Acta Bot. Neerl. 32(1/2), February 1983, p. 97–107.

ON THE INTERRELATIONSHIPS OF CERTAIN SPECIES OF PETUNIA II. EXPERIMENTAL DATA: CROSSES BETWEEN DIFFERENT TAXA

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SUMMARY

In order to investigate taxonomic conclusions based upon herbarium studies, 2n = 14 taxa of *Petunia* have been crossed. Characters identifying hybrids of *P. axillaris* ssp. *axillaris* with *P. axillaris* ssp. *parodii*, as well as of *P. axillaris* (s.l.) with *P. integrifolia* have been established. Length measures show quantitative inheritance, either in subspecies crosses in *P. axillaris* s.l. or in *P. integrifolia* s.l. In the latter, the deflexed pedicel inherits as a dominant character with modifiers. It is argued that both species will have inherited several alleles from a common ancestor.

1. INTRODUCTION

Based on a herbarium study I have shown that for the two ancestral species of *P. hybrida* (Hook.) Vilmorin the names *P. integrifolia* (Hook.) Schinz & Thellung and *P. axillaris* (Lam.) B.S.P. are available (WIJSMAN 1982). In the white-flowering *P. axillaris* two allopatric taxa, ssp. *axillaris* and ssp. *parodii*, are united, in herbarium material not differing except by a non-overlapping difference in the length of the flower tube. The two subspecies are fairly constant in morphology over their large range. However, around the La Plata region where they meet, localities show geographic overlap of the tube length character, which may point to the presence of a hybrid swarm. To determine whether hybridization may actually occur in nature, representatives of both subspecies were crossed to see whether characters typical for hybrids could be found.

In the same paper, the purple-flowered *P. integrifolia* is defined as comprising three population groups (subspecies), viz., spp. *integrifolia*, ssp. *inflata*, and ssp. *occidentalis*. There is geographic contact between the first two and they seem to be the extremes of a cline. In herbarium material this cline could be demonstrated for the main differential character, the position of the pedicel after pollination. The present paper reports on some crosses in which tube length or pedicel condition, respectively, are inspected, as well as on further morphological, electrophoretic, and ecological data based on living material of the taxa mentioned.

Both wild species are known to be diploid and to possess 14 chromosomes (STOUT 1952; own unpublished observations).

2. MATERIALS AND METHODS

2.1 Material

For crosses, inbred lines of the collection of the Institute of Genetics, Amsterdam, were used. Representatives of the different taxa were the lines:

P. axillaris s.s.:	S1 (1954, from the Royal Botanic Gardens, Kew).
	S2 (collected in the wild in Uruquay, inbred since 1958).
P. *axillaris* ssp. *parodii:*	S7 (from Dr. K. C. Sink, Michigan, 1977).
P. *integrifolia* s.s.:	S12 (collected in the wild, Porto Alegre, Rio Grande do Sul, Brazil, by courtesy of Sr. K. Hagelund).
	S13 (collected in the wild, Dom Pedrito, Rio Grande do Sul, Brazil, by courtesy of Dr T. M. Pedersen).
P. *integrifolia* ssp. *inflata:*	S6 (from Botanic Gardens, Stockholm).
	S9 (from Station d'Amélioration des Plantes, Dijon, may be identical to S10).
	S10 (from Dr K. C. Sink, Michigan, under the name of P. violacea).
	S14 (from Mburucuya, Corrientes, Argentina, by courtesy of Dr T. M. Pedersen).

The seed samples from Porto Alegre (near the coast) and Dom Pedrito (300 km more inland) were very limited (the latter derive from a voucher herbarium specimen). From Mburucuya (450 km inland from Dom Pedrito), Dr Pedersen provided us with a considerable number of seeds, part of which, however, did not germinate.

S1 and S7 have an erect aspect, S2 is more rozette-like, with curved up-going stems spreading around a main axis.

