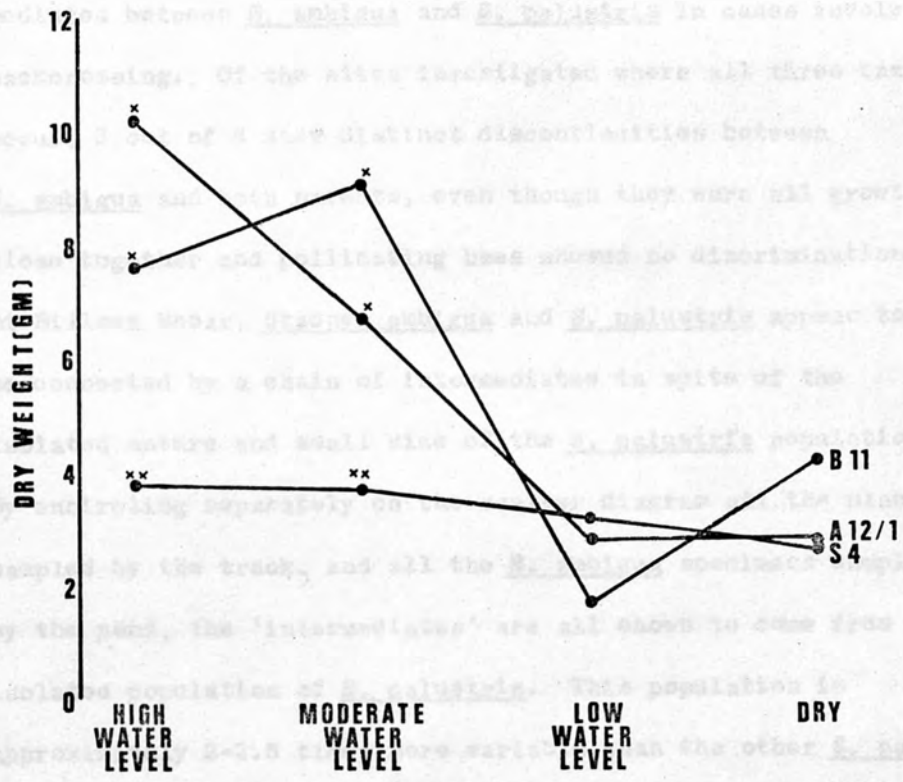
FIG. 49 EFFECT OF FOUR SOIL WATER LEVELS ON RHIZOME PRODUCTION (D.W.)
IN THREE CLONES OF S. PALUSTRIS.

Means and within treatment significant differences at p=0·05 are
plotted.

xx Points differing significantly from the other two means.
x Points differing significantly from one other mean.

occur might be expected to reveal the presence of some inter-
mediates between S. ambigua and S. palustris in cases involving
backcrossing. Of the sites investigated where all three taxa
occur, 3 out of 4 show distinct discontinuities between
S. ambigua and both parents, even though they were all growing
close together and pollinating bees showed no discrimination.
At Billown Mooar, Stachys ambigua and S. palustris appear to
be connected by a chain of intermediates in spite of the
isolated nature and small size of the S. palustris population.
By encircling separately on the scatter diagram all the plants
sampled by the track, and all the S. ambigua specimens sampled
by the pond, the 'intermediates' are all shown to come from the
isolated population of S. palustris. This population is
approximately 2-2.5 times more variable than the other S. palustris
populations, except that at Aylmerton in Norfolk (fig. 33). The
Aylmerton population was sampled as a dry ground form of
S. palustris since it was found growing in a sugar beet field,
and is itself an exceedingly variable population. One factor
that these two populations have in common together with their
high degree of variability is a comparatively dry habitat for a
normally marshland species.

Variation of lamina breadth:length ratio under controlled
soil water regimes of A12/1, B11 and P20/1 (Aylmerton) illustrates
a radical between treatment difference in the response of all
these genotypes. They are 2-3 times more variable in the dry
treatments than in the wet ones, indicating physiological
stress. This would appear to correlate very closely with the
situation existing in nature and indicating that where
S. palustris is growing in dry conditions it is likely to be much

more variable than those populations growing in wet areas.
However, two genotypes of S. palustris, (S4 and X1) investigated
showed no such difference in variability between treatments.
So, on some occasions field populations of S. palustris
growing in dry habitats might not be expected to show any
greater degree of variability than their marshland counterparts.
The relationship of soil water level to variability of leaf
morphology in S. palustris is therefore not a simple one, and
is genotype-dependent.

