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Investigations on an interspecific hybrid involving three species of the genus *Beta*, with special reference to isozyme polymorphism

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Summary. A tetraploid ($2n=36$) interspecific hybrid was obtained involving three species belonging to three different sections of *Beta*. The hybrid was highly sterile and did not show apomixis. At meiosis, up to nine bivalents were observed, most probably resulting from autosyndesis of the chromosomes of *Beta lomatogona*. For nine isozyme systems, individual enzyme expression was investigated in the parental species and in the hybrids. No silencing of genes or genomes was observed. In the case of some polymeric enzymes interspecific heteropolymers could be detected.

Key words: *Beta vulgaris* – Sugar beet – *Beta lomatogona* – *Beta procumbens* – Interspecific hybrid – Isozyme polymorphism

Introduction

Interspecific hybridisation in the genus *Beta* has been carried out in order to broaden the genetic base of sugar beet and for the transfer of genetic characteristics from wild species into the cultivated crop (for reviews see Bosemark 1969; De Bock 1986; Van Geyt 1986).

Species of section *Corollinae* are of interest for monogermity and resistance to curly top, virus yellows, drought and low temperatures. In this section, most polyploid species and hybrids show apomixis, and occasionally unreduced gametes are formed in both diploids and polyploids (Barocka 1966; Cleij et al. 1968, 1976; Jassem and Szota 1978; Jassem 1980; Jassem and Jażdżewska 1980). Rather complicated genomic structures can arise. Species of section *Patellares* are more distantly related to cultivated beet than the other wild *Beta* species. The *Patellares* species are desirable for their round-shaped monogerm seed balls and for resistances to *Cercospora*, curly top and beet cyst nematodes (Savitsky 1975; Speckmann and De Bock 1982; Heijbroek et al. 1983; Löptien 1984; Speckmann et al. 1985).

In breeding programmes involving interspecific hybrids, it is often desirable to identify genomes or individual chromosomes in the hybrids or in their offspring. Dębowski and Trzebiński (1974) demonstrated that several *Beta* species could be distinguished on the basis of electrophorograms of seed proteins, but no data on interspecific hybrids were presented. Studies on chromosome morphology, with or without specific C-banding techniques, might be helpful (De Jong et al. 1985) but chromosomes of *Beta* are small and therefore not easy to distinguish.

Van Geyt and Smed (1984) studied a number of biochemical markers in sugar beet (enzymes and total SDS buffer soluble proteins) and described the necessary techniques. These authors envisaged the use of such markers for detection of taxonomic relationships, control of results of crosses and identification of chromosomes.

This paper describes the development and biochemical characterisation of an interspecific hybrid involving three species of the genus *Beta:* viz. *B. vulgaris* of section *Beta, B. lomatogona* of section *Corollinae* and *B. procumbens* of section *Patellares.* The hybrid was made at the Foundation for Agricultural Plant Breeding in the framework of a much larger programme on interspecific hybridisation in *Beta.* First the interest was focussed on the hybrid itself and on its potential for breeding sugar beet. Later on, this unique material proved to be very useful as a model for genome identification using biochemical markers.

Materials and methods

The original plant material consisted of triploid hybrids between *Beta vulgaris* L. ($2n=2x=18$, male sterile sugar beet) and *B. lomatogona* F. et M. ($2n=4x=36$). The hybrids (genome formula VLL) were obtained from Cleij (SVP, Wageningen) and were described by Cleij et al. (1976). Under isolation as well as in crosses, these hybrids showed a high level of apomixis. For the present study the hybrids VLL were crossed with *B. procumbens* Chr. Sm. ($2n=2x=18$, genome

formula PP) from the collection of the SVP. This species carries resistance to beet cyst nematode (*Heterodera schachtii* Schm.). Part of the offspring of these crosses was tetraploid and showed nematode resistance. These hybrids, with the putative genome formula VLLP, formed the basic plant material of the present study. For further crosses *B. procumbens* (4*x*) and *B. vulgaris* (4*x*) from the SVP collections were also used.

The hybrids were cloned vegetatively from flower buds and were subcultured as described by Van Geyt and Jacobs (1985).

The methods used for screening of nematode resistance and for analysis of mitosis and meiosis were the same as those described by Speckmann et al. (1985).

The procedures for extraction of proteins, and the techniques for vertical polyacrylamide and horizontal starch gel electrophoresis have been described previously (Van Geyt and Smed 1984). The SGE 1 buffer system was used to separate peroxidase, and the enzymes shikimate dehydrogenase, superoxide dismutase and esterase were separated with the buffer system PAGE 1. The staining procedures of Van Geyt and Smed (1984) and Van Geyt (1986) were used for the visualisation of the various enzymes.

