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RESEARCH ARTICLE

Relationship of wild and cultivated forms of *Pisum* L. as inferred from an analysis of three markers, of the plastid, mitochondrial and nuclear genomes

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Abstract Eighty-nine accessions of wild and cultivated peas (12 *Pisum fulvum* Sibth. et Smith., 7 *P. abyssinicum* A. Br., 31 wild and 42 cultivated forms of *P. sativum* L.) were analysed for presence of the variants of three functionally unrelated polymorphic markers referring to different cellular genomes. The plastid gene *rbcL* either contains or not the recognition site for restriction endonuclease *Asp*LEI (*rbcL+* vs. *rbcL–*); the mitochondrial gene *cox1* either contains or not the recognition site for restriction endonuclease *Psi*I (*cox1+* vs. *cox1–*); the nuclear encoded seed albumin SCA is represented by slow (SCA^S) or fast (SCA^F) variant. Most of the accessions possessed either of two marker combinations: 24 had SCA^F *cox1+ rbcL+* (combination A) and 49 accessions had SCA^S *cox1– rbcL–* (combination B), 16 accessions represented 5 of the rest 6 possible combinations. All accessions of *P. fulvum* and *P. abyssinicum* had combination A, the overwhelming majority of cultivated forms of *P. sativum* had combination B while wild representatives of *P. sativum* had both combinations A and B, as well as rare combinations. This pattern indicates that combination A is the ancestral state in the genus *Pisum* L., inherited by *P. fulvum* and *P. abyssinicum*, while combination B seems to have arisen in some lineage of wild *P. sativum* which rapidly fixed mutational transitions of the three markers studied, most probably via a bottleneck effect during the Pleistocene. Then this 'lineage B' spread over Mediterranean and also gave rise to cultivated forms of *P. sativum*. Rare combinations may have resulted from occasional crosses between 'lineage A' and 'lineage B' in nature or during cultivation, or represent intermediate evolutionary lineages. The latter explanation seems relevant for an Egyptian cultivated form '*Pisum jomardii* Schrank' (SCA^F *cox1– rbcL–*) which is here given a subspecies rank. Wild representatives of *P. sativum* could be subdivided in two subspecies corresponding to 'lineage A' and 'lineage B' but all available subspecies names seem to belong to lineage B only. Presently all wild forms would better be considered within a fuzzy paraphyletic subspecies *P. sativum* subsp. *elatius* (Bieb.) Schmalh. s. l.

Keywords
Cleaved amplified polymorphic sequence · Garden pea · Intrageneric phylogeny · Intraspecies taxonomy · Microevolution · Origin of cultivated peas

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Introduction

A regular plant cell contains three types of genomes: the small genomes of plastids and mitochondria and

the huge nuclear genome. The organelle (plastid and mitochondrial) genomes propagate as clones and most frequently are maternally inherited, while the nuclear genome is reshuffled each generation in meiosis by segregation and homologous recombination of chromosomes obtained from both parents. Therefore, the nuclear and cytoplasmic genomes have quite different histories and their analysis may result in quite different phylogenetic reconstructions. Combination of data obtained for all the three genomes allows a better insight into phylogenetic relationships of related forms at the microevolutionary level.

The garden pea (*Pisum sativum* L.) is an ancient and still important cultivated plant of the Old World origin, used as crop, vegetable and fodder. Besides, it is the classical genetic object. Along with a great variety of cultivated peas, a substantial diversity of wild pea forms exists in nature. Although very small and restricted, populations of wild pea are scattered over a great area of the Mediterranean, with the greatest diversity in the Anterior Asia. Some populations are now represented only in germplasm collections.

The existing taxonomy of wild peas is confusing, that results from a great diversification of local populations, which, in turn, is facilitated by self-pollination as the predominant mode of pea reproduction, so that gene exchange between populations is small. Along with a large and very variable species *Pisum sativum* L., all authors recognise a clear-cut and peculiar species *Pisum fulvum* Sibth. et Smith., which is confined to Anterior Asia and reproductively almost completely isolated from *P. sativum*. Recent authors also isolate *Pisum abyssinicum* A. Br. as *bona species*, represented by cultivated and some wild forms from South Arabia and Ethiopia and differing from *Pisum sativum* s. str. by chromosome rearrangements as well as some morphological traits. Other taxa once considered as species are in fact representatives of *Pisum sativum* s. l. Some of them are presently considered as subspecies, although the subspecies concept in case of the pea remains quite vague. Main germplasm collections have accumulated a great number of wild pea accessions, but many of those are doubled under different designations, and some are contaminated by genes of other wild and cultivated forms in the course of reproduction, that poses difficulties for classification and phylogenetic analysis. In fact, comparison of the results of different phylogenetic analyses is difficult due to different sets of accessions used and an incomplete information on taxonomic attribution and provenance of the wild representatives involved.

In our study of inheritance of plastids (Bogdanova and Kosterin 2006) and mitochondria (Bogdanova 2007) in pea using a CAPS (Cleaved Amplified Polymorphic DNA) approach we found polymorphisms for recognition sites of restriction endonucleases in plastid and mitochondrial genomes. In this work, we analyse three polymorphic molecular markers, each belonging to one of the three cellular genomes: nuclear, plastid and mitochondrial. We found that each variant of the three markers analysed is shared roughly by a half of wild pea forms and their occurrence is mostly concordant, while the overwhelming majority of cultivated forms have the same variant. This sheds light onto phyletic relationship and putative origin of different peas.

