Observation of a phenomenon resembling hybrid dysgenesis, in a wild pea subspecies *Pisum sativum* ssp. *elatius*

Bogdanova, V.S. and Berdnikov, V.A. Inst. of Cytology and Genetics, Russian Academy of Sciences
Novosibirsk, Russia

In a series of crosses of wild peas with cultivated forms we found that one line, VIR320, was peculiar in that the characters of hybrid plants produced when VIR320 was used as a female parent differed from those when it was used as the male parent. This line has been assigned to *P. sativum* ssp. *syriacum* (which is a synonym of *P. sativum* ssp. *elatius* var. *pumilio*). It originated from Palestine and was collected on an early expedition of N.I.Vavilov. When VIR320 was used as the female parent in crosses, the majority of the F_1 plants were abnormal to some extent. Hybrid plants produced from the reciprocal cross were normal. Observed abnormalities included chlorophyll pigmentation (sectors of chlorotic tissue on the normal background and vice versa), reduced leaves, and sterile flowers (Fig.1).

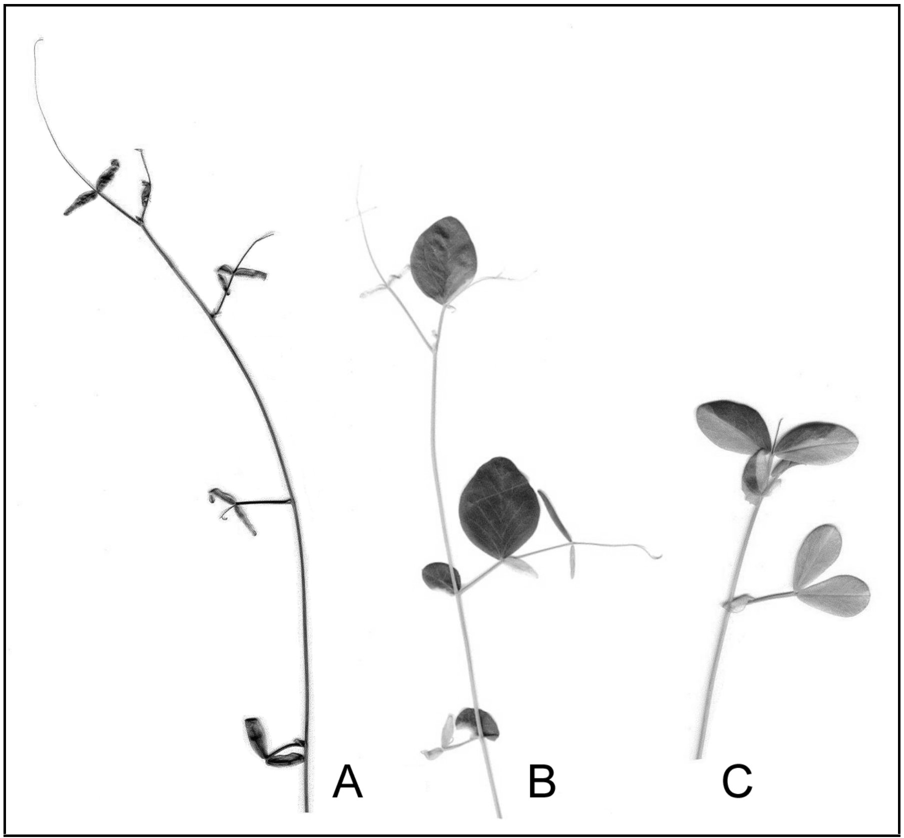

Fig.1. Phenotypes of F_1 plants from the crosses of VIR320 with different lines. A - 320 x CE1; B - 320 x WL1238; C - 320 x af,tac.

We made crosses between VIR320 and 47 accessions, using the former as the female parent. The number of F_1 plants obtained from each cross varied from 1 to 15. Of the alternate parents, 32 were from non-cultivated accessions and 15 from cultivated types. In only six cases (L100, VIR2521, JI1794, L99, P008 and WT301) abnormalities were not observed in the F_1 generation, and in all these cases the alternate parent represented a wild form of *P. sativum* or *P. fulvum* (WT301). The degree of abnormality observed varied, depending on the accession used as the pollen parent. There was a positive correlation between the degree of the abnormality and the fraction of F_1 plants exhibiting an abnormality, with the frequency of abnormal plants varying between 0 and 20%.

