Theoretical and Applied Genetics 39, 17—20 (1969)

Stamina pistilloida: A new Mutation Induced in Pea*

L. M. Monti[1] and **M. Devreux**[2]

Laboratory for Applications in Agriculture of the National Committee for Nuclear Energy, Rome[1],
and Biology Department of European Atomic Energy Community, Bruxelles[2]

Summary. After diethylsulphate treatment of seeds of the pea variety 'Parvus', a new floral mutation was isolated in the second generation. This mutation, named *stamina pistilloida*, is characterized by a partial fusion of the *androecium* with the *gynoecium*; the two marginal stamens of the staminal column are transformed in rudimentary carpels more or less differentiated according to ecoclimatic conditions. The genetic analysis has shown the monogenic and recessive behaviour of the mutation (gene proposed *stp*) and its linkage with the gene *oh* in the chromosome II.

Introduction

In our experiments with physical and chemical mutagens (Monti, 1968) several mutations have been induced, some of which resembling already known gene mutations.

This note describes an induced mutation for a new floral abnormality in pea.

Material and Methods

The M_1 branch progeny P 829c, coming from seed treatment with diethylsulphate in the fodder pea 'Parvus' (Monti and Scarascia-Mugnozza, 1964), segregated in M_2 for 2 plants with floral abnormalities in a total of 14 plants.

The mutation was crossed with the mother line 'Parvus' in order to have segregation data and also crossed with the line no. 851 from Lamprecht's collection (Blixt, 1963) in order to investigate its linkage relationship with other marker genes. Line no. 851 is characterized by the presence of several recessive genes, of which only those reported below have been taken into account in the segregation analysis (in brackets: the linkage group):

i	(I)	green cotyledons in the seeds;
oh	(II)	reddish brown colour of testa of seeds;
s	(II)	seeds sticking together;
wb	(II)	little wax on the plant;
k	(II)	reduced wings of the flowers;
st	(III)	reduced stipules of the leaves;
b	(III)	*clariroseus* flower colour;
le	(IV)	short internodes;
r	(VII)	wrinkled seeds;
tl	(VII)	tendrils transformed into leaflets.

The following percentages of recombination are known among some of those marker genes (Lamprecht, 1961):

chromosome II: *s* (9) *wb*; *s* (20) *k*; *wb* (18) *k*;
chromosome III: *st* (26) *b*;
chromosome VII: *r* (5) *tl*.

The F_1 plants from both the crosses (with the mother line and with line 851) were grown in greenhouse and a meiotic analysis was pursued; the F_2 segregation analysis was performed in the field. A corrected chi square for disturbed segregations (Mather, 1957) was used for detecting linkage and the crossing over values were calculated by the product method using the tables of Stevens (1939).

Data on fertility, expressed as number of ovules and seeds per pod, were collected on 20 mutants and 20 normal plants grown in greenhouse.

For an histological analysis of the mutant, flowers from mutant and normal plants were fixed in FAA, dehydrated in an alcohol xylol series, sectioned at 16 microns, and stained with ferric hematoxylin-fast green.

Results

The histological analysis shows that, in the normal flower, the *androecium* and the *gynoecium* separate from each other, first on the side of the free stamen and afterwards on the opposite side. In the mutant flower, the *gynoecium* disjoins from the *androecium* on the opposite side of the free stamen and remains fused with the staminal column and precisely with both the marginal stamens (Fig. 1—5); the suture at the base of the carpel margins is often incomplete in the mutant because of the fusion above mentioned (fig. 5,6). The two stamens fused with the carpel get thicker and thicker, separate themselves from the staminal column and present reproductive elements with various degrees of differentiation (Fig. 7—10): in fact, those two stamens can show more or less differentiated rudimentary carpels (fig. 11, 12), without or with one or two normal *campylotropous* ovules outside (fig. 13, 14). The most differentiated carpels are like small ovaries but with free margins (Fig. 15). The two pistilloid stamens dissociate from the carpel at different levels and above the carpel-like tissue present rudimentary anthers with degenerate pollen grains (fig. 12, 14). Apparent normal stigma is present on the distal part of each of the pistilloid stamens (fig. 15).

