

THE ALLOPOLYPLOID ORIGIN AND GENOMIC CONSTITUTION OF *POTENTILLA ANGLICA*

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Received 12.i.72

1. INTRODUCTION

Potentilla anglica Laich. (= *P. procumbens* Sibth.) is an octoploid species ($2n = 56$) which is thought to have arisen as the allopolyploid between two tetraploids, *P. erecta* (L.) Räsch. ($2n = 28$) and *P. reptans* L. ($2n = 28$). Over a century ago Grenier (1865) recognised the intermediate morphology of *P. anglica* and identified it as the hybrid between *P. erecta* and *P. reptans*. However, Wolf (1901) pointed out that it is fertile and should be given specific status. The theory of allopolyploidy was not applied to *P. anglica* until 1951, when Stomps showed that its chromosome number is equal to the sum of the chromosome numbers of *P. erecta* and *P. reptans*.

P. anglica and its putative parents are common species and occur sympatrically throughout Europe. Ecological barriers normally separate the three species but they sometimes meet and hybridise. All three possible hybrid combinations have been reported, but it is doubtful whether the tetraploid hybrid between *P. reptans* and *P. erecta* actually occurs in nature (Matfield, 1972). It has recently been synthesised experimentally (Schwendener, 1969; Matfield, 1972) but previous attempts to hybridise the tetraploid species failed as a result of genetic incompatibility barriers (Brückss, 1931; Matfield, Jones and Ellis, 1970). For this reason confirmation of the allopolyploid origin of *P. anglica* by experimental synthesis has proved difficult.

This paper describes a progeny of polyploid hybrids which were obtained from crosses between induced autopolyploid strains of *P. reptans* and *P. erecta*. The hybrids were morphologically similar to and interfertile with *P. anglica* and can therefore be regarded as "synthetic *P. anglica*". Comparisons are made between meiotic behaviour in *P. reptans*, *P. erecta* and their tetraploid hybrid, the induced autopolyploids and natural and synthetic *P. anglica*. The origin and genomic constitution of natural *P. anglica* is also discussed.

2. MATERIAL AND METHODS

P. erecta, *P. reptans* and *P. anglica* were obtained as seed or transplants from wild populations, the localities of which are listed in the appendix. They were maintained in pots sunk into the soil. Auto-octoploid ($2n = 56$) strains of *P. reptans* and *P. erecta* were induced by colchicine treatment as described previously (Matfield, Jones and Ellis, 1970). Autohexaploid *P. erecta* ($2n = 42$) arose by hybridisation between the C_0 generation octoploids and untreated tetraploids.

Meiotic preparations were made by the squash technique for pollen mother cells described by Thomas (1940). Buds were fixed for at least a week in acetic-alcohol containing ferric acetate as a pre-mordant and the anthers were squashed in strong aceto-carmine solution. The techniques for somatic chromosome counts and experimental hybridisations have been described previously (Matfield, Jones and Ellis, 1970).

3. RESULTS

P. erecta and *P. reptans* are morphologically very distinct although Wolf (1908) included both in the subseries *Tormentillae*. The intermediate nature of *P. anglica* is evident from fig. 1 and the polygraph (fig. 2) which shows the relationship between the three species in respect of six quantitative characters. The points on the graph show average values for each species determined from all the plants kept in cultivation. Characters were not recorded until the plants had been in the experimental garden for at least a year in order

TABLE 1
Meiosis in auto-8x P. reptans

Number of multivalents (IIIs or IVs)	Number of P.M.C.s at MI
0	7
1	10
2	16
3	12
4	7
5	4
6	1
7	1
8	1

59

to eliminate environmental effects as far as possible. With the exception of carpel number and flower diameter in *P. reptans*, all values are averages of at least 50 records. *P. anglica* is intermediate in all characters except those directly affected by the difference in chromosome number. Thus the stomata and pollen grains are larger and the length : breadth ratio of the leaflets is lower in *P. anglica* than in the tetraploids.

P. erecta and *P. reptans* are both highly fertile but self-incompatible. *P. anglica*, on the other hand, is self-compatible and has an unusually low fertility for a species. Pollen counts made on plants in the experimental garden and in wild populations showed a mean pollen grain stainability of only 62 per cent. with a range of 30 to 92 per cent.