All *P. integrifolia* lines give few seeds and are best maintained by sib-mating. S12 has a fair fertility; the narrow stems lie flat, or may hang down from a wall-side as a curtain of big reddish-purple flowers.

Tester lines of *P. hybrida* were from the stock collection of the Institute of Genetics.

2.2 Methods

Measurement of flower length (in *P. axillaris*), of total length of the flower (in *P. integrifolia*), and of diameter of the flower (in both species) were taken as indicated in *fig. 1 a, b, c.* For measuring the diameter in *P. integrifolia (fig. 1c)* the flowers were lightly spread.

Colours of hybrids were described according to the Horticultural Colour Chart (HCC) of the British Colour Council. The flower colour genes mentioned are: *An2, Fl, Rt,* and *Hf1* (WIERING 1974). In short, these stand for the following:

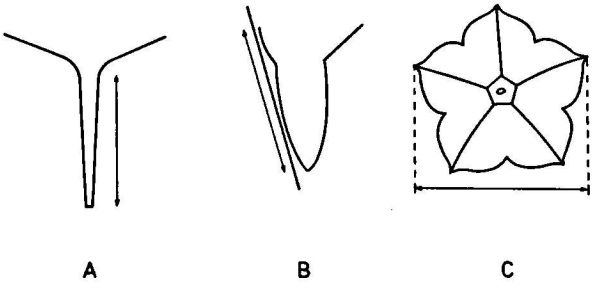

Fig. 1. A. Tube length in *P. axillaris* s.l.; B. total flower length in *P. integrifolia* s.l.; C. diameter of the flower.

An2 is a basis factor for colour (*an2an2* is albino); *Fl* stimulates flavonol synthesis (*flfl*: poor in flavonols); *Rt* is a glycosidation gene with, in the presence of other factors, a corresponding change in the aglycon (*rtrt*: delphinidin 3-glucoside instead of malvidin 3-rutino-5-glucoside); *Hf1* is a hydroxylation gene (changing, in the flower limb, peonidin into malvidin). The allele *hf1–1* (or *Hf1'*) is active in the flower tube, but the effect in the flower limb is much weaker; the dominance relation is *Hf1* > *hf1–1* > *hf1*. *An4* and *Po* are genes involved in development of the blue pollen colour: genotype *an4an4popo* gives yellow pollen (WIERING et al. 1979). Electrophoresis was carried out as described in VAN DEN BERG & WIJSMAN (1981). Prx genes are involved in peroxidase isoenzymes; gpi in glucose phosphate isomerase (WIJSMAN and VAN DEN BERG 1982).

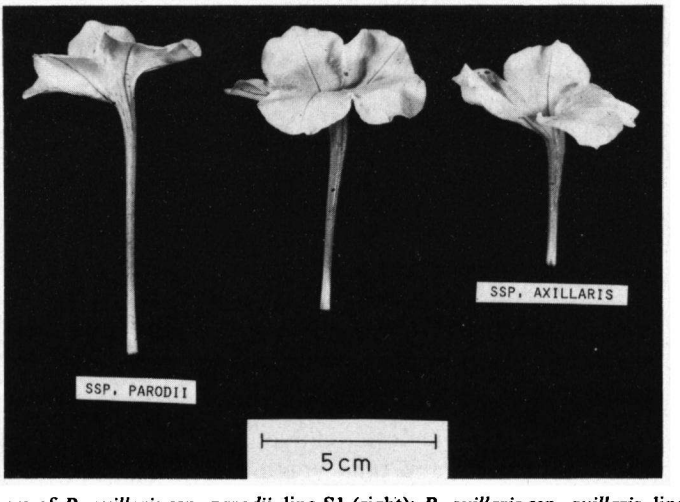

Fig. 2. Flowers of *P. axillaris* ssp. *parodii*, line S1 (right); *P. axillaris* ssp. *axillaris*, line S7 (left); and the F1 hybrid S1 × S7 in the middle to show the intermediate tube length.