---

* Contribution No. 187 from the Laboratorio per le Applicazioni in Agricoltura, C. N. E. N., S. Maria di Galeria, Roma, Italy and No. 406 from the Biology Department of European Atomic Energy Community.

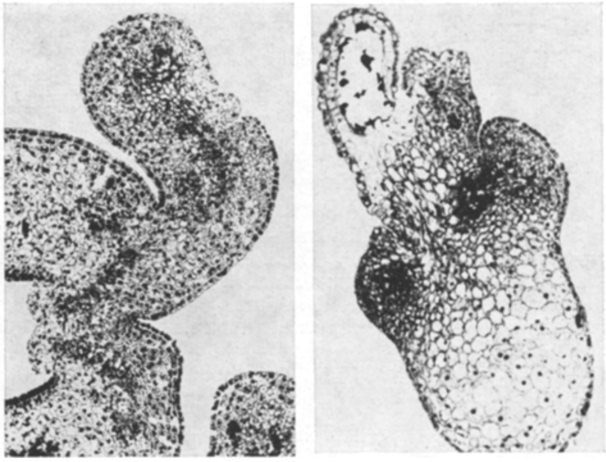

Fig. 11—12. Transversal sections in a *stp* mutant flower

Fig. 11. One "pistilloid" stamen fused with the carpel

Fig. 12. At a higher level, the "pistilloid" stamen with its rudimentary anther and degenerate pollen grains

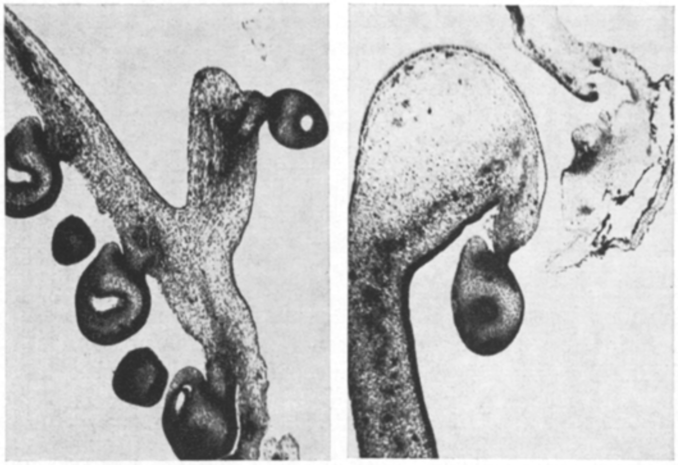

Fig. 13—14. Supernumerary ovules out of the carpel

Macroscopically, the main differences between the mutant and the normal flowers are that the two petals forming the keel are not fused but overlapping and that, instead of the diadelphous condition, there are in the mutant 8 normal stamens (7 connate, one free) and two pistilloid stamens connate at their base with the staminal column and with the normal unicarpellate pistil (fig. 16). On the ripe pods, the two pistilloid stamens remain as rudiments on the ventral suture (fig. 17). Furthermore, it seems that the environmental growing conditions influence the mutation phenotype.

No difference was found between the mutant and the mother line 'Parvus' as to number of ovules per pod (5.85 ± 0.13 for the mutant against 5.92 ±