Material and methods

Material

Many accessions of wild and cultivated peas were received from the Vavilov All-Russian Institute of Plant Breeding, St.Petersburg (designated here with prefix 'VIR') the Weibullsholm collection, Landskrona (designated 'WL') via the courtesy of Ms. Brigitte Lund. A valuable set of accessions of wild peas from different collections, including John Innes Centre, Norwich (designated 'JI'), Pullman Institute, Pullman (designated 'PI') and those collected by Dr. F. Muehlbauer with colleagues in Turkey (designated 'P') were obtained through the courtesy of Dr. N. Weeden (Boseman, USA). The samples collected by Ben-Ze'ev and Zohary (1973) (numbers without prefix) were kindly provided by Dr. N. O. Polans (De Kalb, Illinois), and some of them (designated as 'L') by Dr. N. Weeden. Accessions CE1 and CE2 were collected in Crimea by ourselves. To make our result comparable with those of other research teams and to exclude material doubling we provide in Table 1 information concerning the accessions studied including their origin and, if known, designations of the same stock in other germplasm collections.

VIR320 is a highly heterogeneous accession and might have resulted from a spontaneous cross of a

Table 1 Presence of *Psi*I recognition site in the mitochondrial *cox1* gene, *Asp*LEI recognition site in the plastid *rbcL* gene, and allelic variants of the SCA protein in accessions of wild and cultivated peas

Accession number[a]	Provenance, as labelled on the samples or indicated in literature	Wild or cultivated	Original taxonomic attribution	Here accepted taxonomic attribution	*Psi*I recognition site present	*Asp*LEI recognition site present	SCA variant
VIR2523; =JI2203 (=WL1256; =PI595947)	Palestine, 1925	Wild	*Pisum fulvum* var. *nitidum* Makasheva	*Pisum fulvum*	Yes	Yes	F
VIR3397 (=WL2030)	Palestine, 1925	Wild	*Pisum fulvum* var. *incisum* Post subvar. *inciso-brunneum* Makasheva	*Pisum fulvum*	Yes	Yes	F
VIR6070	Palestine, foothills approx. 30 km SW of Jerusalem, 1960	Wild	*Pisum fulvum* var. *integrifoliatum* Makasheva et var. *virescens* Makasheva	*Pisum fulvum*	Yes	Yes	F
VIR6071	Palestine, foothills approx. 30 km SW of Jerusalem, 1960	Wild	*Pisum fulvum* var. *striatum* Makasheva	*Pisum fulvum*	Yes	Yes	F
WL2140; =Wt303 (=WL2029; =JI224; =PI 560061)	Israel, the valley of Cross	Wild	*Pisum fulvum*	*Pisum fulvum*	Yes	Yes	F
Wt301	Palestine	Wild	*Pisum fulvum*	*Pisum fulvum*	Yes	Yes	F
701; =L93 (=JI3265; =PI560062)	Israel, Jerusalem, Valley of the Cross, among trees	Wild	*Pisum fulvum*	*Pisum fulvum*	Yes	Yes	F
702 (=L94; =JI3266; =PI560063)	Israel, Upper Galilee, 2 km E of Safed, Mt. Canaan	Wild	*Pisum fulvum*	*Pisum fulvum*	Yes	Yes	F
703 (=L95; =JI3267; =PI560064)	Israel, ca.15 km SW of Jerusalem, env. Of Bar Gyyora, shrubs	Wild	*Pisum fulvum*	*Pisum fulvum*	Yes	Yes	F
706; =L96 (=JI3268; =PI560065)[d]	Israel, Ruhama, a coastal plain, on calcareous sandstone, in dwarf-shrub formation	Wild	*Pisum fulvum*	*Pisum fulvum*	Yes	Yes	F
707 (=L97; =JI3269; =PI560066)	Israel, W Galilee, Adamit, opening in maquis and roadside	Wild	*Pisum fulvum*	*Pisum fulvum*	Yes	Yes	F
708 (=L98; =JI370; =PI560067)	Israel, 2 km W of Jerusalem, Jerusalem forest, on stone mounds and abandoned fields	Wild	*Pisum fulvum*	*Pisum fulvum*	Yes	Yes	F
Wt6	Unknown	Cultivated	*Pisum abyssinicum*	*P. abyssinicum*	Yes	Yes	F
WL808	Unknown	Cultivated	*Pisum abyssinicum*	*P. abyssinicum*	Yes	Yes	F