The results suggest that at least two forms of S. palustris
occur in nature and may be recognized by their morphological
response to wet and dry enviromnental conditions. The so-called
dry ground form has therefore some experimental justification when
strictly applied to genotypes that are adapted to dry ground
conditions. Both forms are found in dry conditions and the dry
ground ecotype may be distinguished by its greater uniformity and
proportionately longer petioles.

Apart from differences in variability, the lamina breadth:
length ratio only occasionally varies significantly between
treatments for all three taxa. It is therefore particularly
useful as a taxonomic character when comparing plants from a
range of soil moistures. Unfortunately no satisfactory method
has been devised of analyzing in the same way the petiole:total
leaf length ratio also used on the scatter diagrams. The intra-
individual variation of the character is so large as to render
between-treatment comparisons on the basis of means and
confidence limits inappropriate. This variation is associated
with the position of the node on the stem. From the highest
vegetative node on a flowering shoot downwards the petiole:total

leaf length ratio increases. The ratio is also positively correlated with leaf size ($r = +0.70$, $p < 0.001$, 14 plants from all taxa). The positional effect of the node is not completely removed when this is taken into account by dividing the total leaf length by the ratio. As the petiole:total leaf length ratio is an important taxonomic character it would be useful to have some information on its variation under differing soil water treatments. Clearly, studies of several ramets of a clone grown in growth cabinets, at various soil water levels with between-treatment analysis of the character for each node, will have to be made to obtain this information.

The intraspecific variation of the lamina breadth:length ratio within S. ambigua (fig. 34: groups A and B) suggests that some population differentiation may have occurred in Britain on a geographical basis. Perring et al (1968) has pointed out that unusually broad-leaved forms of S. ambigua occur in Orkney and were originally mistaken for specimens of S. sylvatica. While specimens of S. ambigua from Orkney do not have broader leaves than those from other areas in Scotland (Perth and Argyll) the northern populations do have broader leaves than those further south (group A).

The variation in flower production (fig. 37) for all three taxa illustrates the more 'floriferous' nature of S. palustris and S. ambigua. Plants of S. sylvatica show marked constancy in the number of flowers (6) at one verticil. Any specimen exhibiting more than 6 flowers at a whorl is far more likely to be S. palustris or S. ambigua. The highest numbers of flowers at one whorl are exhibited by S. palustris. Low numbers (down to 6) may also be encountered in S. palustris, sometimes in the same population as high ones (for example, Kilchrenan).

The variation of corolla length and corolla tube length measurements suggests some taxonomic significance for the two characters. _S. sylvatica_ populations are significantly larger but there is no discontinuity in the variation with _S. palustris_ and _S. ambigua_. The corolla lower lip measurements A and B show no taxonomic usefulness. In all these corolla characters, _S. palustris_ is inherently more variable than the other two species.

The Kilchrenan population of _S. palustris_ exhibits a high degree of intrapopulation variability in floral characters (figs. 43 and 44 and table 13). The data show that at least two forms are recognizable in the population. One with 'open' flowers and low numbers at the second whorl of the inflorescence, the other with large numbers of 'closed' flowers at each whorl. This hypothesis is supported by the discovery of two independent. Variables associated with the same number of characters.

From the results, there is evidence to suggest that the two forms intergrade. The transect (fig. 43) shows a gradual gradation (plants 44-60) from one form to another. Within the population, though, both forms may be found in close proximity.

The origin and maintenance of the variability may be dependent on the production and isolation of the 'closed'-flowered form. At Kilchrenan two types of visiting insect were observed, bumble bees (_Bombus spp_) and flies (_Rhingia spp_). Whilst the flies visited both the open and closed forms, the bees never penetrated closed flowers, so that they worked their way through the population selecting the open flowers to visit.

If both the flies and bees are effective pollinators, there will then be a tendency for the closed form to remain isolated to some extent, being pollinated only by flies. The

lack of discrimination of _Rhingia_ ensures that both forms belong to the same breeding population.

While hybridization of the two forms appears to occur at Kilchrenan, the existence of intermediate types suggests a significant degree of seedling establishment in the population. However, the relative role of vegetative reproduction compared with seedling establishment in maintaining natural populations of _Stachys spp._ is not known.

The simplest explanation of the intrapopulation variation observed at Kilchrenan is that the closed-floweredform arose as a rare recombinant or mutant within the open, 6-flowered type and has been maintained by immediate exclusion of the commonest pollinating insects in _Stachys_ populations, _Bombus spp._ Intermediates have been produced by inter-form pollination by _Rhingia spp._ Vegetative spread by rhizomes would lead to a general mixing of forms within the site.