Results

The crosses VLL×PP

The first attempt to hybridize the triploid hybrid VLL, from *B. vulgaris* (2*x*, VV)×*B. lomatogona* (4*x*, LLLL), with *B. procumbens* (2*x*, PP) was carried out in 1974. Nineteen plants yielded 430 g of seed. Most seed balls were empty, and only slightly over 0.6% of the seed germinated. The majority of the offspring was triploid and had the same appearance as the female parent. These plants are considered to have originated from apomixis. However, 56 plants (about 8% of the offspring of the cross VLL×PP) differed in appearance, were tetraploid and showed resistance to the beet cyst nematode. Such plants were thought to be hybrids, with genome formula VLLP.

In 1976 the cross VLL×PP was repeated with 330 female plants. Again an abundance of seed was formed but for unknown reasons the germinability was much lower (about 0.04%), and no hybrid plants were recovered.

The interspecific hybrid VLLP

Plant morphology showed strongest resemblance with that of *B. lomatogona,* but plant vitality was rather low. The leaves were somewhat leathery, triangular, and had pointed tips (Fig. 1). In younger leaves the basal parts showed a yellowish discoloration, which also was observed in hybrids between *B. vulgaris* and *B. procumbens.*

Meiosis was studied in young anthers. Unfortunately it proved to be very difficult to obtain a good spreading of the chromosomes in the pollen mother cells. Therefore, only a few complete cells could be analysed at first metaphase. Most cells showed 9 bivalents and 18 univalents (Figs. 2 A, B), but in several PMCs the number of bivalents was even lower (down to five bivalents). Because of the high number of univalents meiosis was disturbed, resulting in irregularities in later meiotic stages (Fig. 2 C).

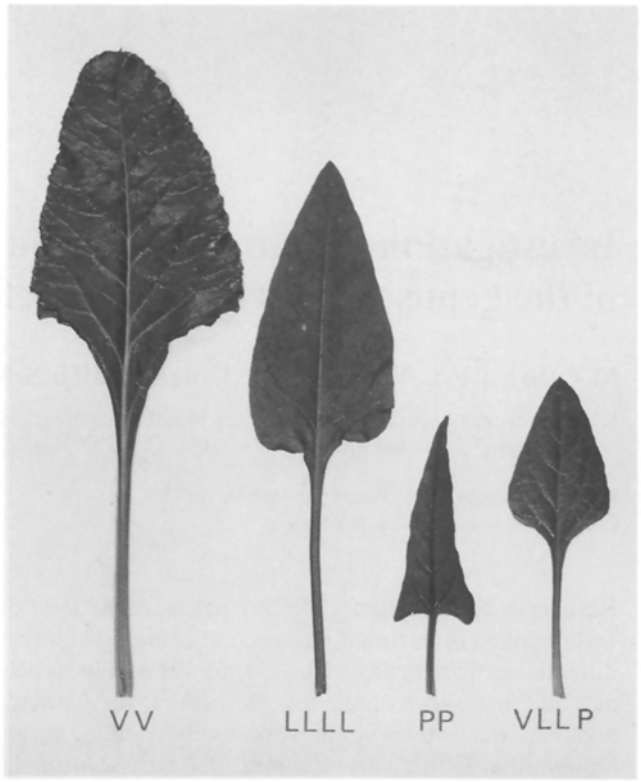

Fig. 1. Leaves of *Beta vulgaris* (2*x*, VV), *B. lomatogona* (4*x*, LLLL), *B. procumbens* (2*x*, PP), and their interspecific hybrid (4*x*, VLLP)

The hybrid plants were fully male sterile. Female fertility was tested in various crosses. In 1976, hybrids were pollinated with either *B. procumbens* (4*x*) or *B. vulgaris* (4*x*). Both crosses yielded some seed, but this did not germinate at all. In 1977, the cross with *B. vulgaris* (4*x*) was repeated and yielded seed that showed some germinability and gave rise to 12 plants. Unexpectedly all plants had 18 chromosomes, were susceptible to beet cyst nematodes and looked completely like the male parent.

Isozyme analysis

The banding patterns of nine isozymes were investigated in the hybrid VLLP and in the three parental species (Fig. 3).