Table 1 continued

Accession number[a]	Provenance, as labelled on the samples or indicated in literature	Wild or cultivated	Original taxonomic attribution	Here accepted taxonomic attribution	*Psi*I recognition site present	*Asp*LEI recognition site present	SCA variant
VIR2759 (=WL1491; WL2042)	Aethiopia	Cultivated	*P. sativum* subsp. *abyssinicum* var. *vavilovianum* Govorov	*P. abyssinicum*	Yes	Yes	F
WL1445	Unknown	Cultivated	*Pisum abyssinicum* var. *vavilovianum*	*P. abyssinicum*	Yes	Yes	F
WL1446	Unknown	Cultivated	*Pisum abyssinicum* var. *viridulogriseum* Govorov	*P. abyssinicum*	Yes	Yes	F
JI1876 (=PI 358610)	Aethiopia, Makale, Adishen market	Cultivated	*P. sativum* subsp. *abyssinicum*	*P. abyssinicum*	Yes	Yes	F
VIR3567	Yemen, Dammar	Cultivated	*P. sativum* subsp. *abyssinicum*	*P. abyssinicum*	Yes	Yes	F
VIR320	Palestine (received from Sutton, France, by N. I. Vavilov in 1922)	Wild	*P. sativum* subsp. *syriacum* var. *syriacum*	*P. sativum* subsp. *elatius*	Yes	Yes	F
VIR320*	- " -	Wild (?), probably a result of an occasional cross	*P. sativum* subsp. *syriacum* var. *pulchristipulatum* Makasheva	*P. sativum* subsp. *elatius*	***Yes***	***No***[b]	F
VIR2521 (=WL2034)	Palestine, Kinereth, 1923	Wild	*P. sativum* subsp. *syriacum var. marginatifoliatum* Makash.	*P. sativum* subsp. *elatius*	Yes	Yes	F[c]
711; =L99 (=JI3272; =PI560068)	Israel, 2 km W of Jerusalem, Jerusalem forest, edges of an abandoned terrace field	Wild, weed	*Pisum humile*, 'southern'	*P. sativum* subsp. *elatius*	Yes	Yes	F
712; =L100 (=JI3273; =PI560069)	Israel, 10 km S of Be'er Sheva; wadi bed with loess deposit, as a weed in barley	Wild, weed	*Pisum humile*, 'southern'	*P. sativum* subsp. *elatius*	Yes	Yes	F
713; =L101 (=JI3274; =PI560070)	Israel, Beit Kama, southern coastal plain, field edges and road sides	Wild, weed	*Pisum humile*, 'southern'	*P. sativum* subsp. *elatius*	Yes	Yes	F
714 (=L102; =JI3275; =PI560071)	Israel, between Bet Shemesh and Bet Gurvin, field edges and roadsides.	Wild, weed	*Pisum humile*, 'southern'	*P. sativum* subsp. *elatius*	Yes	Yes	F

Table 1 continued

Accession number[a]	Provenance, as labelled on the samples or indicated in literature	Wild or cultivated	Original taxonomic attribution	Here accepted taxonomic attribution	*Psi*I recognition site present	*Asp*LEI recognition site present	SCA variant
JI1794; ?=716[e]	Golan Heights, ca. 3 km NW of Quneitra, Tel Abu Nida, on volcanic ash	Wild	*Pisum humile*, 'northern'	*P. sativum* subsp. *elatius*	No	No	S
VIR7327	Turkey	Wild	*P. sativum* subsp. *syriacum*	? *P. sativum* subsp. *elatius*	***No***	***No***	***F***
VIR7328	Turkey	Wild	*P. sativum* subsp. *syriacum*	? *P. sativum* subsp. *elatius*	***No***	***No***	***F***
VIR7329	Turkey ("received from USA")	Wild	*P. sativum* subsp. *syriacum*	*P. sativum* subsp. *elatius*	No	No	S
PI343993; =82-15 (=WL2035)	Turkey, 5 km SW of Kale, dry farm, dark clay soil from limestone: 100 m above sea level, 1969	Wild	Unknown	*P. sativum* subsp. *elatius*	***No***	***Yes***	F
JI261 (=WL2038)	Turkey, Gilindire	Wild	*Pisum humile*	*P. sativum* subsp. *elatius*	Yes	Yes	F
P002; 050689-0302	Turkey, Mardin Prov,, 1050 m (coll. by F. Muehlbauer and colleagues in 1989)	Wild	*P. sativum* subsp. *humile*	*P. sativum* subsp. *elatius*	No	No	S
P008; 120689-0202	Turkey, Siirt Prov., 630 m (coll. by F. Muehlbauer and colleagues in 1989)	Wild	*P. sativum* subsp. *humile*	*P. sativum* subsp. *elatius*	***Yes***	***No***	S
P015; 090785-03	Turkey, Diyarbakir Prov., 1250 m (coll. by F. Muehlbauer and colleagues in 1985)	Wild	*P. sativum* subsp. *humile*	*P. sativum* subsp. *elatius*	No	No	S
WL2123	Jordan Valley	Wild	*Pisum elatius*	*P. sativum* subsp. *elatius*	***Yes***	***No***	S
VIR2524 (WL1488)	North Galilea, in maccia Tarschich et Peccia	Wild	*P. sativum* subsp. *elatius* var. *palestinicum* Makash. et *galilaeicum* Makash.	*P. sativum* subsp. *elatius*	Yes	Yes	F[c]
721; =L104 (=JI3262; =PI560058)	Israel, Mt. Carmel, 5 km NE of Zikhron Ya'akov, in maquis.	Wild	*P. sativum* subsp. *elatius*	*P. sativum* subsp. *elatius*	Yes	Yes	F
722 (=L105 (=JI3263; =PI560059)	Israel, W Galilee, Adamit, opening in maquis and roadside	Wild	*P. sativum* subsp. *elatius*	*P. sativum* subsp. *elatius*	Yes	Yes	F
WL805	Turkey, Anatolien	Wild	*Pisum elatius*	*P. sativum* subsp. *elatius*	***No***	***Yes***	S