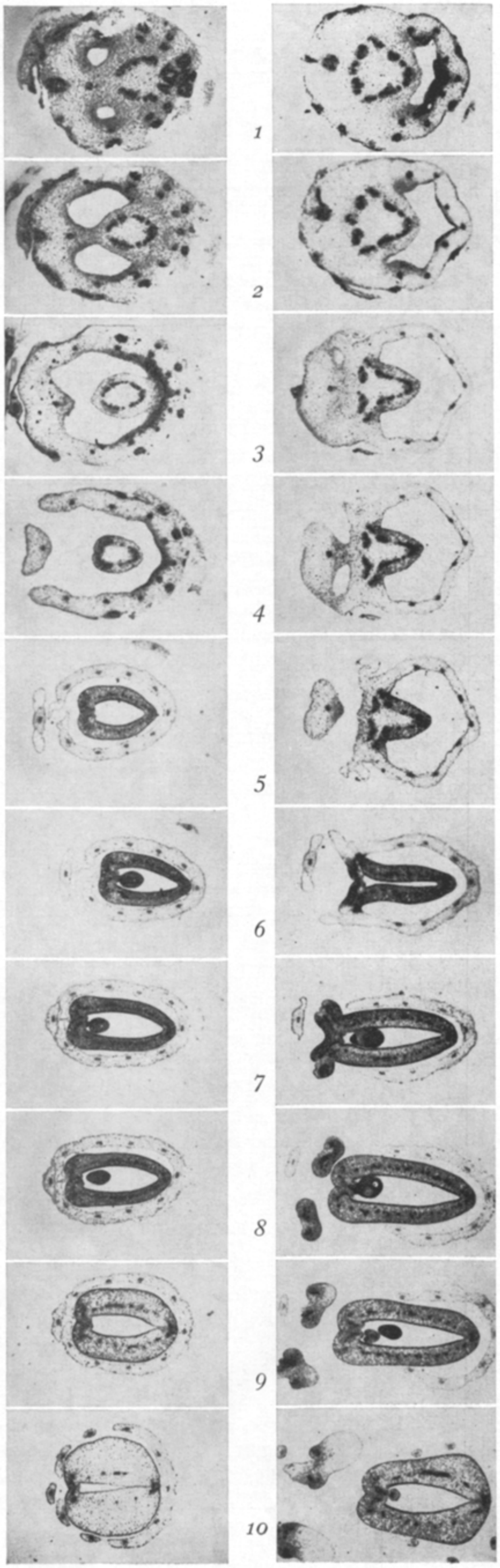

Fig. 1—10. Flower transversal sections in control (left) and *stp* mutant (right).

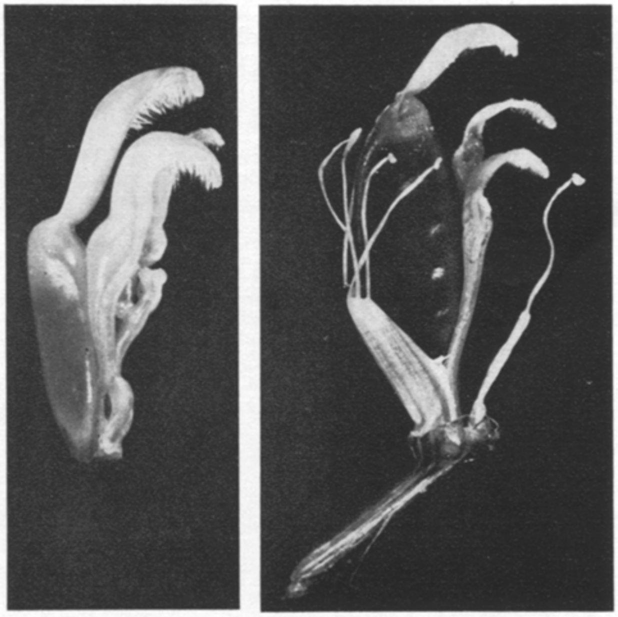

Fig. 15—16. The *gynoecium* of a flower and a young pod of the *stp* mutant where the abnormalities of the mutation are clear

0.06 of 'Parvus') and number of seeds per pod (3.99 ± 0.36 and 4.22 ± 0.18 respectively).

The F_1 plants after backcross with 'Parvus' were normal and had a good seed set; no chromosome aberration was found at meiosis . In F_2, on a total of 156 plants a segregation of 25.64% was found (chi square for $3:1 = 0.034$; $0.75 < P < 0.90$), showing a recessive and monogenic behaviour of the mutation. The symbol *stp* (*stamina pistilloida*) is proposed to design this mutation.

For linkage studies, 500 seeds, coming from 6 F_1 plants after the cross between the mutant line and the line *851* were sown; they gave 452 plants, 21 of which did not set seeds due to disease attack.

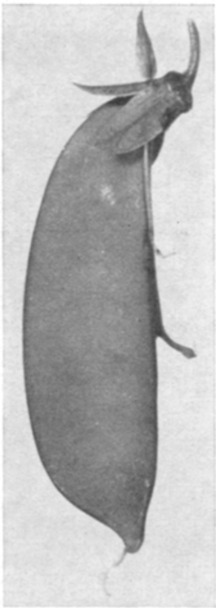

Fig. 17
A ripe pod of the *stp* mutant: the two "pistilloid" stamens remain as rudiments on the ventral suture.