Both tetraploids regularly formed 14 bivalents at metaphase I, tetrad formation was normal and the resultant pollen grains were uniform in size, giving no evidence for non-reduction. The induced autopolyploids formed a proportion of multivalents, as shown in tables 1 and 2. Auto-8x *P. reptans* can form a theoretical maximum of 14 quadrivalents, but the observed multivalent frequency was much lower than this, ranging from 0 to 8 with an average of 2.5 per cell. The majority (about 75 per cent.) of the multivalents were quadrivalents, the remainder being trivalents. Secondary



FIG. 1.—A, *P. reptans*. B, *P. anglica*. C, *P. erecta*.

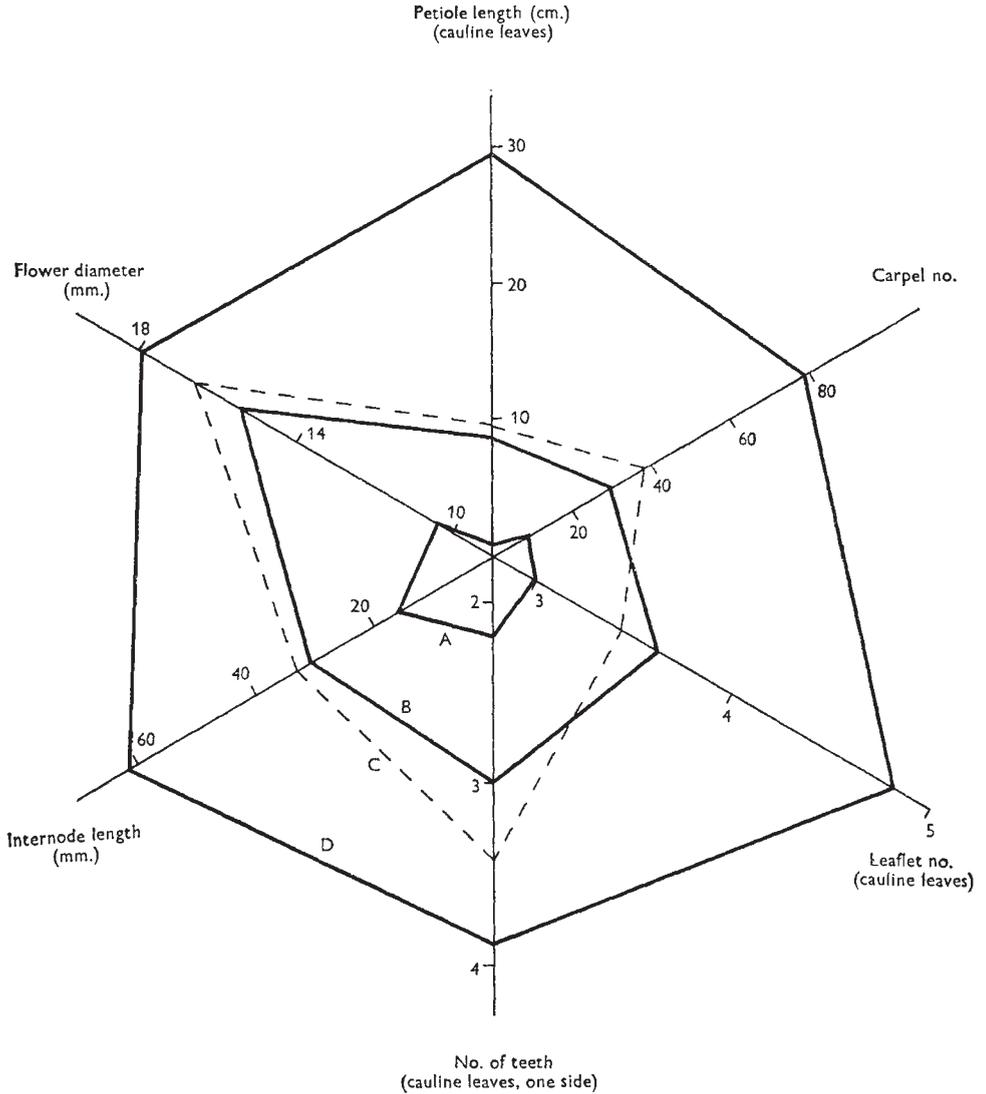


FIG. 2.—Polygraph showing the comparative morphology of A, *P. erecta*; B, natural *P. anglica*; C, “synthetic *P. anglica*”; and D, *P. reptans*.