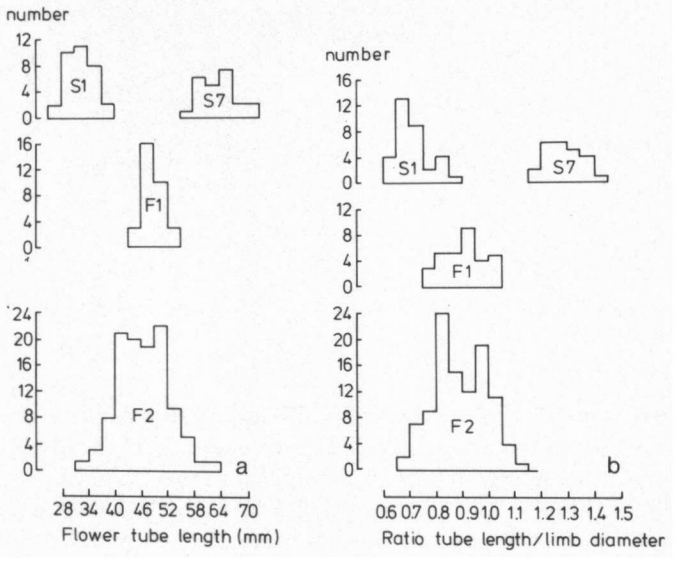

Fig. 3. Flower tube length (a) and the ratio of tube length and limb diameter (b) in the F2 of S1 (ssp. *axillaris*) × S7 (ssp. *parodii*).

3. RESULTS

3.1. Inter-subspecific crosses in Petunia axillaris

The inbred lines S1 (ssp. *axillaris* from Kew) and S7 (ssp. *parodii* from Sink) were crossed. S1 was preferred to S2 (ssp. *axillaris* from Uruquay) because S1 is carrying the electrophoretic allele for one of the peroxidases, prxB1. Compared to all inbred lines of *P. hybrida* and to line S2, S1 has a translocation neighbouring to prxB (VAN DEN BERG & WIJSMAN 1982; VAN DEN BERG, DE JONG, MAIZONNIER & WIJSMAN, in prep.) S7 has also prxB1 and, naively, it was assumed that it might carry the same chromosome complement as S1, potentially giving rise to a biassed segregation in the F2. As it is, from studies of the meiosis in hybrids, it can be concluded that S7 has, instead, the standard complement (de Jong, pers. comm.).

The F1 S1 × S7 has intermediate flower tube length *(fig. 2)*.

In the F2 the segregation for tube length *(fig. 3)* follows the same pattern as the classical case in short-tubed × long-tubed Tobacco (EAST 1916). The range of variation resembles an unbiassed normal distribution, indicating that, on the one hand, many polymeric characters are involved, and, on the other, that only an insignificant part of these can be located on the translocated chromosome segment.

Because the diameters of the flower limb of ssp. *axillaris* and ssp. *parodii* do not differ significantly, the ratio tube length/limb diameter can be used as a diagnostic character separating the parent species and identifying hybrids. In our samples in ssp. *axillaris* the ratio varied between 0.65 and 0.80, while in

Fig. 4. Position of the pedicel after pollination (or wilting) of the flower. Left: inflexed, P. *integrifolia* ssp. inflata (line S10), as in all lines of *P. hybrida*. Right: completely deflexed pedicels in *P. integrifolia* ssp. *integrifolia* (Porto Alegre population).

ssp. *parodii* between 1.18 and 1.44. In the hybrids the ratio overlaps with the *axillaris* one; but a ratio between 0.85 and 1.15 was only found in hybrids.