It proved useful to divide all accessions into two groups, those 'compatible' with VIR320 (producing only normal progeny in crosses) and those 'incompatible' with VIR320 (producing abnormal progeny in crosses). The phenotypes resulting from crosses with these two types of alternate parent are schematically presented in Fig.2. One of the accessions that caused the most severe anomalies in the progeny was VIR1451 (Arkhangelsk region) which was used as a tester in the experiments described below.

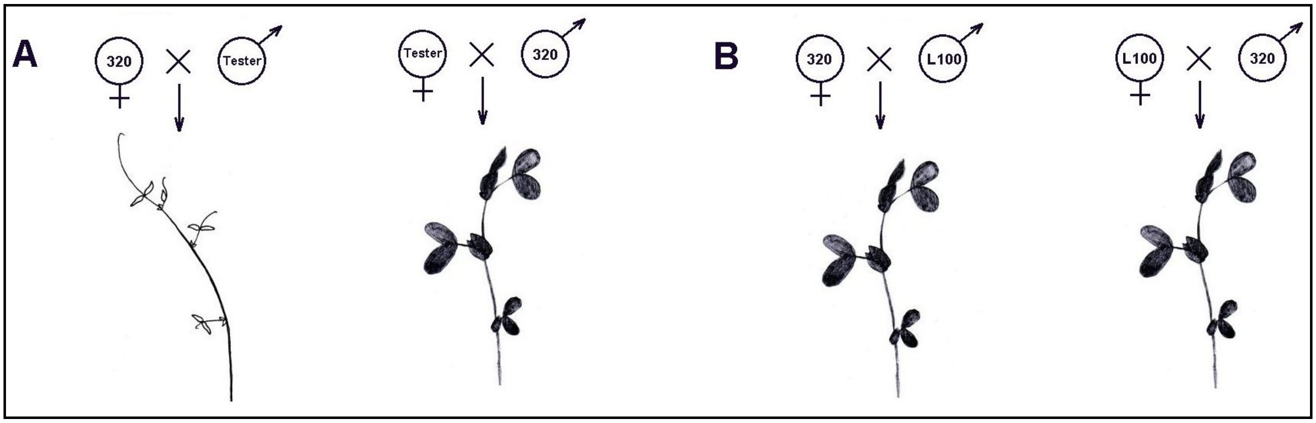

Fig.2. Schematic model of phenotypes observed in seedlings from crosses with incompatible (A) and compatible (B) lines.

We investigated the mode of inheritance of the ability of line VIR320 to induce aberrations in the progeny using two types of crosses: 1) crosses with incompatible lines; 2) crosses with compatible lines. We investigated segregation in F_2, and the trait we scored was the ability of an F_2 plant to produce aberrant progeny when pollinated with foreign pollen. In the presentation of results we use the ♀ and ♂ symbols to denote maternal and paternal lines, respectively. The line VIR320 is abbreviated to 320. In all crosses, the maternal parent is indicated first.

Crosses with incompatible lines

Each plant from an F_2 population of 120 plants derived from the cross WL1238 x 320 was pollinated with VIR1451. None of the 821 plants produced from these crosses was phenotypically abnormal. We can summarize these results in the following scheme:

F_2[♀ x 320] x VIR1451
—> normal

Table 1. Results of pollination of line VIR320 with pollen from 43 F_2 plants from the cross WL1238 x 320

Only abnormal progeny	Number of F_2 producing Both abnormal and normal	Only normal progeny
5 (9 progeny)	13 (19 norm. + 33 abnorm.)	25 (46 progeny)

When VIR320 plants were pollinated using 43 F_2 plants from the cross WL1238 x 320 the F_2 plants appeared to resolve into three classes (Table 1), one that produced only abnormal progeny, one that produced both normal and abnormal progeny, and one that produced only normal progeny.

It appears that in incompatible crosses either the presence of VIR320 genes in the pollen or the presence of VIR320 cytosol is the factor responsible for the appearance of the observed abnormalities.

Crosses with compatible lines

In this type of cross the ability of VIR320 to induce aberrations in the progeny was approximately the same regardless of the direction of the cross (Table 2). The line L100 was used as the compatible parent.

Table 2. Results of pollinations of F_2 plants from compatible crosses with VIR1451 pollen.