The experimental investigation of rhizome production in _Stachys palustris_ and _S. ambigua_ shows important differences between genotypes (figs. 46-49). The three genotypes of _S. ambigua_ studied respond differently. R3 and Q2 are shown to be soil water-dependent, while L1 is relatively water-independent. In _S. palustris_, rhizome production appears to be closely related with the degree of variability in lamina breadth:length ratio. B11, A12/1 and P23/1 are all soil water-dependent genotypes producing greater quantities of rhizomes in wet conditions, when they are 2-3 times more stable in lamina breadth:length ratio. S4 and X1 are not soil water-dependent, with no significant differences in rhizome production in wet or dry soil conditions. This genotypic difference supports the existence of two forms

suggested by the variation in lamina breadth:length ratios.
The two 'dry-ground' genotypes do not produce significantly
greater quantities of rhizome material in dry soil conditions
than in wet ones and these are generally not significantly
different from B11, A12/1 and P20/1 when grown in dry soils.
The dry ground ecotype does not, then, appear to be better
adapted to dry soils than the other genotypes in terms of
rhizomes production but, rather, not so well adapted to wet
soils. They are, however, distinguished by a greater degree
of stability of the lamina breadth:length ratio in dry soils
than their water-dependent counterparts.

A concluding remark on the overall variation in morphology
exhibited by S. palustris and S. sylvatica in all aspects
investigated is the strikingly greater degree of variability,
both between and within populations, existing in S. palustris.

6.   GENERAL DISCUSSION

From studies of the morphology, chromosome number and
pollen inviability of the population samples of Stachys it is
possible to refer the majority of them to one of the three taxa
investigated.   Samples with hybrid combinations of characters -
intermediate morphology, intermediate chromosome number and high
pollen inviability ($>10\%$) - may be referred to Stachys ambigua
Sm.   They are H1A, H2, H4, H6, H7, H8, H9, H10, H11, H12 and H13.
Three population samples, whose chromosome numbers are not
known, show intermediate morphology and high pollen inviability -
H5, H14 and H15 - i.e. possess Wagner's (1968) two criteria for
the detection of hybridization (see Reproductive Isolation p.6)

The population samples referable to S. sylvatica L. show no
overlap in leaf morphology with S. ambigua.   Samples further
investigated show other features consistent with their taxonomic
treatment, such as low chromosome number and low pollen inviability.
Two population samples - P11 and P14 - are referable to
S. palustris L. on the basis of their leaf morphology, high
chromosome number and low pollen inviability.   P11 is distinctive
for two reasons: it has large flowers (see figs. 38-42) and a
chromosome number of between 96 and 99.   The P11 population
itself is very constant, perhaps representing one genotype, but
is unusually large, covering approximately 0.5 hectare of
marshland.   The chromosome number of two plants from the
population was found to be well below the number found in British
S. palustris ($2n = 102$) but approaching the number reported for
that species in N. America ($2n = 96$).

P6 and P13 are not immediately referable to any of the

three taxa, possessing conflicting character combinations - leaf
morphology of S. palustris, high chromosome number but with high
pollen inviability. While these populations may represent back-
cross populations, only the high level of pollen inviability is
inconsistent with their treatment as S. palustris. Male-sterile
plants have been repeatedly observed in many populations of
species of Labiatae - for example by Willis (1891) and Hedge
(1968) - and these commonly have smaller flowers than the male-
fertile plants. P13 shows distinctly smaller corolla and lower
lip measurements than the other Stachys populations sampled.
The flowers of P6 are not significantly smaller than other
S. palustris populations. The chromosome number of 2n=103
recorded in P13 may be a causal factor in the sterility of the
plants. The P6 population, when re-examined at a slightly later
time the next year, was producing mature fruits, indicating a
post-meiotic mechanism operating in stamens only. However, no
information on the level of viability of the fruits is known,
which may not have contained any seeds. P13 produced no mature
fruits.

The scatter diagram (fig. 27) of the Billowa Mooar
population samples - P3, H2 and S3 - shows an apparently classic
example of one-way backcrossing of a hybrid. The S. sylvatica
population sample is unusually variable in many of the
morphological characters, but shows no overlap with S. ambigua
in leaf morphology. S. ambigua and the sample P3 from the small
and isolated population nearby, appear to intergrade. While
the greatest degree of variability in leaf data is shown by P3,
the population sample of the hybrid (H2) shows somatic chromosome
numbers ranging from 81-86. This has been the most intensively

studied of all the populations for chromosome number and they range both sides of 84. However, this sort of range is not likely to arise from backcrossing. Because the plant is essentially a vegetative apomict small variations in chromosome number are likely to occur within plants and hence accumulate in populations.