Flower colour markers as defined by WIERING (1974) and WIERING et al. (1979) have been studied in the inbred lines used by crossing these to different *P. hybrida* tester lines. Of particular interest is the genotype for the gene *Fl*. When a dominant allele *Fl* is present, much flavonol (quercetine) is found; but when the gene is recessive, flavonol is found only in small quantity. The presence of the strongly fluorescent flavonols may be important for insects visiting the flowers in the evening; the flower seems to be of the moth-visited type (cf. STOUT 1952). Though in our lines of *P. axillaris* s.s. *Fl* has been found, line S7 (ssp. *parodii*) is of genotype *flfl*. Granting that the samples are limited, one is tempted, in view of the postulated pollination by moths, to correlate the presence of flavonols with odour. The odour of S7 is sweet; in *P. axillaris* s.s. scent is virtually absent. This in itself might indicate that different pollinating insect species are attracted with reproductive isolation as a result. Odour does not inherit as a simple mendelian trait; hybrids of S7 with several non-odoriferous *Petunia* lines

were odoriferous, but in the F2 studied no more than 25% could be classified into that category.

No other consistent differences between ssp. *axillaris* and ssp. *parodii* could be found. It has already been reported that our line S7, in contrast to the *parodii* material described by Steere (original description) has only two longer stamina, which has been interpreted as an indication for hybridization in nature (WIJSMAN 1982).

3.2. Inter-subspecific crosses in Petunia integrifolia

The deflexed pedicel after pollination or wilting has been described as the main character separating ssp. *integrifolia* from ssp. *inflata* where, as in *P. axillaris,* the pedicel stays erect (inflexed); see *fig. 4.* Moreover, the diameter of the flower limb in ssp. *integrifolia* is greater than in ssp. *inflata* (WIJSMAN 1982).

In an F1 of *P. integrifolia* (S12) × *P. axillaris* (S2), the pedicel position is intermediate: it stands out at an angle of 90°. The same has been reported by STOUT (1952). Even so, a thick "knee" is formed, indicating that the partial deflexion is the result of active growth. However, in crosses between different isolates of *P. integrifolia* s.s. (plants of the same provenance as lines S12 and S13, i.e., the Brazilian province of Rio Grande dol Sul) and ssp. *inflata* (from the same populations as S14, i.e., the Argentinian province of Corrientes) consistently the pedicel is deflexed in the F1.

It was next to impossible to study a normal F2. F1 plants can be obtained from certain capsules only, and even then normally in low numbers. Selfing these in all cases gave rise to few seeds and of these only very few plants germinated. Crossing different F1 plants gave not much better results. Finally, 49 plants could be pollinated to study their pedicel morphology.

The distribution of fully inflexed and fully deflexed over form classes *(fig. 5b)* suggests the involvement of modifying genes (MATHER 1943a) because the parental, completely deflexed type, barely comes back. On the other hand some nearly inflexed plants, though few in number, have been found. The latter class may have formed the basis for the present uniformly inflexed *P. hybrida* sorts. Therefore, my interpretation is that here we see a biassed mendelian segregation for a major gene; the over-presented allele is the dominant deflexus allele. Biassed segregations are rule rather than exception in *Petunia* and can in general be ascribed to certation (BIANCHI 1961; own unpublished observations).

From the same plants the total length of the flower has been measured. The flowers of both subspecies sampled are isomorphic: the ratio of greatest length/diameter is 1.0. The total length shows a similar variational distribution as tube length in *P. axillaris*, and polygenic inheritance is indicated *(fig. 5a)*. As in the herbarium study, ssp. *integrifolia* has larger flowers than ssp. *inflata*; of course the absolute value obtained under greenhouse conditions should not be compared directly with herbarium data nor with field measurements.