Table 1 continued

Accession number[a]	Provenance, as labelled on the samples or indicated in literature	Wild or cultivated	Original taxonomic attribution	Here accepted taxonomic attribution	*Psi*I recognition site present	*Asp*LEI recognition site present	SCA variant
P012; 050785-0109	Turkey, Adiyamen Prov., 380 m (coll. by F. Muehlbauer and colleagues in 1985)	Wild	*P. sativum* subsp. *elatius*	*P. sativum* subsp. *elatius*	No	No	S
P013; 190785-0105	Turkey, Tokat Prov., 750 m (coll. by F. Muehlbauer and colleagues in 1985)	Wild	*P. sativum* subsp. *elatius*	*P. sativum* subsp. *elatius*	Yes	Yes	F
P016; 290685-01	Turkey, Denizli Prov., 16 km from Samandag on the road to Yaybdagi, 430 m (coll. by F. Muehlbauer and colleagues in 1985)	Wild	*P. sativum* subsp. *elatius*	*P. sativum* subsp. *elatius*	No	No	S
P017; 270685-0105	Turkey, Mersin Prov., 790 m (coll. by F. Muehlbauer and colleagues in 1985)	Wild	*P. sativum* subsp. *elatius*	*P. sativum* subsp. *elatius*	No	No	S
VIR1851	Georgia, Tbilisi, Botanical Garden	Wild	*P. sativum* subsp. *elatius* var. *elatius* subvar.*elatius-maculatum* Makash.	*P. sativum* subsp. *elatius*	No	No	S
VIR2998	Georgia (from the Riga Botanical Garden)	Wild	*P. elatius* subsp. *caspicum* Govorov	*P. sativum* subsp. *elatius*	No	No	S
VIR4014	Azerbaijan, Lenkoran, a winter wheat field	Wild, weed	*P. sativum* subsp. *elatius* var. *elatius* subvar. *elatius-marmoratum* Makash. et var. *brevipedunculatum* Davis et Meikle	*P. sativum* subsp. *elatius*	No	No	S
CE1	Crimea, Simeiz (coll. by Y. Trusov and O. Kosterin in 1990).	Wild	*P. sativum* subsp. *elatius*	*P. sativum* subsp. *elatius*	No	No	S
CE2	Crimea, Kara-Dag, Svyataya Mt. (coll. by Y. Trusov and O. Kosterin in 1990).	Wild	*P. sativum* subsp. *elatius*	*P. sativum* subsp. *elatius*	No	No	S
PI344008	Greece, 1 km S of Daphne, Mt. Athos peninsula	Wild	*P. sativum* subsp. *elatius*	*P. sativum* subsp. *elatius*	***No***	***Yes***	F
PI344537	Italy, Sicily	Wild	*P. sativum* subsp. *elatius*	*P. sativum* subsp. *elatius*	***No***	***No***	***F***

Table 1 continued

Accession number[a]	Provenance, as labelled on the samples or indicated in literature	Wild or cultivated	Original taxonomic attribution	Here accepted taxonomic attribution	*Psi*I recognition site present	*Asp*LEI recognition site present	SCA variant
723 (=JI3271; =560060; =L106)	Italy, Sardinia, env. of Caligari	Wild	*Pisum elatius*	*P. sativum* subsp. *elatius*	Yes	Yes	F
82-20 (=PI273209)	Unknown	Wild	*P. sativum* subsp. *elatius*	*P. sativum* subsp. *elatius*	***Yes***	***Yes***	***S***
L90	Unknown	Wild	*P. sativum* subsp. *elatius*	*P. sativum* subsp. *elatius*	***Yes***	***No***	S
VIR3424	Egypt	Cultivated	*P. sativum*	*P. sativum* subsp. *jomardii*	***No***	***No***	***F***
VIR3429	Egypt	Cultivated	*P. sativum*	*P. sativum* subsp. *jomardii*	***No***	***No***	***F***
VIR3439	Egypt, Assuan	Cultivated	*P. sativum*	*P. sativum* subsp. *jomardii*	***No***	***No***	***F***
VIR3171	Unknown (Madrid, Botanical Garden)	Cultivated?	*P. sativum*	*P. sativum* subsp. *jomardii*	***No***	***No***	***F***[c]
VIR3249	Georgia, Gori	Cultivated	*P. sativum* subsp. *transcaucasicum* var. *mitabicum* Makasheva	*P. sativum* subsp. *transcaucasicum*	No	No	S
VIR4871	Georgia	Cultivated	*P. sativum* subsp. *transcaucasicum*	*P. sativum* subsp. *transcaucasicum*	No	No	S
VIR3115 (=WL2028)	Italia, Catania	?[f]	*P. sativum* subsp. *elatius* var. *catanicum* Makash. et var. *italicum* Makash.	*P. sativum* subsp. *sativum*	No	No	S
VIR1884	Afghanistan, Maymene, 2860 m above sea level, market	Cultivated	*P. sativum* subsp. *asiaticum*	*P. sativum* subsp. *sativum*	No	No	S
VIR1915	Afghanistan, Katagan, Tli kishlak, 3050 m	Cultivated	*P. sativum* subsp. *asiaticum* var. *candagaricum* Makash. subvar. *candagarico-fuscum* Makash.	*P. sativum* subsp. *sativum*	No	No	S