The only other population that shows a range of variation of leaf morphology consistent with backcrossing is at Stonethwaite in the Lake District (fig. 29). Here the morphological variation of population samples P19 and H23 approach one another but do not overlap. The occurrence of mature fruits in one or two of the broader leaved petiolate specimens is unusual in 'ambigua-like' plants and may indicate introgression. It is not known whether any of these mericarps contained viable seed, but most of the ones looked at were empty. This population has not been investigated for chromosome number. The unusual variability of leaf ratios in these two taxa may be dependent on the availability of water, as occurs in some genotypes. But even if this population is ignored, the scatter diagram (fig. 34) showing the range of leaf morphology of all Stachys populations sampled still indicates an intergradation between S. ambigua (A and B) and S. palustris (C). This inadvertently gives the impression of extensive backcrossing between the taxa, although no definite evidence for backcrossing has been found in any of the individual populations studied.

The range of leaf morphology of the S. ambigua population samples (fig. 34) appears much closer to S. palustris than S. sylvatica. This is also true of the other characters studied. Only the occasional broad-leaved plants are more or less intermediate

between parents. If the population samples of <u>S. ambigua</u> presumed to contain largely F_1 specimens are considered, then the characters of <u>S. palustris</u> obviously predominate in the hybrid. This is most likely to be caused either by matrocliny or dominance as a result of hybridization between parents with unequal chromosome numbers. If matrocliny is involved either the majority of successful interspecific crosses must result from those where <u>S. palustris</u> is the maternal parent, or matriclinous inheritance occurs only in the cross <u>S. palustris</u> ♀ x <u>S. sylvatica</u> ♂ and not in <u>S. sylvatica</u> ♀ x <u>S. palustris</u> ♂.

Baker (1951) gives several examples of interspecific crosses where the F_1 hybrid is not morphologically intermediate between the parents. Many of these occur in crosses between parents with different chromosome numbers and in these cases the F_1 more closely resembles the parent contributing the greater number of chromosomes. However, in some cases of interspecific hybridization between parents of unequal chromosome number, the F_1 may be intermediate. In diploid x tetraploid crosses in <u>Paeonia</u> spp, Saunders and Stebbins (1938) found that triploids in some cases were intermediate and in others resembled the tetraploid. The difference was apparently dependent on the origin of the tetraploid. Autotetraploids (with a high number of multivalents, and a homogeneous morphology) were involved in crosses where the F_1 triploid resembled the tetraploid. Allotetraploids (with low numbers of multivalents and a high level of morphological variability) were involved in crosses where the F_1 triploid was more or less intermediate between the parents. The same conclusion was reached by East (1935) with <u>Nicotiana</u> hybrids and Anderson (1936) with <u>Tradescantia</u> <u>canaliculata x subaspera</u>.

S. palustris shows little indication of having originated
by autopolyploidy, being a highly polymorphic species and, as
shown by Lang (1940), exhibiting only low numbers of multi-
valents. As an allopolyploid, interspecific hybrids would
therefore be expected to possess a more or less intermediate
morphology. However, the F_1 hybrids raised by Lang (1940) were
reported to show a morphology most closely resembling
S. palustris. Since these plants were obtained from crosses
involving S. palustris as the maternal parents, either matrocliny
or chromosome number differences may be the causal factor.
Neither hypothesis entirely fits the observed facts. The
chromosome number difference appears to be important when the
higher-numbered parent possesses two or more sets of similar
genomes. In matrocliny some characters of the F_1 may be expected
to show normal inheritance and thus show a range more or less
intermediate between the parents. No good examples of such
characters have been found in S. ambigua. The chromatographic
spot patterns of S. ambigua more closely resemble those of
S.palustris than S. sylvatica. The corolla length characters
(figs. 38 and 39) may represent examples where S. ambigua
approaches a more or less intermediate condition. However, the
high degree of variability of S. palustris in this as in other
characters obscures the trends.

A genetic model is proposed that fits the morphological
variation and chromosome associations at metaphase observed in
S. palustris, S. sylvatica, and S. ambigua. With the base
number x = 17 in Stachys, S. sylvatica represents a tetraploid
($4x$ = 68, 2n = 68-2) and S. palustris a hexaploid ($6x$ = 102,
2n = 102).

Three different genomes are involved in the model — A_1, A_2 and B. Chromosomes of the A_1 and A_2 genomes are homoeologous. The parental genome complements are:-

S. sylvatica : A_2A_2BB

S. palustris : $A_1A_1A_1A_2BB$

S. sylvatica therefore represents an allotetraploid. S. palustris shows both allopolyploid and autopolyploid characteristics. It is an autopolyploid with respect to A_1, and an allopolyploid by virtue of possessing all three genomes. Chromosome pairing is normal, the 'diploidization' possibly being under genetic control by one or more chromosomes similar in effect to the VB chromosome of hexaploid wheat (Riley and Chapman, 1958).