The few plants germinating from the few seed samples collected in the wild (or from non-fumigated herbarium specimens) do not allow proper population genetic treatment. Even so, the presence of several peroxidase electrophoretic

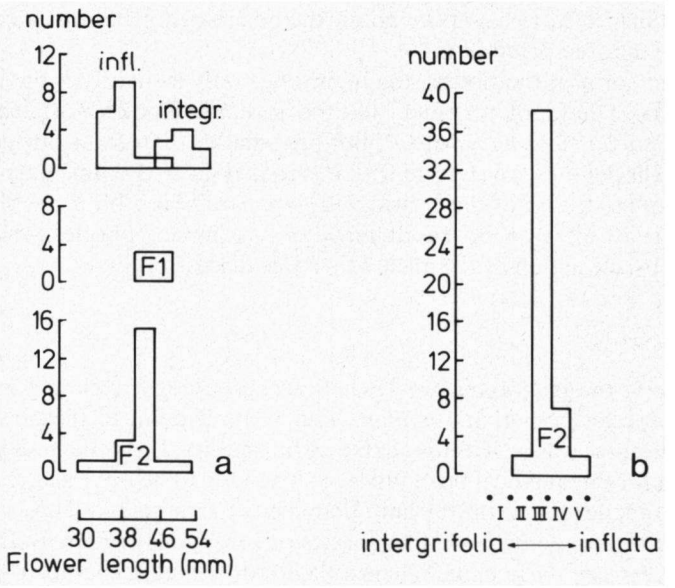

Fig. 5. a. Flower diameter in *P. integrifolia* ssp. *integrifolia* × *P. integrifolia* ssp. *inflata*: parents, F1, F2. b. Position of the pedicel, classified as I (deflexed like ssp. *integrifolia*), V (inflexed like ssp. *inflata*), III (intermediate, more or less at right angle), and II (intermediate between I and III) and IV (intermediate between III and V).

Table 1. Genotypes with respect to the peroxidase isozyme genes prxA and prxB of plants grown from seed collected in the wild for the two subspecies of *P. integrifolia*.

	prxA								prxB			
	A5A5	A4A5	A2A5	A2A4	A4A4	A1A2	A2A2	A1A4	B3B3	B2B3	B2B2	B2B4
ssp. *integrifolia*	3	1	2	1					4	3		
ssp. *inflata*			4	5	2	4	24	1	3	3	31	3

mobility alleles was demonstrated (VAN DEN BERG & WIJSMAN 1981; see *table 1*). It can be deduced that the same four alleles of prxA occur in both populations, though their frequency is likely to differ; the allele prxB4 is only found in ssp. *inflata* and unknown in *P. hybrida* lines (WIJSMAN 1983).

Both subspecies are grassland taxa. The population of Dom Pedrito originated from "dry rough grassland" (Pedersen, personal communication).

3.3. Crosses between P. integrifolia and P. axillaris

It has already been mentioned that *P. axillaris* seems to be rich in modifiers counteracting the deflexus character, so that the F1 *P. integrifolia* × *P. axillaris* has an intermediate pedicel position (class III in *fig. 5b*). Too few F2 plants

have been inspected to say more about the postulated genes involved. Several had nearly inflexed pedicels.

As to the form of the flower, the hybrid has only a slightly wider tube than *P. axillaris* s.s.; they look very much like the flowers in Hooker's original description of *"Petunia violacea hybrida"*, but are smaller than most normal garden petunias. The colour of the hybrid with *P. axillaris* s.s. is "Petunia purple" (HCC 632); by contrast, that of the hybrid with ssp. *parodii* is a bit more bluish than magenta (HCC 629 or 630, "rhodamine" or "cyclamen" purple), which can be explained by the action of the allele *hf1–1* (see discussion).

4. DISCUSSION

In *P. axillaris*, the geographic overlap in flower tube length around Buenos Aires of the subspecies *parodii* and *axillaris* may in itself point to the presence of a hybridization zone as well as to overlap of full species. From the present report, a criterion for unequivocal recognition of hybrids can be obtained, viz., a ratio of flower tube length to flower limb diameter of around 1.0. Thus, it must be possible to test in the field whether species or subspecies are involved.