Crossing over (%) values of 31.3 ± 2.7 and of 8.8 ± 1.4 were calculated respectively between *st* and *b* and between *r* and *tl*. The values found for *s*, *wb* and *k* are reported in table 1. As to the gene *stp*, linkage was ascertained between it and *oh* located in chromosome II (chi square = = 36.45; $P < 0.001$), with a crossing over value of 16.95 ± 4.65 (Table 1).

Discussion

Pistillody of any of the floral organs is a very frequent floral abnormality in higher plants (Meyer, 1966). More recently, mutations showing conversion of some stamens to pistils, have been described in different species of *Gramineae* (Kihara, 1951; Moh and Nilan, 1953; Barabas, 1962; Bathia and Swaminathan, 1963; Fejer, 1966); Meyer and Buffet (1962) have found external ovules on carpel-like tissue at the top of the *androecium* in *Gossypium* interspecific hybrids. In *Pisum*, cases of pistillody of sepals (Laxton, 1866; Lamprecht, 1934; Gottschalk, 1961) and leaves (Marx and Mishanec, 1964) are known. Also in pea, Gottschalk (1961) has described an induced mutant in which extra carpels are formed from anther and calyx tissue. The abnormality reported in this paper represents the first case of a clear conversion of some stamens to pistil-like structures.

The main effect of the mutation seems to be the partial fusion of the *androecium* with the *gynoecium*.

Table 1. F_2 *segregation and recombination values* (%) *for the markers oh, s, wb, k of the chromosome II and the mutant gene stp*

Factors		Phase *	number of plants					chi-squared			Recombination value %
			Phenotype				Total				
Xx	Yy		X Y	X y	x Y	x y		Segregation of x	Segregation of y	Joint Segregation**	
Oh oh	*Stp stp*	R	218	84	126	3	431	5.59	5.32	36.45	16.95 ± 4.65
S s	*Stp stp*	R	303	80	41	7	431	44.18	5.32	1.05	—
Wb wb	*Stp stp*	R	270	79	82	21	452	1.18	1.99	0.23	—
K k	*Stp stp*	R	267	72	85	28	452	0.00	1.99	0.62	—
Oh oh	*S s*	C	272	30	111	18	431	5.59	44.18	1.47	—
Oh oh	*Wb wb*	C	240	62	94	35	431	5.59	1.43	2.26	—
Oh oh	*K k*	C	227	75	98	31	431	5.59	0.04	0.03	—
S s	*Wb wb*	C	332	51	2	46	431	44.18	1.43	166.53	6.68 ± 1.25
S s	*K k*	C	315	68	10	38	431	44.18	0.04	86.74	17.62 ± 2.05
Wb wb	*K k*	C	306	43	33	70	452	1.18	0.00	131.31	18.81 ± 2.08

* R = Repulsion; C = Coupling
** The chi square for disturbed segregations was used (Mather, 1957)

According to HESLOP-HARRISON (1957), the growth of stamen and pistil primordia is governed by the auxin level and „the concentration promoting maximal stamen growth is lower than that promoting maximal pistil growth". The transition forms of stamino-carpels of our mutant could probably be due to an alteration of the relative concentration of auxins present in the flower primordium and the effect of the ecoclimatic conditions in the manifestation of this mutation could also be explained with the influence that those conditions have on the auxins available at the differentiating apex.

The mutation behaves as a simple recessive Mendelian character, and it does not seem to be due to, or accompanied by chromosome aberration. The quite good correspondance between the crossing over found by us and those reported by LAMPRECHT (1961) and BLIXT (1965), confirms that no translocation is present in our material, at least in the analyzed chromosomic regions.

The genetic analysis has shown that the gene responsible for the mutation *stamina pistilloida* is located in chromosome II at a distance of about 17% of crossing over from the *locus oh*. Further studies are needed for more exact localization of the mutation.

Acknowledgement

The authors are greatly indebted to Prof. F. D'AMATO and Prof. G. T. SCARASCIA-MUGNOZZA for the critical review of the manuscript and to Mr. P. VITALE and Mr. L. ANSELMI for their precious technical assistance.