Hybridization between the two species will result in the production of two genome complements in S. ambigua, as shown below (pairing relations of chromosomes shown in brackets):-

S. palustris   S. sylvatica

$A_1A_1A_1A_2BB(3\varkappa II)$ x $A_2A_2BB(2\varkappa II)$

gametes A_1A_1B
         A_1A_2B A_2B

S. ambigua   1. $A_1A_1A_2BB$ $(2\varkappa II + 1\varkappa I)$
             2. $A_1A_2A_2BB$ $(2\varkappa II + 1\varkappa I)$
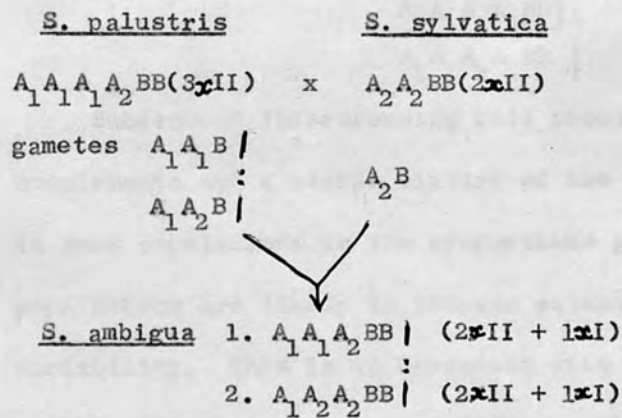
Pairing in the hybrid, in either case, will therefore involve all the chromosomes of two genomes, the remaining genome set being unpaired. Homologous chromosomes will preferentially pair leaving a single homoeologous genome set unpaired. At least 33 bivalents at metaphase of meiosis in S. ambigua have been recorded, in this investigation and by Lang (1940).

Meiosis in $\underline{S.\ sylvatica}$ is reported by Lang to be regular, consisting of 33 bivalents.

The first hybrid genome complement ($A_1A_1A_2BB$) is likely to give rise to plants morphologically closer to $\underline{S.\ palustris}$ than genome complement 2. The br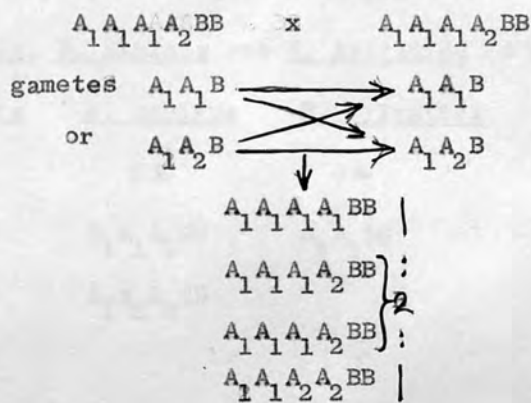oad- and narrow-leaved forms of $\underline{S.\ ambigua}$ may, therefore, possess distinct genome complements. Smith's original specimen from the Orkneys was a broad-leaved form more or less intermediate between the parents. Specimens nearer $\underline{S.\ palustris}$ in morphology possess genome complement 1.

Outcrossing in $\underline{S.\ palustris}$ will produce the following genome complements:-

$$A_1A_1A_1A_2BB \quad x \quad A_1A_1A_1A_2BB$$

gametes $A_1A_1B \longrightarrow A_1A_1B$

or $A_1A_2B \longrightarrow A_1A_2B$

$$A_1A_1A_1A_1BB \quad |$$
$$A_1A_1A_1A_2BB \quad \Big\} : $$
$$A_1A_1A_1A_2BB \quad \Big\} : 2$$
$$A_1A_1A_2A_2BB \quad |$$

Subsequent interbreeding will produce no new genome complements and a stable mixture of the three genomes will exist in some populations in the proportions given above. Such populations are likely to possess extensive phenotypic variability. This is in agreement with the high level of both within-- and between - population variability recorded in some populations of $\underline{S.\ palustris}$, for both the morphological and biochemical characters studied in this investigation. Populations with either genome complement $A_1A_1A_1A_1BB$ or $A_1A_1A_2A_2BB$ will be self-perpetuating and less variable than the mixed populations.