TABLE 2
Meiosis in auto-6x P. erecta

Chromosome pairing	Number of P.M.C.s at M I
8 III+6 II+6 I	1
9 III+5 II+5 I	0
10 III+4 II+4 I	3
11 III+3 II+3 I	8
12 III+2 II+2 I	15
13 III+1 II+1 I	15
14 III	8

associations were quite commonly observed (plate I, A). The multivalent frequency in auto-6x *P. erecta* was much closer to the theoretical maximum of 14 trivalents per cell, with a range of 8 to 14 trivalents and a mean of 12.26 per cell. Plate I, B shows a cell with 12 trivalents, two bivalents and two univalents. As a consequence of the high trivalent frequency chromosome segregation was more irregular in auto-6x *P. erecta* than in auto-8x *P. reptans*.

Meiosis in *P. anglica* was fairly regular. The majority of cells formed 28 bivalents, but a small proportion contained a single quadrivalent (table 3). Failure of pairing with the formation of two, or in one case four, univalents was seen in 4 per cent. of cells. Plate I, C shows a cell at diakinesis with one chain quadrivalent and a secondary association between two bivalents. It is of interest that Schwendener (1969) observed a much greater degree of meiotic irregularity in *P. anglica* from a British source. Of a total of 59 cells examined, only 22 (37 per cent.) contained 28 bivalents. The remainder contained one to five multivalents and cells with two, four, six and even ten

TABLE 3
Meiosis in P. anglica

Chromosome pairing	Number of P.M.C.s		
	at diakinesis	at metaphase I	Totals
26 II+4 I	1	0	1
27 II+2 I	5	0	5
28 II	95	28	123
26 II+1 IV	12	0	12
	113	28	141

univalents were observed. The reason for this difference in meiotic behaviour between members of the same species is unknown.

Previous attempts to hybridise the tetraploid species failed because of incompatibility barriers (Matfield, Jones and Ellis, 1970). *P. reptans* pollen did not stimulate seed set in *P. erecta*, and although the reciprocal cross produced many seeds germination was very rare: a total of more than 3000 seeds yielded only one inviable seedling. Subsequently four more pollinations were made using a different clone of *P. reptans* as female parent. Two hundred and twenty-three seeds were obtained and two germinated, but only one seedling survived. This developed into a weak plant, smaller than either parent but otherwise intermediate in morphology.* It was highly, but not completely, sterile. An average of 12 per cent. of the pollen grains stained with aceto-carmine and only one seed was obtained from eight open pollinated flowers. The chromosome number was the same as that of the parental species, $2n = 28$. Meiosis was observed in 103 pollen mother cells (table 4) and more than half of these had regular bivalent pairing. The remainder contained two, four or six univalents, and one cell included a quadrivalent. The mean bivalent number was 13.5. Plate I, D shows a cell with 13 bivalents and two univalents.

Schwendener (1969) obtained three tetraploid hybrids from crosses between Swiss specimens of *P. reptans* and *P. erecta* and examined metaphase

* A full description of this hybrid is published elsewhere (Matfield, 1972).

I in a total of 202 pollen mother cells. The bivalent number ranged from 3 to 14 and the mean bivalent numbers for the three plants were 13.1, 12.6 and 10.9. He also observed 11 cells with a single quadrivalent and one cell with two quadrivalents. The meiotic behaviour of the British and Swiss hybrids was therefore very similar.

In an attempt to synthesise the allopolyploid, reciprocal crosses were made between induced auto-octoploid strains of *P. reptans* and *P. erecta*,

TABLE 4
Meiosis in the 4x P. reptans × 4x P. erecta F₁ hybrid

Chromosome pairing	Number of P.M.C.s at diakinesis or MI
11 II+6 I	3
12 II+4 I	6
13 II+2 I	34
14 II	59
12 II+1 IV	1