F_2 population	N	Number of F_2 producing Only abnormal progeny	Both abnormal and normal	Only normal progeny
320 x ♂	29	4 (12 progeny)	13 (44 norm. + 22 abn.)	12 (61 progeny)
♀ x 320	52	14 (72 progeny)	24 (100 norm. + 98 abn.)	14 (115 progeny)

In the experiment with the ♀ x 320 F_2 population, the segregation of phenotypes is very close to a 1:2:1 ratio, suggesting that there is one (or several closely linked) nuclear factor responsible for aberrations observed. We conclude that the ability of the line VIR320 *Pisum sativum* ssp. *elatius* to cause aberrations in the progeny is determined by the nuclear genome, but this effect can be observed only in the presence of compatible cytoplasm. Abnormal progeny occurs in crosses where maternal plants refer to the line VIR320 or its hybrids with compatible lines.

Phenotypic aberrations observed by us are very similar to those described by Lutkov (4) in distant hybrids of pea. However, in experiments of Lutkov, abnormal plants were observed in F_2 and F_3 generations, while we could observe the phenomenon only in the F_1 after pollination of VIR320 (or its hybrids) with a tester pollen.

There are some types of crosses where phenotypes of the resulting progeny depend on the direction of the cross. For example, cytoplasmic male sterility (CMS), manifested as inability to produce fertile pollen or functional anthers, is maternally inherited although it is influenced by nuclear genes. CMS is commonly associated with changes in mitochondrial gene expression (reviewed in 6). In fact, VIR320 line acts like a source of sterility-inducing cytoplasm, whereas compatible lines perform as if carrying nuclear fertility restorer genes. However, segregation in the F_2 of compatible crosses indicates that we are dealing with a nuclear factor that can be transmitted both maternally and paternally, although the source of cytoplasm plays an important role. The issue appears to be involved in the phenomenon of cytoplasmic incompatibility (CI) described for some arthropod species and associated with endocellular bacteria of *Wolbachia* species. CI is maternally transmitted and appears as reproductive aberrations when an infected male is crossed with a female either devoid of *Wolbachia* or harboring another strain of bacteria (see for example, 1). This aberration can be overcome by antibiotic treatment against microbial invasions. We treated seeds of VIR 320 and tester lines with doxycycline (25 μg/ml and 40 μg/ml). The resulting seedlings were pale-green indicating that the treatment was effective; however, the treatment did not abolish aberrations in the progeny.

Thus, we suppose that the phenomenon observed most closely resembles hybrid dysgenesis described in *Drosophila* (3). In flies, the dysgenic syndrome appears in hybrid progeny when mobile genetic elements of certain type come from the male parent and the hybrid genome conflicts with cytoplasm of the oocyte produced by the female lacking active copies of this mobile element. The resulting mobilization of the transposon in the hybrids causes multiple genetic disorders including complete or partial sterility. It is known that plant genomes may contain numerous copies of transposable elements (5). By analogy with *Drosophila*, we can suppose that the genomes of the majority of pea samples are saturated with some type of transposons which are, however, inactive due to accumulation in the cytoplasm of a repressor encoded on the DNA of the mobile element. Male gametes lack the repressor or contain very little of it. A cross involving two such lines does not cause dysgenesis because mobile elements delivered by the male gamete are controlled by the repressor produced by transposons of the egg. We suppose that the genome of the line VIR320 is devoid of mobile elements capable of repressor production, therefore, when an egg of this line meets with a male gamete of an incompatible line, transposons of the latter begin active transpositions and repressor production. As for compatible wild samples, we suppose that their genome harbors defective mobile elements not capable of active transposition but producing a factor of repression which is accumulated in cytoplasm thus making it non-reactive. Therefore, crosses of these lines with common testers produce normal progeny, and the offspring from the crosses with VIR320 also does not suffer from genetic disorders caused by transpositions of mobile elements.

It should be noted that crosses of *Pisum sativum* with other pea taxa such as *P. sativum* ssp. *abyssinicum* and *P. fulvum* are successful (with rare exceptions) only when *P. sativum* is used as the female plant (2, 7). In addition, an F_2 may segregate for aberrant plants (4) similar to those described in the present study. It is possible, therefore, that nuclear-cytoplasmic conflict contributes to interspecies isolation and, thus, to speciation.

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