Hybridization of the two less common genome complements with

S. sylvatica will produce hybrid genome complements identical
with the two previously derived, as follows:-

$$A_1A_1A_1A_1BB \quad x \quad A_2A_2BB$$

gametes $\quad A_1A_1B \qquad A_2B$

$$A_1A_1A_2BB \ (2 \times II + 1 \times I)$$

and,

$$A_1A_1A_2A_2BB \quad x \quad A_2A_2BB$$

gametes $\quad A_1A_2B \qquad A_2B$

$$A_1A_2A_2BB \ (2 \times II + 1 \times I)$$
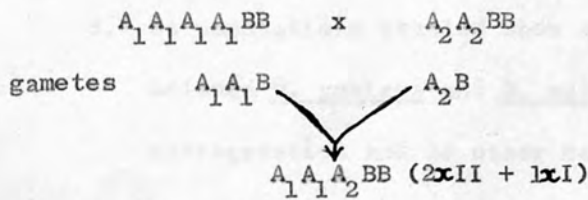
A summary of the 6 genome complements postulated in
S. palustris, S. ambigua and S. sylvatica is given:-

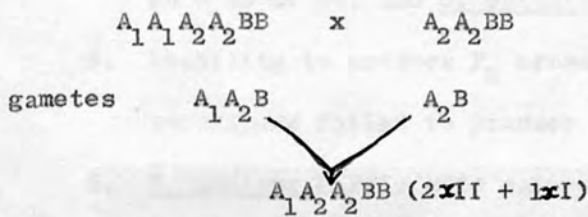| S. palustris	S. ambigua	S. sylvatica
$6 x$	$5 x$	$4 x$
$A_1A_1A_1A_1BB$	$A_1A_1A_2BB$	A_2A_2BB
$A_1A_1A_1A_2BB$	$A_1A_2A_2BB$	
$A_1A_1A_2A_2BB$		

A list of the points favouring and those against free gene-
exchange between S. ambigua and S. palustris is given.

Evidence favouring introgression

1. S. palustris intergrades morphologically with S. ambigua.

2. A small but significant amount of fertile pollen occurs
   in some population samples of S. ambigua.

3. Pollinating Bombus spp. show no discrimination between
   the three taxa.

4. S. ambigua occasionally forms mature fruit (proportion
   of viable seeds produced not known).

Evidence against introgression

1. S. palustris is a highly polymorphic species with many
   characters exhibiting a wide phenotypic range.

2. F_1 <u>S. ambigua</u> is not intermediate but closer to
   <u>S. palustris</u> than <u>S. sylvatica</u>.

3. No populations studied show a spectrum of intermediates
   between <u>S. ambigua</u> and <u>S. palustris</u> attributable to
   introgression and no other cause.

4. Lack of aneuploid chromosome numbers between <u>S. ambigua</u>,
   $2n = 83$ or 84, and <u>S. palustris</u> $2n = 102$ or 96.

5. Inability to produce F_2 crosses (however, the same
   techniques failed to produce any F_1 plants).

6. <u>S. ambigua</u> rarely sets mature fruit in nature or
   cultivation.

The distribution of <u>Stachys ambigua</u> suggests that it may be
a relic of cultivation in some parts of Britain (Perring, 1968).
It is very common in the north and western parts of Britain. In
Orkney the hybrid is a pernicious garden weed and is commoner
than either parent (<u>S. sylvatica</u> is rare and is found in
association with trees which have been introduced in the last
200 years - for example, at Kirk Burn Bu' on Hoy). The chances
of hybridization prior to the nineteenth century on Orkney must
therefore have been very small, although Smith reported that the
hybrid was very common in potato fields in 1810. Cultivation
techniques must have tended to fragment the rhizomes and spread
them more widely. Smith may have been referring to 'plantecrues'
which are areas set aside close to farm steadings for growing
household vegetables. These are equivalent to the gardens of
other householders. <u>S. ambigua</u> is very common in these areas
today and this is particularly notable in neglected gardens (see
fig. 50). Spread occurs very easily by accidental transmission
of rhizome fragments with other garden plants, gravel etc. and

Fig. 50. <u>Stachys</u> <u>ambigua</u> growing in a neglected garden at
Lyness, Hoy, Orkneys. Gardens form one of the
commonest habitats for the hybrid on Orkney.

even rubbish as seen in fig. 32. At Netherhouse Farm an
S. ambigua population was sampled (H13) that appears identical
with one (H14) found growing in soil overlaying the top of the
farm's rubbish tip. S. ambigua, almost certainly brought down
with rubbish from the farm, has become established and colonizes
new covering soil when the pit is full.

Attempts to determine how the hybrid originally became
established in Orkney gardens have been unsuccessful. It may
perhaps have originally had some herbal use and was deliberately
introduced. This must be the most likely explanation as the
majority of garden sites exist in areas where S. palustris and
S. sylvatica are not in the vicinity. The existence of S. ambigua
in gardens is not restricted to Orkney, being recorded in such
situations in Caithness, Sutherland, Mull, Skye and the Outer
Hebrides.