Table 1 continued

Accession number[a]	Provenance, as labelled on the samples or indicated in literature	Wild or cultivated	Original taxonomic attribution	Here accepted taxonomic attribution	*Psi*I recognition site present	*Asp*LEI recognition site present	SCA variant
VIR1975	Afghanistan, Gerat	Cultivated	*P. sativum* subsp. *asiaticum* var. *ivanovii* Makash. subvar *ivanovii-marmoratum* Makash.	*P. sativum* subsp. *sativum*	***No***	***Yes***	S
VIR3513	Afghanistan, Vahan	Cultivated	*P. sativum* subsp. *asiaticum*	*P. sativum* subsp. *sativum*	No	No	S
VIR178	Tadjikistan, the Pamirs	Cultivated	*P. sativum* subsp. *asiaticum*	*P. sativum* subsp. *sativum*	No	No	S
VIR261	Tadjikistan, the Pamirs	Cultivated	*P. sativum* subsp. *asiaticum*	*P. sativum* subsp. *sativum*	No	No	S
VIR1448	Tadjikistan, Darvaz, Tobi-Dara	Cultivated	*P. sativum* subsp. *asiaticum*	*P. sativum* subsp. *sativum*	No	No	S
VIR3940	Tadjikistan, the Shakh-Dara River, Shitam kishlak	Cultivated	*P. sativum* subsp. *asiaticum*	*P. sativum* subsp. *sativum*	No	No	S
VIR3954	Tadjikistan, Shugnan District, Emch kishlak	Cultivated	*P. sativum* subsp. *asiaticum*	*P. sativum* subsp. *sativum*	No	No	S
VIR4974	Tadjikistan, Gornyi Badakhshan, Ishkashim District	Cultivated	*P. sativum* subsp. *asiaticum*	*P. sativum* subsp. *sativum*	No	No	S
VIR5195	Tadjikistan	Cultivated	*P. sativum* subsp. *asiaticum*	*P. sativum* subsp. *sativum*	No	No	S
VIR7335	Tadjikistan, Gorno-Badakhshan Autonomous Region, Rushan District, Sipondzh kishlak, 25000 m	Cultivated	*P. sativum* subsp. *asiaticum*	*P. sativum* subsp. *sativum*	***No***	***No***	***F***
VIR4911	Tibet, Lhasa, 4200 m a. s. l.	Cultivated	*P. sativum* subsp. *asiaticum*	*P. sativum* subsp. *sativum*	No	No	S
VIR990	Inner Mongolia, Tsagan-Mugzhan on Bara-Gol	Cultivated	*P. sativum* subsp. *asiaticum*	*P. sativum* subsp. *sativum*	No	No	S
VIR1464	China, Manshuria	Cultivated	*P. sativum* subsp. *asiaticum*	*P. sativum* subsp. *sativum*	No	No	S
VIR5166	China, Shenxi, Uchun	Cultivated	*P. sativum* subsp. *asiaticum*	*P. sativum* subsp. *sativum*	No	No	S
VIR1007	Mongolia, a Chinese farm in the Usu-Sere valley	Cultivated	*P. sativum* subsp. *asiaticum*	*P. sativum* subsp. *sativum*	No	No	S

Table 1 continued

Accession number[a]	Provenance, as labelled on the samples or indicated in literature	Wild or cultivated	Original taxonomic attribution	Here accepted taxonomic attribution	*Psi*I recognition site present	*Asp*LEI recognition site present	SCA variant
VIR6191	Belorussia	Cultivated	*P. sativum* subsp. *sativum*	*P. sativum* subsp. *sativum*	No	No	S
VIR1120	Belorussia, Zhubilovo	Cultivated	*P. sativum* subsp. *sativum*	*P. sativum* subsp. *sativum*	No	No	S
VIR5078; =cv. Vinko	Holland	Cultivated	*P. sativum* subsp. *sativum* var. *pseudoalbiflorum* Makash.	*P. sativum* subsp. *sativum*	No	No	S
VIR5432	France	Cultivated	*P. sativum* subsp. *sativum*	*P. sativum* subsp. *sativum*	No	No	S
VIR1937	Austria	Cultivated	*P. sativum* subsp. *sativum*	*P. sativum* subsp. *sativum*	No	No	S
VIR3188	Spain	Cultivated	*P. sativum* subsp. *sativum*	*P. sativum* subsp. *sativum*	No	No	S
VIR6144	Greece, a 'winter pea'	Cultivated	*P. sativum* subsp. *sativum*	*P. sativum* subsp. *sativum*	No	No	S
VIR2593	Cyprus, Nikosia	Cultivated	*P. sativum* subsp. *sativum*	*P. sativum* subsp. *sativum*	No	No	S
VIR2501	Turkey, Denizli	Cultivated	*P. sativum* subsp. *sativum*	*P. sativum* subsp. *sativum*	No	No	S
VIR3262	Turkey	Cultivated	*P. sativum* subsp. *sativum*	*P. sativum* subsp. *sativum*	No	No	S
P014; 190785-02	Turkey, Tokat Prov., 900 m (coll. F. Muehlbauer in 1985)	Cultivated	*P. sativum* subsp. *arvense*	*P. sativum* subsp. *sativum*	No	No	S[b]
VIR6103	Morocco, Rabat	Cultivated	*P. sativum* subsp. *sativum*	*P. sativum* subsp. *sativum*	No	No	S
VIR7163	Lebanon	Cultivated	*P. sativum* subsp. *sativum*	*P. sativum* subsp. *sativum*	No	No	S
VIR7006	Syria, Damask	Cultivated	*P. sativum* subsp. *sativum*	*P. sativum* subsp. *sativum*	No	No	S
VIR2516	Palestine	Cultivated	*P. sativum* subsp. *sativum*	*P. sativum* subsp. *sativum*	No	No	S
VIR2172	Palestine, Prosch-Pina Agelet Hoschahas	Cultivated	*P. sativum* subsp. *sativum*	*P. sativum* subsp. *sativum*	No	No	S