103

but no viable seed was obtained (Matfield, 1968). However, a progeny was raised from crosses using auto-8x *P. reptans* as female parent and auto-6x *P. erecta* as pollen parent. Thirty-six seeds were obtained and 24 germinated, but 13 died at the seedling stage or soon after. The remaining 11 plants were vigorous and their chromosome numbers ranged from $2n = 43$ to $2n = 56$ (table 5). Plate I, F shows a somatic metaphase from the individual with $2n = 53$. The single octoploid ($2n = 56$) showed no evidence of hybridity and may have resulted from accidental self-pollination or from the apomictic

TABLE 5
Chromosome numbers of the progeny from crosses between auto-8x P. reptans and auto-6x P. erecta

Number of plants	Chromosome number						Total
	43	52	53	54	55	56	
	1	2	1	3	3	1	11

development of an unreduced ovule. The other 10 plants were morphologically intermediate between the parents which confirmed their hybrid origin. The individual with $2n = 43$ differed from the others in morphology and fertility and resembled the natural hybrid *P. × mixta* (Matfield, Jones and Ellis, 1970). Its pollen grain stainability was less than 1 per cent. and no seed was set. In contrast, the nine hypo-octoploids ($2n = 52-55$) were all fertile. Their average pollen stainability ranged from 55 to 83 per cent. and the average number of carpels developing into seeds from 39 to 80 per cent. (table 6). There was no correlation between chromosome number and fertility. Like *P. anglica*, they were self-compatible. The nine plants were quite uniform in morphology and were strikingly similar to natural *P. anglica*. This can be seen from the polygraph (fig. 2) which shows the values for several quantitative characters averaged over all nine plants. Fig. 3 shows a flowering stem from the individual with $2n = 53$ alongside one from natural *P. anglica*.



FIG. 3.—A, "Synthetic *P. anglica*" with $2n = 53$. B, Natural *P. anglica*.

TABLE 6
*Fertility of the hypo-octoploid progeny from the cross between auto-8x P. reptans
 and auto-6x P. erecta*

Plant no.	2n	Average percentage of stainable pollen	Average seed number per flower	Average percentage of carpels forming seeds
1	52	71	11	39
2	52	83	39	80
3	53	57	17	56
4	54	66	34	70
5	54	60	25	70
6	54	72	24	68
7	55	74	21	53
8	55	72	20	67
9	55	55	20	44

Meiosis was examined in two plants, with $2n = 53$ and $2n = 54$. Since both were aneuploids, with less than the full octoploid chromosome complement, their pollen mother cells were expected to contain three and two univalents respectively. Table 7 shows that, apart from these univalents, pairing was very regular and very similar to that in *P. anglica*. A few cells contained a trivalent or a quadrivalent and one cell had two univalents in

TABLE 7
Meiosis in hybrids between auto-8x P. reptans and auto-6x P. erecta

	Chromosome pairing	Number of P.M.C.s
$2n = 53$	24 II+5 I	1
	25 II+3 I	45
	23 II+1 IV+3 I	3
$2n = 54$	26 II+2 I	35
	24 II+1 IV+2 I	4
	25 II+1 III+1 I	3
		91

addition to the expected three. Plate I, E shows diakinesis in the plant with $2n = 54$, with 26 bivalents and two univalents.

The nine hypo-octoploids were so similar to *P. anglica* in morphology, fertility and meiotic behaviour that they could be regarded as "synthetic *P. anglica*". One of them, with $2n = 53$, was crossed reciprocally with the natural species. The pollinations were all successful (table 8) and about

TABLE 8
Crosses between "synthetic P. anglica" with $2n = 53$ and natural P. anglica

♀	♂	Number of flowers pollinated	Number of successful pollina- tions	Average seed number per flower	Total seed number	Percentage germination
"Synthetic <i>P. anglica</i> "	Natural <i>P. anglica</i>	4	4	32.2	129	43
Natural <i>P. anglica</i>	"Synthetic <i>P. anglica</i> "	4	4	9.7	39	69

half of the seeds had germinated at the time of writing. The progeny were vigorous and fairly uniform in appearance although their chromosome numbers varied (table 9). When the aneuploid "synthetic *P. anglica*" was

TABLE 9
Chromosome numbers of hybrids between "synthetic P. anglica" with
2n = 53 and natural P. anglica

	Chromosome number					Total
	52	53	54	55	56	
Number of plants with "synthetic <i>P. anglica</i> " as ♀ parent	1	6	8	3	0	18
Number of plants with natural <i>P. anglica</i> as ♀ parent	0	1	2	3	3	9

used as seed parent, the chromosome numbers of the offspring ranged from $2n = 52$ to $2n = 55$, but when the same plant acted as pollen parent the range was $2n = 53$ to $2n = 56$. The functional pollen grains must therefore have contained more chromosomes than the ovules, presumably as a result of stronger selection pressures on the independent male gametophyte.