In *B. vulgaris,* the pattern for leucine aminopeptidase (LAP) system is characterised by one or two major bands. *B. procumbens* only showed one band, migrating

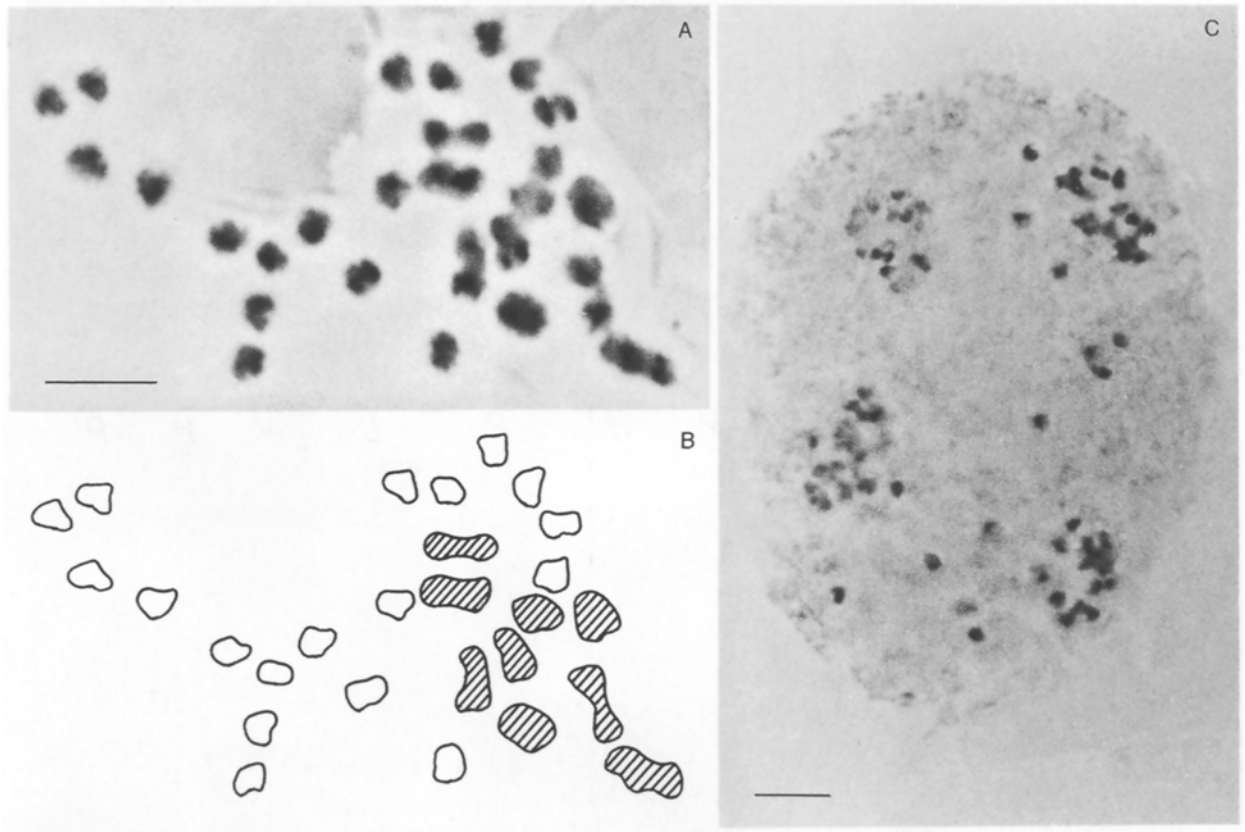

Fig. 2A–C. Meiosis in an interspecific hybrid (VLLP) involving three species of the genus *Beta*. **A and B** first metaphase (photograph and elucidating drawing), showing 9 bivalents (hatched) and 18 univalents; **C** second anaphase, showing many lagging chromosomes. Bars equal 5 μm

slower than the bands previously mentioned. *B. lomatogona,* on the other hand, is characterised by three bands. In the zymogram of VLLP, all bands are expressed at the corresponding positions. Although the pattern of the minor bands is less clear, the respective bands expressed in the different species are also present in the interspecific hybrid.

A similar observation was made for the cathodal peroxidases (POD), shikimate dehydrogenase (ShDH), and phosphoglucomutase (PGM), which are known to be active as monomers. The zymogram of VLLP is the sum of the single bands expressed in the parental species.

At present, the genetics of superoxide dismutase (SOD) and esterase (EST) isozymes is unknown. However, it is clear that both systems are under polygenetic control. All the bands expressed in the parental species were expressed in the interspecific hybrid. No hybrid proteins were found that originated from the combination of proteins of the parental species.