Table 1 continued

Accession number[a]	Provenance, as labelled on the samples or indicated in literature	Wild or cultivated	Original taxonomic attribution	Here accepted taxonomic attribution	*Psi*I recognition site present	*Asp*LEI recognition site present	SCA variant
VIR1853	Ethiopia	Cultivated	*P. sativum* subsp. *sativum*	*P. sativum* subsp. *sativum*	No	No	S
VIR5414	Ethiopia, Godzhan Province, Takhr-Dar	Cultivated	*P. sativum* subsp. *sativum*	*P. sativum* subsp. *sativum*	No	No	S

[a] Where known, identity to accessions of other collections is indicated: in parentheses if the synonymous accession was not studied here and without parenthesis if the synonymous accession has been studied as well. In the latter case, the accession number published in an available paper, eg. By Ben-Ze'ev and Zohary (1973) is indicated first and this designation is used further in the text

The prefices used with the accession numbers refer to the following collections:

VIR—All Russian Institute of Plant Breeding, St. Petersburg, Russia;

JI—John Innes Centre, Norwich, United Kingdom;

WL—Weisbullholm collection, Landscrona, Sweden;

PI—USDA, Dept. Agronomy, Washington State University, Pullman, Washington, USA;

P—accessions collected by Dr. F. Muehlbauer from Washington State Univ. made available to us through the courtesy of Dr. N. Weeden;

Wt—Wiatrowo collection, Poland;

L—H. Lamprecht's collection (Dr. M. Ambrose, pers. comm.);

701–723 without prefices—the lines by Ben-Ze'ev and Zohary (1973) made available to us through the courtesy of Dr. N. Polans

The table may still harbor some synonyms

[b] Unusual ('recombinant') combinations of markers are bold italicized

[c] In these accessions both variants of SCA were found during a large-scale survey (see Smirnova et al. 1999), but the individual plant analysed in the present study had the indicated variant

[d] 706 and L96 acquired from different sources, although claimed to be synonyms, appeared in fact very dissimilar (while in other cases of synonymy L lines and those of Ben-Ze'ev and Zohary were identical indeed)

[e] Dr. M. Ambrose kindly informed us that JI1794 was received by John Innes Centre from the Hebrew University of Israel, where Ben-Ze'ev and Zohary worked, with a label "Ramat haGolan", that means "Golan Heights" in Hebrew. Its identity with 716 is not proved but highly probable, provided they are identical in all available traits of the phenotype

[f] Accession VIR3115 is claimed to represent a wild form from Catania, Italia, but the plants grown from the seeds we obtained from VIR lack any trait of a wild pea

wild and cultivated pea with further segregation. Due to a special attention to this line (Bogdanova and Kosterin 2006), here we treat it as two accessions, VIR320 and VIR320*, representing the most contrasting variants. WL2123 is also heterogeneous for many characters. We isolated the most wild-looking subline and investigated it under designation WL2123.

Plant growing and DNA extraction

Seeds were sown into a claydite/vermiculite mixture. From one seedling of each accession about 100 mg of leaves were rubbed with a teflon pestle through a stainless steel grid (1 × 1 mm) into a vessel containing 1.5 ml of 0.15 M NaCl. After centrifugation at 1500 *g* for 5 min in an 1.5 ml Eppendorf tube, the pellet was resuspended in 200 µl of a buffer containing 100 mM Tris–HCl (pH 8.0), 100 mM NaCl, 5 mM EDTA, 0.5% SDS (w/v), stirred and left for 30 min at room temperature for extraction. After centrifugation at 6000 *g* for 5 min, the supernatant was collected and, for DNA purification, added with an equal volume of 5 M LiCl solution, stirred and left for 15 min on ice. Then the mixture was centrifuged at 6000 *g* for 5 min, the supernatant collected, added with 1 ml of 96% ethanol and left for an hour on ice for precipitation. The precipitate was collected by centrifugation at 8000 *g* for 10 min, washed with 100 µl of 75% ethanol and centrifuged. The supernatant was discarded, the pellet dried at 50°C for 5 min and dissolved in 50 µl of deionised water. Optionally, insoluble contaminants were removed by centrifugation at 6000 *g* for 10 min, and the supernatant transferred to the fresh tubes.