The hybrids between "synthetic *P. anglica*" and the natural species were as fertile as their parents. Meiosis was examined in 27 cells from two plants with $2n = 56$. Quadrivalents and univalents were occasionally present but otherwise there was regular bivalent pairing (table 10). Seeds

TABLE 10
Meiosis in octoploid hybrids between natural and synthetic P. anglica

Chromosome pairing	Number of P.M.C.s
27 II+2 I	1
28 II	24
26 II+1 IV	2
	27

collected after open pollination gave a high frequency of germination and all the seedlings were vigorous.

4. DISCUSSION

The hybridisations reported in this paper have shown that fertile hybrids similar in morphology to the octoploid species *P. anglica* can be synthesised from the tetraploid species *P. erecta* and *P. reptans*. The chromosome numbers of these hybrids ranged from $2n = 52$ to $2n = 55$, but a euploid, with $2n = 56$, was not obtained in the small progeny raised. The experimental

Schwendener (1969) obtained two hexaploids from crosses between $4x$ *P. reptans* and $4x$ *P. erecta* which he considered had arisen by spontaneous non-reduction in *P. reptans*. Morphologically similar hexaploid hybrids known as *P. × mixta* also occur naturally. All these $6x$ hybrids are vigorous, spread by runners and are highly but not completely sterile. Schwendener (1969) pollinated one of the natural hexaploids with *P. erecta* and obtained three offspring one of which was octoploid. This plant, which resulted from non-reduction in the hybrid, was very similar to *P. anglica* in morphology, fertility and meiotic pairing. This demonstrates the feasibility of the evolutionary pathway shown in fig. 4.

However, it cannot be assumed that all the natural hybrids assigned to *P. × mixta* have originated by hybridisation between *P. reptans* and *P. erecta*. This is because genomically equivalent hybrids could arise subsequent to the origin of *P. anglica* by backcrossing between *P. anglica* and *P. reptans*.

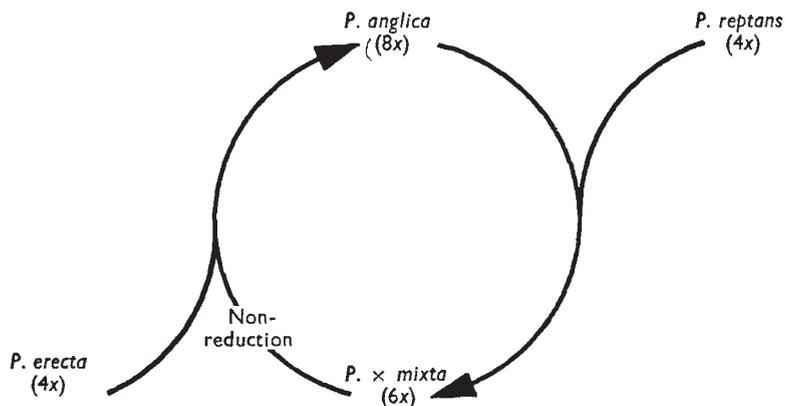


FIG. 5.—A cycle showing the sequence in which the genomes of *P. reptans* and *P. erecta* may be incorporated into *P. anglica*.

This hybrid has been synthesised by Valentine (personal communication) and Matfield (unpublished data) although many previous attempts to hybridise *P. anglica* and *P. reptans* failed (Matfield, Jones and Ellis, 1970). A novel situation may therefore exist in the European *Tormentillae* in that the same hybrid, *P. × mixta*, may arise from different parental species (*P. reptans* × *P. anglica* or *P. reptans* × *P. erecta*). In addition, the allopolyploid may not be genetically isolated from the progenital species. The experimental results have demonstrated the feasibility of the cyclical process shown in fig. 5 which would result in *P. anglica* repeatedly incorporating fresh genomes from the tetraploids. This would account for the observed lack of differentiation between the genomes of *P. erecta* and *P. reptans* and the corresponding genomes in the allo-octoploid.