Glutamate dehydrogenase (GDH) is characterised by one single band in each of the parental types. The bands of *B. vulgaris* and *B. lomatogona* migrate to a similar position, while the band of *B. procumbens* moves faster. In VLLP, a hybrid protein is found to be formed between the proteins encoded by *B. procumbens* and one or both of the proteins formed by the other species. Studies of interspecific hybrids of *B. vulgaris* and *B. procumbens* have shown that the subunits of GDH of both species can form active heteromers (Van Geyt 1986).

The pattern for glutamate oxaloacetate transaminase (GOT) is characterised by three bands in *B. vulgaris* as well as in *B. procumbens.* In *B. lomatogona,* bands were visualised at five different positions from which one band overlaps with a *B. vulgaris* band and one with a *B. patellaris* band. In the VLLP plants all bands were found at corresponding positions. In addition, two new bands were detected.

In the hybrid VLLP, the pattern of NAD specific malate dehydrogenase (MDH) showed the two bands of *B. procumbens,* the slowest migrating band of *B. vulgaris* and the single band of *B. lomatogona.* Interspecific heterodimers, viz. proteins that originated from the

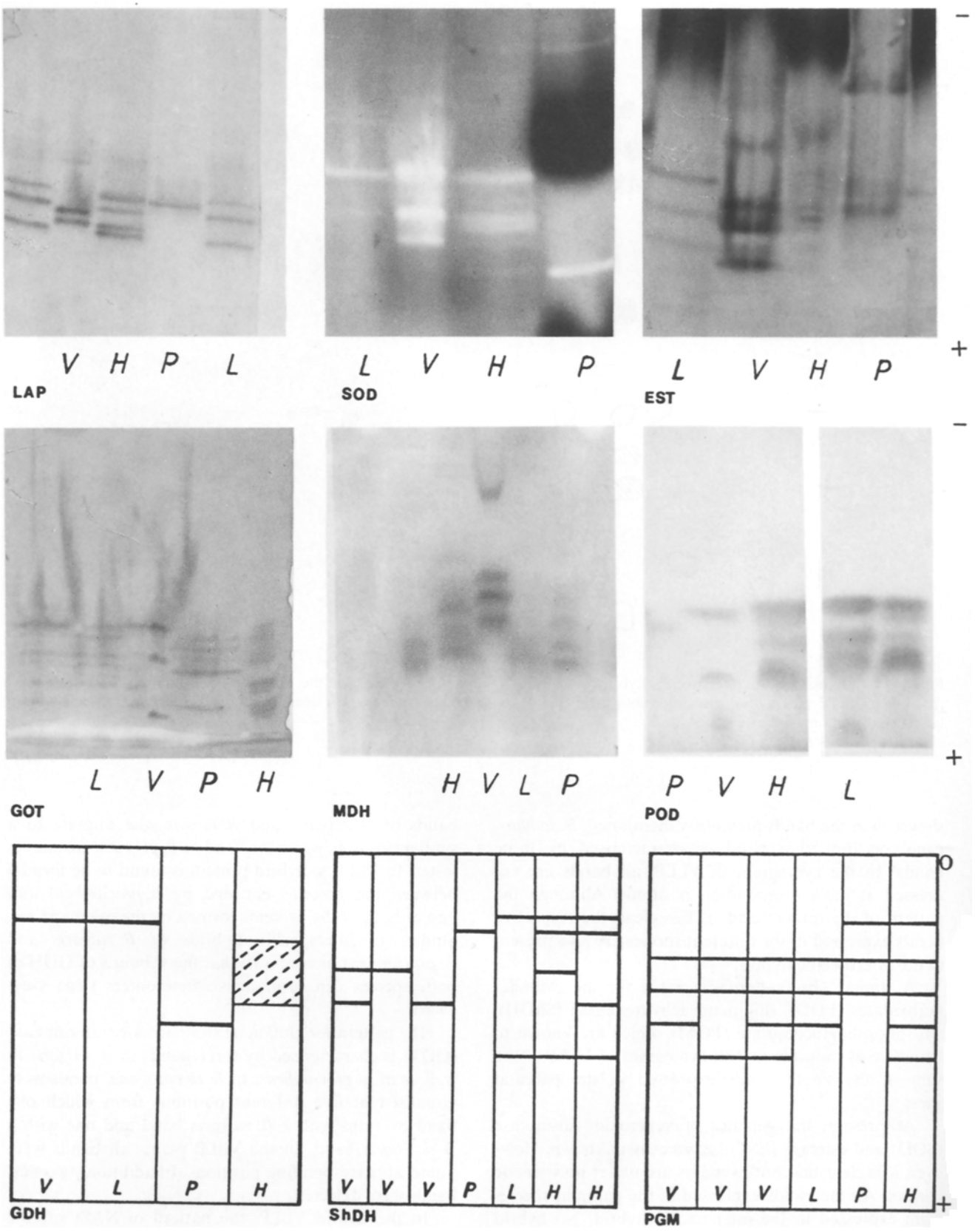

Fig. 3. The zymograms of *Beta vulgaris* (V), *B. lomatogona* (L), *B. procumbens* (P) and the interspecific hybrid VLLP (H) regarding leucine aminopeptidase (LAP), superoxide dismutase (SOD), esterase (EST), glutamate oxaloacetate transaminase (GOT), malate dehydrogenase (MDH), peroxidase (POD), glutamate dehydrogenase (GDH), shikimate dehydrogenase (ShDH) and phosphoglucomutase (PGM)

combination of subunits of two or all three parental species, are formed. In several cases, the bands overlap.

Discussion

Allopolyploids are suitable material for studying interactions between related genomes. The present study revealed information about apomixis, chromosomal association and expression of isozyme loci in a hybrid involving three species of *Beta*.

The results of the crosses between the hybrid VLL and diploid *B. procumbens* confirmed the data of Cleij et al. (1976) regarding the high level of apomixis in the triploid hybrid between *B. vulgaris* (2*x*) and *B. lomatogona* (4*x*). The tendency towards apomixis appears to be a general phenomenon in the polyploid species of section *Corollinae* (Barocka 1966) and in polyploid hybrids between these species (Jassem 1980). Therefore, it was rather unexpected that in the hybrid VLLP, no apomixis was observed. Instead of this, the hybrids were highly sterile, and the few offspring that were obtained could only be explained by assuming in vivo androgenesis.