PCR amplification and endonuclease restriction

A 1129-bp part of the coding region of the plastid *rbcL* gene was PCR-amplified. Primers were designed to match the X03853 accession: 5′-TTAT TATACTCCTGACTATCAAACC and 5′-TACAGA ATCATCTCCAAATATCTCG. The cycling parameters used: 95°C for 1 min followed by 35 cycles including denaturation at 94°C 59 s, annealing at 58°C 59 s, elongation at 72°C 1 min. 5 µl of the reaction were digested with 5 units of *Asp*LEI endonuclease according to manufacturer's recommendations, incubated for 2 h at 37°C, the products were analysed in 1.5% agarose gel in TAE buffer.

A 1200-bp part of the coding region of the mitochondrial *cox1* gene was PCR-amplified using primers designed to match the X14409 accession: 5′-TGGTAATTGGTCTGTTCCGATTCT and 5′-CCA-CTGCTTGAAGTGATTGTTACG. The cycling parameters used: 95°C for 1 min followed by 38 cycles including denaturation at 94°C 59 s, annealing at 56°C 45 s, elongation at 72°C 1 min. 5 µl of the reaction were then treated with 1 unit of *Psi*I endonuclease for 2 h at 37°C and the products analysed in 1.5% agarose gel in TAE buffer.

Isolation and electrophoresis of the SCA albumin

About 30 mg of dry cotyledons were powdered in a mortar and homogenised in 1 ml of 5% $HClO_4$. After centrifugation at 1500*g* for 5 min the protein was recovered from supernatant by adding 6 volumes of acetone and sulphuric acid to final concentration of 0.5 M and precipitated at 4°C for 2 h. The precipitated protein was centrifuged and dissolved in 0.2 ml of a medium containing 0.9 M acetic acid, 8 M urea and 15% (w/v) sucrose (Smirnova et al. 1992). The preparations were electrophoresed in slabs of 15% polyacrilamide/methylenbisacrylamide gel containing 6.25 M urea and 0.9 M acetic acid, according to a modified method of Panyim and Chalkley (1969). After electrophoresis, the gels were stained in 0.01% (w/v) Coomassie Brilliant-Blue R250 in 0.9 M acetic acid and destained by diffusion in 0.9 M acetic acid.

Results

The amplified portion of the plastid gene *rbcL* is 1129 bp long and either contains or not the recognition site for the *Asp*LEI endonuclease, which cuts it into two fragments of about 800 and 300 bp (Fig. 1). Sequencing of the part of the *rbcL* in VIR320 showed that the restriction site is conditioned by a synonymous nucleotide substitution T- > C in the position 325 from the beginning of the primer used. Further in the text, we designate the variant with the restriction site as *rbcL+* and without the site as *rbcL–*. The PCR-amplified portion of the coding region of the

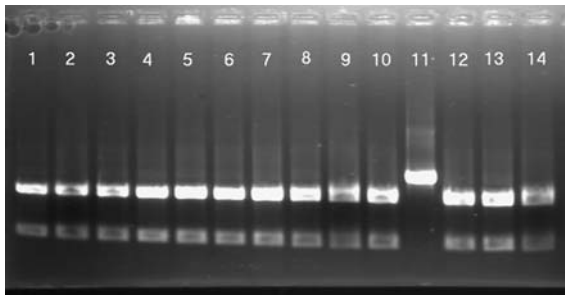

Fig. 1 Products of *Asp*LEI digestion of PCR-amplified part of the plastid *rbcL* gene from the wild pea accessions from the collection of Ben-Ze'ev and Zohary (1973). Lanes 1–14 correspond to the following accessions: 1, 701; 2, 702; 3, 703; 4, 706; 5, 707; 6, 708 (*Pisum fulvum*); 7, 711; 8, 712; 9, 713; 10, 714; 11, 716 ('*P. humile*'); 12, 721; 13, 722; 14, 723 ('*P. elatius*'). (The original designation of taxa by the cited authors is here retained)

mitochondrial *coxI* gene is about 1200 bp and eihter contains or not a recognition site for *Psi*I endonuclease which, provided the site is present, digests it into two fragments of about 260 and 940 bp (Fig. 2). The seed albumin SCA has two well recognisable electromorphs (Fig. 3), SCA^S and SCA^F, containing 9 and 10 lysine residues, respectively (Smirnova et al. 1992). Table 1 shows which variants of the three markers are present in the accessions studied.

We assayed 89 accessions of wild and cultivated peas (Table 1), each represented by one plant. Since

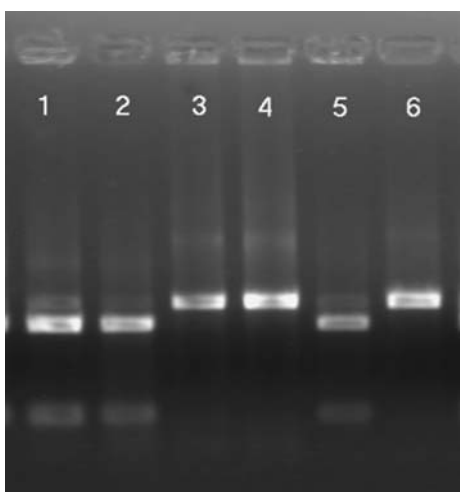

Fig. 2 Products of *Psi*I digestion of the PCR-amplified part of the mitochondrial *cox1* gene from some wild pea accessions. Lanes 1–6 correspond to the following accessions: 1, WL2140 (*Pisum fulvum*); 2, WL2123; 3, CE1; 4, P002; 5, P008; 6, P016 (*P. sativum* subsp. *elatius* s. l.)