The high degree of meiotic pairing in the tetraploid hybrid between *P. reptans* and *P. erecta* indicates that the genomes of the two species are homoeologous, provided that pairing is allosyndetic. This assumption is justified by earlier observations on meiotic pairing in hexaploid hybrids between *P. reptans* and *P. anglica* (Matfield, unpublished data) and between *P. anglica* and *P. erecta* (Matfield, 1968) which showed that little or no autosyndesis occurs in either the *P. reptans* or the *P. erecta* genomes. The

constituent genomes of *P. anglica* are therefore homoeologous, but at meiosis bivalents are predominantly formed and quadrivalents occur with a low but variable frequency. *P. anglica* therefore behaves as a segmental allopolyploid as defined by Stebbins (1947) and exhibits preferential pairing. *Primula kewensis* is a well-known example of this type of polyploid. The F_1 hybrid, *Primula verticillata* \times *P. floribunda*, has a more irregular meiosis than the corresponding *Potentilla* hybrid, with 2-4 unpaired chromosomes in each cell. However, the allopolyploid *Primula kewensis* has a higher multivalent frequency than *P. anglica*, forming 1-3 quadrivalents per cell together with an occasional trivalent (Upcott, 1940). The homology between the *P. reptans* and *P. erecta* genomes therefore appears greater than that between the two *Primulas*, as shown by bivalent formation in the F_1 hybrid. Despite this, multivalent formation is less frequent in *Potentilla anglica* than in *Primula kewensis*. The reason for the high incidence of preferential pairing in *P. anglica* is not known. The low quadrivalent frequency in the raw auto-octoploid *P. reptans* suggests that multivalent formation may be limited by factors such as small chromosome size or low chiasma frequency. However, it is difficult to reconcile this explanation with the very high trivalent frequency in auto-6 \times *P. erecta*. Furthermore, Morrison and Rajhathy (1960) found no correlation between quadrivalent frequency and chromosome size in a survey of the meiotic behaviour of a number of autopolyploids. Thus *P. anglica* provides an example of a segmental allopolyploid with preferential pairing, though the mechanism by which this is achieved is unknown.

5. SUMMARY

1. Crosses between autopolyploid strains of *P. reptans* and *P. erecta* have produced fertile hybrids similar in morphology and meiotic behaviour to *P. anglica* and interfertile with it. The allopolyploid origin of *P. anglica* has thus been confirmed.

2. Meiotic observations have shown that *P. anglica* is a segmental allopolyploid with a low incidence of multivalent formation.

3. A two stage evolutionary origin is proposed for *P. anglica* in which the hexaploid hybrid *P. \times mixta* is formed as an intermediate step.

4. It is suggested that the allo-octoploid, *P. anglica* may not be genetically isolated from its tetraploid progenitors.

6. REFERENCES

- BRÜKSS, Z. 1931. Untersuchungen über die hybridogene Herkunft der *Potentilla procumbens* Sibth. *Acta Horti Bot. Univ. Latv.*, 5, 194-198.
- GRENIER, M. C. 1865. *Flore de la Chaîne Jurassique*. Part 1. Paris.
- MATFIELD, B. 1968. Ph.D. Thesis. University of London.
- MATFIELD, B. 1972. *Potentilla reptans* L.—Identification of its hybrids. *Watsonia* (in the press).
- MATFIELD, B., JONES, J. K., AND ELLIS, J. R. 1970. Natural and experimental hybridization in *Potentilla*. *New Phytol.*, 69, 171-186.
- MORRISON, J. W., AND RAJHATHY, T. 1960. Frequency of quadrivalents in autotetraploid plants. *Nature*, 187, 528-530.
- MUNTZING, A. 1930. Über Chromosomenvermehrung in *Galeopsis*—Kreuzungen und ihre Phylogenetische Bedeutung. *Hereditas*, 14, 153-172.
- NEWTON, W. C. F., AND PELLEW, C. 1929. *Primula kewensis* and its derivatives. *J. Genet.*, 20, 405-462.

Plate I

A, Metaphase 1 in auto-8x *P. reptans* showing one quadrivalent (arrowed) and several secondary associations.