As to *P. integrifolia*, a cline was postulated for the main character separating ssp. *inflata* from ssp. *integrifolia*, typical *P. integrifolia* having the pedicel after flowering deflexed (like most other species of *Petunia*), while typical *P. inflata* has them inflexed (like *P. axillaris*) (WIJSMAN 1982). In crossing extremes of the cline, we find that the expression of the difference is influenced by many modifying minor genes; a similar polygenic difference is found in flower morphology. This means that the subspecies are more than the extremes of a series of simple mendelian polymorphism.

As to crossability, in the past (MATHER 1943b, STOUT 1952, SINK 1981) incompatibility barriers have been stressed and these sometimes have been equalled to the reproductive barriers between species. As far as they limit intraspecific reproduction just as well, they rather can be envisaged as side products of pollen tube growth factor action, promoting cross-fertilisation in general. Therefore, the fact that ssp. *inflata* and ssp. *integrifolia* do not readily intercross definitely does not indicate the presence of a reproductive barrier as between species; rather, any cross within the species has a limited chance of success.

The number of plants involved in the F2 analysis of *P. integrifolia* is regrettably small, but the only result of the seeds sown, obtained from about 15 capsules. As to electrophoretic analysis I have pooled the two ssp. *integrifolia* populations (300 km apart) and contrasted these to the ssp. *inflata* population (450 km from Dom Pedrito). There is no significant difference between the populations; for quantitative comparison the numbers are too low. The allele prxB4 is only found in ssp. *inflata* and unknown in lines of *P. hybrida*. The alleles prxB3 and prxA2 occur in one *P. hybrida* line only, each; all other lines carry prxA1 and prxB2 (WIJSMAN 1983). At present Van den Berg & Wijsman are determining possible linkages between regulatory mutations in plants from wild populations and electrophoretic mutations in the structural genes (in preparation).

The purple colour of the F1 hybrid *P. integrifolia* s.l. × *P. axillaris* s.s. can be explained by the action of three main flower colour factors (WIERING 1974; WIERING et al. 1979): *Hf1, Fl,* and *Rt. Fl* stems from *P. axillaris* and *Hf1* from *P. integrifolia;* both species bring in *Rt. Fl* and *Rt* are widespread among *P. hybrida* cultivars, as are their recessive alleles *fl* and *rt.* Recessive *rt* seems to originate form mutation during cultivation; magenta *(rtrt)* is known as a flower colour in *P. hybrida* since 1861 (Countess of Ellesmere = Gloire de Segrez, le Texnier, 1908; may be identical to the modern cultivar Rose of Heaven). In the same way *Hf1* and *hf1* can be found in *P. hybrida.* However, for the dark-coloured tubes of *P. axillaris* (s.l.) the allele *hf1–1* is responsible; in the limb only a faint colour is formed because the allele *hf1–1* is only active in the tube. In the limb, the potential anthocyanin is transformed by the factor *Fl* into the flavonol quercetin. The allele *hfl-1* is practically unknown in *P. hybrida;* it was present in only one inbred line of the Institute of Genetics (De Vlaming, pers. comm.).

The combination of *Rt, Hf1,* and *Fl* in the hybrids leads in the limb to the aglycon pigments malvidin and quercetin. The latter gives the blue hue. Hooker's figure (1837) does not relate to the first generation; he shows purple *(Hf1–)* and light venation *(hf1–1hf1–1)* types as from a backcross to *P. axillaris,* and, in fact, in the text mentions white *(an2an2)* as a third colour. MATHER & EDWARDES (1943) found that the colour differences result from genes *W* and *M*; apparently, their *M* is gene *Fl,* their *W* is gene *An2;* but at least some other monogenic differences are postulated, though not named.

I have considered the colour "Petunia purple" (HCC32) a reliable character for the hybrid *P. integrifolia* s.l. × *P. axillaris* s.s. Absence of the colour from specimens collected in the wild (WIJSMAN 1982) is interpreted as a complete lack of hybridization in nature, *P. integrifolia* being pollinated by bumble-bees and bees, *P. axillaris* by moths. (In Europe, *P. hybrida* is visited by both groups of insects).