In these areas it possibly represents an introduction at the
time of the Norse invasions from the late 8th Century, being
brought by invaders as a 'famine' crop or medicinal herb. While S. ambigua has
not been reported to occur in the Faroes or Iceland it may be
that Norse settlers in these areas came from the northern parts
of Norway where both S. palustris and S. sylvatica are rare, and
S. ambigua is absent. S. ambigua is reported to occur widely
further south, on beaches and by lakes, in both Norway and Sweden.
Furthermore, plants from the Orkneys, the N.W. Scottish mainland
and the Western Isles, have broader leaves than those further
south in Argyll, the Lake District, Isle of Man and Wales
indicating morphological distinctness of the north and western
Scottish plants.

South of Caithness and Sutherland and the Western Isles,

the hybrid becomes rarer, though still common in Argyll, Lake
District, and the Isle of Man. In East and S.E. England it is
much rarer than the distribution map in the Critical Supplement
(1968) to the Atlas of the British Flora suggests. In England
and Wales the hybrid also often occurs in the absence of one or
both parents. In many of these situations it is unlikely that
the hybrid has been introduced by man (accidentally or desired)
and other explanations must be sought. Competition between the
hybrid and, in particular, its maternal parent may be an
important factor, but the results of competition experiments
conducted through two growing seasons, have not positively
substantiated this view. In competition, the environmental
responses of the genotypes involved are likely to have such a
strong influence on the results as to make general taxon-taxon
inferences largely meaningless.

An important consideration is the disappearance of
S. palustris from previously occupied localities by the drainage
of marshland areas and their reclamation for agricultural use.
This has become an increasingly significant factor, especially
in the last 50 years as shown by Perring (1970). Another
consideration in S. ambigua sites where one parent is missing is
the area covered by the pollinating agent. It is almost certainly
a more effective agent of gene dispersal than the seed, but no
information is yet available on the range pollinating insects
cover between Stachys plants.

The longevity of hybrid genotypes at a locality is emphasized
by the plants growing on the ayre at the Loch of Carness,
Orkney. In the Magnus Spence herbarium at Stromness are
specimens dating from 1st October 1883. In the remarks on a

specimen collected at Carness on 25th July 1933 the corolla is
noted to have a rich dark purple colour. This unusual corolla
colour was still present in all the flowering specimens in
September 1971.

A tabular comparison of the three taxa based on the
populations sampled in this study is presented below:

CHARACTER	S. palustris	S. ambigua	S. sylvatica
A. CHARACTERS OF GENERAL DIAGNOSTIC UTILITY			
1. Petiole: total leaf length ratio	0.02-0.09	0.09-0.16	0.30-0.44
2. Fruit production	Mature fruits usually produced	Mature fruits rarely produced	Mature fruits always produced
3. Level of pollen inviability	Usually $<10\%$, but $\male$ sterile plants occur	$>10\%$; generally $>50\%$	$<10\%$
4. Somatic chromosome number	(97-)102(-103)	(78-)84(-86)	$(62-)66(-68)$
5. Corolla colour	Usually pale pink	Usually bright red	Dark mauve
6. Lamina breadth: length ratio	0.16-0.40	0.28-0.52	0.60-0.85
B. CHARACTERS OF LIMITED DIAGNOSTIC UTILITY			
7. Number of flowers at the second whorl of inflorescence	(1)-6-(18)	(1)-6-(10)	(1)-6
8. Corolla length cms.	0.90-1.7	1.2-1.5	1.45-1.7
9. Corolla tube length cms.	0.63-1.0	0.74-0.96	0.93-1.1
10. Lower lip measurement A	0.49-0.97	0.51-0.74	0.50-0.78
11. Lower lip measurement B	0.61-1.4	0.66-0.95	0.71-0.93
C. OTHER FEATURES			
12. Habitat preferences in Britain	Marshland, banks of canals and rivers.	By streams and rivers, but often	Generally dry habitats in hedgerows, thickets,

	Sometimes as a weed in dry places.	in disturbed ground by roadsides etc. Very common in gardens in N.W.Scotland and Scottish islands off the N. and W. coast.	edges of woods and gardens
13. Distribution in the British Isles	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 South and East England	Very common and widely distributed. Absent or rare in islands off the N. and W. coast of Scotland.

The field identification of plants is not usually difficult as at a site the range of variation of the taxa encountered is greatly restricted. A combination of the/first/two characters will generally be sufficiently discriminating in the field. Critical specimens may be confirmed by the level of pollen inviability but the only single discriminating character so far discovered is chromosome number.