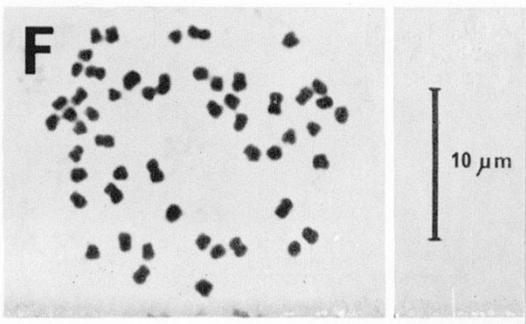
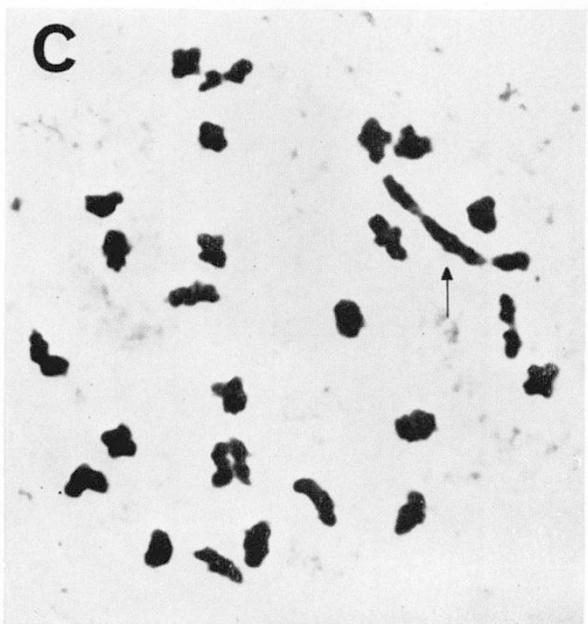
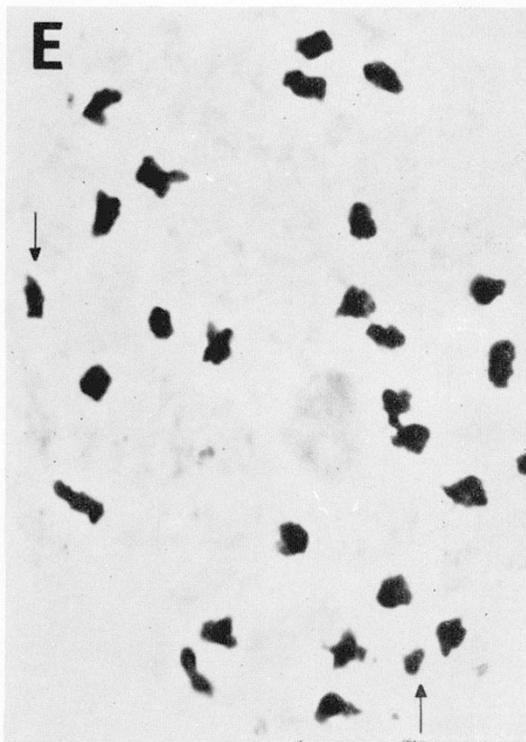
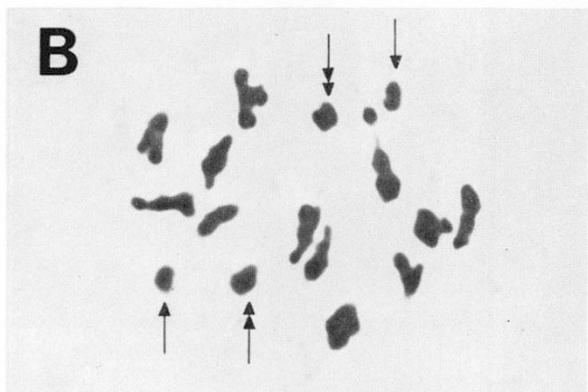
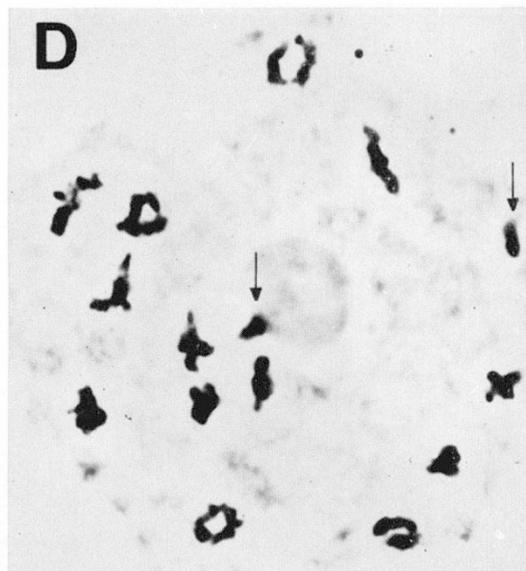
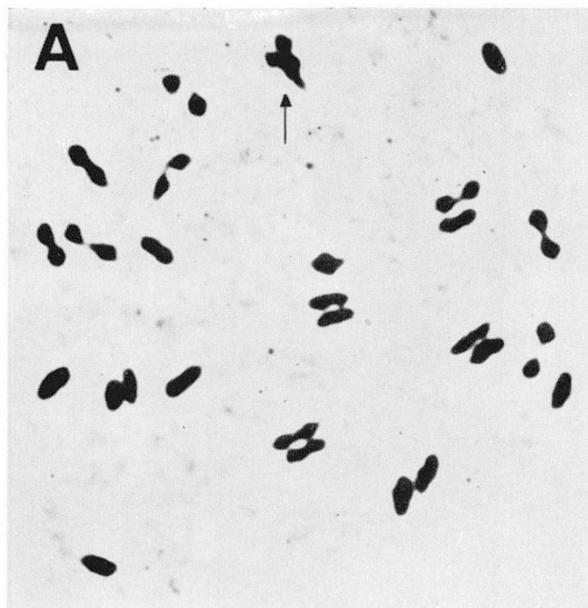
B, Metaphase 1 in auto-6x *P. erecta* with 12 trivalents, 2 bivalents (double arrows) and 2 univalents (single arrows).