Zusammenfassung

Nach Behandlung von Samen der Erbsensorte 'Parvus' mit Diäthylsulfat wurde in der 2. Generation eine neue Blütenmutation ausgelesen. Diese Mutation, die *stamina pistilloida* benannt wurde, ist durch eine teilweise Verschmelzung des Androezeums mit dem Gynaezeum gekennzeichnet. Die beiden marginalen Staubblätter der Staubblatt-Röhre sind zu rudimentären Karpellen umgebildet, die entsprechend den ökoklimatischen Verhältnissen mehr oder weniger differenziert sind. Die genetische Analyse ergab, daß sich die Mutation (Gensymbol *stp*) monogen und rezessiv verhält und mit dem Gen *oh* im Chromosom II gekoppelt ist.

Literature

1. BARABAS, F.: Observation on sex differentiation in *Sorghum* by use of induced male-sterile mutants. Nature **195**, 257–259 (1962). — 2. BATHIA, C. R., and M. S. SWAMINATHAN: An induced multiple carpel mutation in bread wheat. Genetica **43**, 58–65 (1963). — 3. BLIXT, S.: A presentation of the Lamprechtian *Pisum*-material. Report from Weibullsholm Plant Breeding Institution (1963). — 4. BLIXT, S.: Linkage studies in *Pisum* I linkage of the genes Chi_2 Chi_3 and Chi_4 causing chlorophyll deficiency. Agri Hort. Genet. **22**, 26–42 (1965). — 5. FEJER, S. O.: A floral mutation in *Lolium perenne*. Canad. Jour. Genet. and Cyt. **8**, 115–121 (1966). — 6. GOTTSCHALK, W.: Über die zeitliche Aufeinanderfolge bestimmter Gestaltungsprozesse bei der Ausdifferenzierung von Vegetationskegeln zu Blüten. Naturwissenschaften **49**, 59 (1961). — 7. HESLOP-HARRISON, J.: The experimental modification of sex expression in flowering plants. Biol. Rev. (Cambridge Phil. Soc.) **32**, 38–90 (1957). — 8. KIHARA, H.: Substitution of nucleus and its effects on Genome Manifestation. Cytologia **16**, 177–193 (1951). — 9. LAMPRECHT, H.: Das Gene *Uni* und seine Koppelung mit anderen Genen bei *Pisum*. Hereditas **18**, 269–296 (1934). — 10. LAMPRECHT, H.: Die Genenkarte von *Pisum* bei normaler Struktur der Chromosomen. Agri Hort. Genet. **19**, 360–401 (1961). — 11. LAXTON: Double-blossomed peas. Gard. Chron., p. 901 (1866). — 12. MARX, G. A., and W. MISHANEC: The „Spontaneous" Formation of Rudimentary Carpels on Leaf Tissue. Journal paper no. 1938 (N. Y. State Agricultural Exp. Station), 40–41 (1964). — 13. MATHER, K.: The measurement of linkage in heredity. London: Methuen and Co. Ltd. 1957. — 14. MEYER, V. G.: Flower abnormalities. The Botanical Review **32**, 165–218 (1966). — 15. MEYER, V. G., and M. BUFFET: Cytoplasmic effects on external-ovule production in cotton. J. Hered. **43**, 251–253 (1962). — 16. MOH, C. C., and R. A. NILAN: Multi-ovary in barley. A mutant induced by atomic bomb irradiation. J. Heredity **44**, 183–188 (1953). — 17. MONTI, L. M.: Mutations in peas induced by diethylsulphate and X-rays. Mutation Res. **5**, 187–191 (1968). — 18. MONTI, L. M., and G. T. SCARASCIA MUGNOZZA: Effetti mutageni del dietilsolfato nel pisello. Atti Assoc. Genet. Ital. **9**, 115–125 (1964). — 19. STEVENS, W. L.: Tables of the recombination fraction estimated from the product ratio. J. Genetics **39**, 171–180 (1939).

L. M. MONTI
Laboratory for Applications in Agriculture
of the National Committee for Nuclear Energy,
Rome, Italy

M. DEVREUX
Biology Department of European Atomic
Energy Community,
Bruxelles, Belgium.