The sterility may be the result of irregular meiosis, leading to unbalanced gametes, but other causes cannot be ruled out. The little data obtained on meiosis in the hybrid in general is in accordance with what could have been expected on the basis of earlier studies (for references see Bosemark 1969; Cleij et al. 1976; Speckmann and De Bock 1982). Thus, the bivalents most probably resulted from autosyndesis of the chromosomes of *B. lomatogona,* indicating the autotetraploid nature of this parental species. The number of univalents point out the absence or perhaps little association between chromosomes of *B. vulgaris* and *B. procumbens.*

Due to the codominant expression of isozymes, the effect of the combination of various sources of genetic information within one organism can be studied – as for instance the effect of heterozygosity on expression or preferential silencing of genes or parts of genomes.

From the interaction between the different gene products new isozyme types may be formed, indicating structural resemblance between the protomers of the different species. These events can cause changes in the physiological control of biochemical pathways or in the kinetic properties of the newly formed isozymes.

Evolutionary studies on duplicated gene loci in the *Salmonidae* (Allendorf et al. 1975; Ferris and Whitt 1977) indicated that for about 100 million years half of the gene loci were silenced. In plants, only a limited number of studies have been presented with regard to the influence of unrelated genomes on the expression of the genetic information. Polyploid wheat has been studied most extensively. Hart (1979, 1983) found that only few loci are silenced during the evolution. This could reflect the recent speciation of hexaploid wheat (Hart and Langston 1977).

With regard to the enzyme systems LAP, POD, PGM, ShDH, EST and SOD, the interspecific hybrid showed bands at corresponding positions as in the parental species. No isozyme system of either of the three parents seems to be repressed. In polymeric systems as GDH, GOT and MDH, heterodimers are formed between proteins of the parental species. Unfortunately, in these cases, the various isozyme bands overlap. It is not clear if such hybrid proteins are formed between the protomers of all three species. Studies of hybrids between *B. vulgaris* and *B. procumbens* demonstrated that in such material hybrid protein formation is possible in the case of GDH and MDH (Van Geyt 1986). No evidence for the silencing of isozyme genes could be found.

The present study showed that there is a very low crossability between the three species as well as probably no chromosomal affinity. This indicates a low degree of genomic homology. However, at the level of structural proteins, a certain homology can be found, as was indicated by the formation of heterodimers between parental proteins. In terms of gene expression or regulation, no genes were found to be silenced. Also, no evidence for competition or suppression of genes or parts of genomes could be obtained.

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