C, Diakinesis in natural *P. anglica* with one quadrivalent (arrowed).

D, Diakinesis in the 4x hybrid between *P. reptans* and *P. erecta*, showing 13 bivalents and 2 univalents (arrowed).

E, Diakinesis in "synthetic *P. anglica*" with $2n = 54$ showing 26 bivalents and 2 univalents (arrowed).

F, Somatic metaphase in "synthetic *P. anglica*" with $2n = 53$.



- SCHWENDENER, J. 1969. Experimente zur Evolution von *Potentilla procumbens* Sibth. *Ber. der Schweiz. Bot. Ges.*, 79, 49-92.
- STEBBINS, G. L. 1947. Types of polyploids: their classification and significance. *Advances in Genetics*, 1, 403-429.
- STEBBINS, G. L. 1950. *Variation and Evolution in Plants*. New York.
- STOMPS, T. J. 1951. *Potentilla anglica* Laich. ein neues Beispiel eines allopolyploiden Bastards. Abstract in *Proc. Bot. Soc. Brit. Isles*, 1, 197.
- THOMAS, P. T. 1940. The aceto-carminic method for fruit material. *Stain Tech.*, 15, 167-172.
- UPCOTT, M. 1940. The nature of tetraploidy in *Primula kewensis*. *J. Genet.*, 39, 79-100.
- WOLF, T. 1901. *Potentillen-Studien*. I. Dresden.
- WOLF, T. 1908. *Monographie der Gattung Potentilla*. Stuttgart.

7. APPENDIX

Sources of material:

Grid ref.

<i>P. erecta</i>	{	Northwood, Middlesex	TQ 097898
		Thursley, Surrey	SU 899416
		Langdale, Westmorland	NY 284056
		Aberystwyth, Cardiganshire	SN 629837
		Lisdoonvarna, Co. Clare	Ireland 1196
<i>P. reptans</i>	{	Pinner, Middlesex	TQ 127885
		Chesil Bank, Dorset	SY 495885
		Lea Valley, Essex	Unknown
		Newcastle upon Tyne, Northumberland	Unknown
		Monks Wood, Huntingdonshire	TL 188805
		County Durham	Unknown
<i>P. anglica</i>	{	St John's Wood, London	TQ 272828
		Arley, Cheshire	SJ 673810
		Aberystwyth, Cardiganshire	SN 629837
		Cumberland	Unknown