The ease with which the wild species are crossed to *P. hybrida* and give mono-factorial segregation for several genes (SINK 1975; present study) points to a strong genetic similarity. Meiosis in certain hybrids is irregular, but this can be attributed to chromosome mutations; in others meiosis is normal.

What MATHER & EDWARDES (1943) called *P. axillaris* (s.s.) seems to have concerned ssp. *parodii* (Stout 1952). It may be that our line S7 *(flfl)* is not typical for all *parodii* since MATHER & EDWARDES (1943) found that the hybrid with *P. integrifolia* (s.s.) had "Petunia purple" as its colour. If *P. axillaris* of genotype *flfl* occurs in nature, the vivid bluish magenta colour of the hybrid would not differ much from the reddish purple of *P. integrifolia,* but the form of the tube would be much more narrow than in the latter species. In nature, the taxa *parodii* and *integrifolia* probably never met.

P. axillaris has yellow pollen in contrast to *P. integrifolia* (blue pollen), because of a recessive genotype *an4an4popo* for pollen colour factors. The distribution of the highly linked genes gpiB and *An4* in a number of cultivars still has the alleles linked in the parental situation: *P. axillaris,* gpiB2B2*an4an4; P. inte-*

grifolia, gpiB1B1*An4An4* (WIJSMAN & VAN DEN BERG 1982). On the other hand, both species share other alleles, e.g., several lapB alleles (l.c.), prxB2 (in practically all *P. hybrida* cultivars, but also in lines S2, S9, S10 (see data from populations studies above). Futhermore, prxF occurs in line S2, S9 and S10, but not in any other line or plants of the wild species grown; it is present in a very few inbred lines of *P. hybrida* only (WIJSMAN 1983). These polymorphisms may point to inheritance from a common ancestor, as is the remarkable fact that *P. axillaris* behaves as an *an2an2* albino mutant, having all other factors necessary to allow flavonoid synthesis in the flower.

Apart from recombination of allele pairs mentioned (for the genes *Hf1, Fl, An2, Po* and *An4*) most other types in *P. hybrida* seem to be due to mutations during cultivation; for instance, in wild material there is no evidence for the presence of recessive alleles *hf1* or *rt*, or several *an* mutations (albino); cf. WIERING 1974.

In no case evidence has been found for the theoretical possibility of *P. hybrida* having two genes of the same function – where the parents each would have only one – which could have been brought about by translocation having occurred and having been fixed in the parents before the artificial crossing started.

Summarizing, I postulate the following evolutionary history. An originally 2n = 14 *Petunia* species split into one coloured, and one albino species, with yellow pollen. The albino species split into two subspecies. Where they meet secondary intergradation may occur, but a field study is needed for certification. The purple species split into two geographic isolates (apparently based on the same refugia as in the *P. axillaris* case, see WIJSMAN 1982): *occidentalis* and *inflata/integrifolia*. The latter is a series of populations with a number of differences between the extreme inland form *(inflata)* and the more coastal populations. Around the Rio de la Plata *P. integrifolia* s.s. (as figured by HOOKER 1831) and *P. axillaris* s.s. (as figured by SIMS 1825) occur, and these have been transported to Britain, where their hybridization and further selection gave rise to *P. hybrida*.

Support for the above concept may be obtained by:

a. A study on hybridization between the subspecies of *P. axillaris:*

b. By sampling electrophoretic types, and, in particular, by

c. A detailed study of allelic enzymes with the same electrophoretic mobility but other differences like temporal programme of development. In these ways it can be ascertained whether some alleles are really shared by both species and, therefore, likely to be inherited from a common ancestor with the same number of chromosomes as both present wild species.

ACKNOWLEDGEMENTS

The present study required the use of living material and I thank everyone who provided it. In particular, I acknowledge the advices and the supply of seed samples by Dr T. M. Pedersen, who, moreover, brought me into contact with Sr K. Hagelund, and even undertook to inspect "his" plants in our greenhouses himself.

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