A few critical herbarium specimens exist which appear to possess a bewildering combination of S. palustris and S. ambigua characters. While these may with reason be considered back-crosses, without information of the population structure and variability, and knowledge of the range of chromosome numbers, this supposition cannot be confirmed. In particular, this refers to specimens morphologically close to S. ambigua but exhibiting a high degree of fruit set. Specimens morphologically close to S. palustris with a very low level of fruit set may represent F_1 plants, backcrosses or female-associated male sterile plants of

S. palustris. Herbarium specimens of both types cannot be
determined with any certainty.

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APPENDIX I

Habitat, site and grid references for _Stachys_ population samples.

Population Sample Number			Habitat and Site	Grid reference
P	S	H		
	2	1A;1B	Streamside (H) and by path (S). Silverburn, Isle of Man.	SC 266682
3	3	2	In marshland surrounding a stream (H) and the edge of a pond (H), by the side of a track near pond (S), on track by bridge (P). Billown Mooar, Isle of Man.	SC 263697
5;6	9	4	In marshland and by the side of R. Derwent. Grange-in-Borrowdale, Lake District.	NY 253170
7			In wet ground by road, near Bothel, Cumberland.	NY 203364
10	10	5	Roadside verge, near Inverary, Argyll.	NN 114098
8			Marshland by a stream, Kilchrenan, Argyll.	NN 023209
9	11;12	6	Margins of a ditch, Glendaruel, Argyll.	NS 033906
10	13;14	7;8;9	In wet fields, Dalmally, Argyll.	NN 167275
11			Marshland and nearby field, near Inchnadampf, Sutherland.	NC 253183
		10	By railway bridge and roadside, Crianlarich, Perth.	NN 383254
		11	In a neglected garden, White Glen, Hoy, Orkneys.	HY 243024
12	15	12	In ridge of ayre (S and H), P only a few non-flowering specimens in wet ground nearby, Loch of Carness, Orkney.	HY 465138
		13	In garden, Netherhouse Farm, Orkney.	HY 372185
		14	On rubbish tip in mire outside field, near Netherhouse Farm, Orkney.	HY 374185
	16;17		Shady bank near stream, Kirk Burn Bu', Hoy, Orkneys.	HY 235046

Population P	S	H	Habitat and Site	Grid reference
		18	Near ayre, Loch of Scockness, Rousay, Orkneys.	HY 449331
		15	Neglected garden, Rousay, Orkneys.	HY 445320
13			Streamside, Waulkmill Bay, Orkney.	HY 386062
14			In ditch at edge of field, Wideford Hill, Orkney.	HY 397117
		16	In neglected garden, Lyness, Hoy, Orkneys.	ND 303939
		17	House tip on beach edge, S. Ronaldsay, Orkneys.	ND 488932
		18	Streamside in sand dunes, Deerness, Orkney.	HY 589068
		19	In plantecrue, Scarwell, Orkney.	HY 244213
		20	On midden, Isbister, Orkney.	HY 392186
		21	By loch, Isbister, Orkney.	HY 394189
		22	Mill of Firth, Finstown, Orkney.	HY 355143
15			By R. Thames, near Wallingford.	SU 608890
16			By R. Thames, Wallingford Yacht Marina.	SU 608887
17			By R. Thames, Carmel College.	SU 606877
18			By R. Thames near Carmel College.	SU 606870
19		23	In ditch, Stonethwaite, Lake District.	NY 144258
20			In sugar beet field, Aylmerton, Norfolk.	TG 184400
	19		In woodland, Royal Holloway College grounds.	SU 999705
	20		By roadside, Botany Department, Royal Holloway College.	SU 995696
21	21	24	By roadside, Cae-glas, near Ruthin, Denbigh.	SJ 149595

APPENDIX II

Habitat, site and grid references of the genotypes used in the experiments.

Taxon and code	Habitat and site	Grid reference
S. palustris		
A12/1 A7	In marshland, Eaton marshes, Norfolk.	TG 313076
B11 B8	In marshland, Wheatfen Broad, Norfolk.	TG 199059
X1	In dry ground, South of Kidwelly, Carmarthen	SN 411058
S4 S5	In allotment plot, Greasby, Wirral, Cheshire.	SJ 264875
S. ambigua		
L1	By road, Scalloway, Shetlands.	HU 397391
R3	Roadside, Cae-glas, near Ruthin, Denbigh.	SJ 149595
Q2 Q8	By R. Derwent, Lake District.	
S. sylvatica		
K5 K1	By bridge, Lydford Station, N. Devon.	SX 504833
M4	By railway bridge, Corwen, N. Wales	SJ 089435
V2)	By Slapton ley, S. Devon.	SX 824428
V3) P3	Allotment, Greasby, Wirral, Cheshire	SJ 264875

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