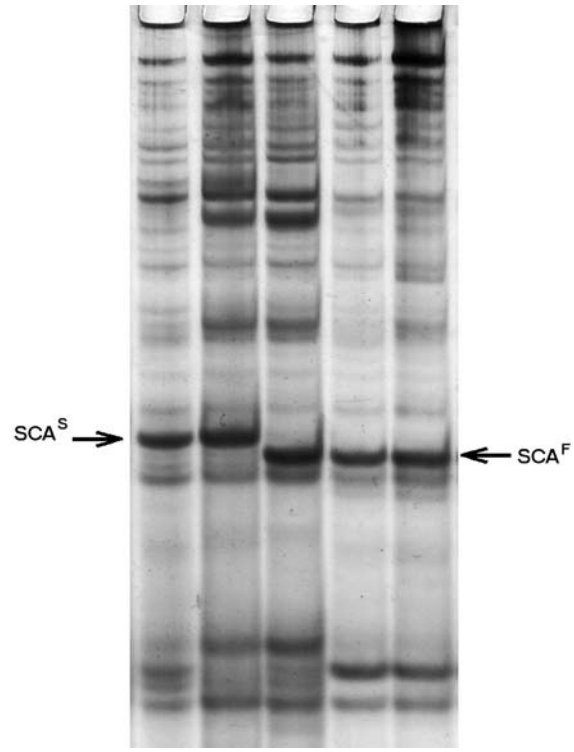

Fig. 3 Electrophoretic pattern of albumin SCA isolated from seeds of a heterogenous accession VIR2521 (*P. sativum* subsp. *elatius* s. l.) containing either SCA^S (two left lanes) or SCA^F variants (three right lanes)

the accessions were obtained from different sources and their originators and providers had different taxonomic concepts and sometimes used the botanical names not critically, the original taxonomic attribution is indicated along with the taxonomic attribution according to a conventional system accepted in this work (see Discussion).

The majority of accessions appeared to possess either of two combination of the three markers: 24 accessions have SCA^F *cox1*+ *rbcL*+ (let us call it combination A) and 49 accessions have SCA^S *cox1*– *rbcL*– (combination B). Other combinations of the eight possible are rare (combination SCA^F *cox1*– *rbcL*+ not found at all) and can be considered as exceptional 'recombinants', they are found in 16 accessions. Seven of them represent discordance in the variants of the plastid and mitochondrial markers:

SCA^F *cox1* + *rbcL*– found in VIR320* (originally identified as '*Pisum sativum* subsp. *syriacum* (Boiss. et Noë) Berger')

SCA^S *cox1* + *rbcL*– found in P008 and WL2123 (‘*Pisum elatius* Bieb.’);
SCA^S *cox1*– *rbcL*+ found in WL805 (‘*P. elatius*’), PI343993 (‘*Pisum humile* Boiss. et Noë’), PI344008 (wild) and VIR1975 (cultivated).

The rest nine unusual combinations represent ‘recombinants’ between two organelle markers on one side and an SCA variant on the other side:

SCA^S *cox1* + *rbcL*+ found in 82-20 (‘*P. elatius*’)
SCA^F *cox1*–, *rbcL*– found in VIR7327, VIR7328 (‘*P. s.* subsp. *syriacum* (Boiss. et Noë) Berger’), PI344557 (‘*P. elatius*’); VIR3424, VIR3429, VIR3439, VIR7335, VIR3171 (cultivated).

All available peas classified as *Pisum fulvum* Sibth. et Smith. (12 accessions) and *Pisum abyssinicum* A. Br. (7 accessions) possess combination A without exceptions. Of 42 analysed accessions of cultivated *Pisum sativum* L., 36 have combination B and 6 are ‘recombinants’. Among 31 tested wild representatives of *P. sativum* (in the here accepted taxonomic concept, see below), 21 belong to either of the conventional lineages A and B and 10 are ‘recombinants’. Among accessions which were originally designated as ‘*Pisum elatius* Bieb.’ or ‘*P. sativum* subsp. *elatius* (Bieb.) Schmalh.’, 3 have combination A, 8 have combination B, and 4 are ‘recombinants’. Of the accessions originally identified as ‘*Pisum humile* Boiss. et Noë’ or ‘*Pisum sativum* subsp. *syriacum* (Boiss. et Noë)’ Berger (that is the same taxon), 5 have combination A, 4 have combination B and 5 are ‘recombinants’. We have no information on the original identification for the two rest accessions of wild *Pisum sativum*.

Of the ‘recombinant’ combinations of markers, the most frequent (8 accessions) is SCA^F *cox1*– *rbcL*–. It is found in two accessions originally identified as *Pisum sativum* subsp. *syriacum*, one as *Pisum sativum* subsp. *elatius* (that is the same wild subspecies), three accessions representing locally cultivated peas of Egypt, one accessions from ‘Madrid Botanical Garden’ and one met among a collection of peas cultivated at high elevations in the Pamirs (Table 1). The Egyptian accessions represent the so-called ‘*Pisum jomardii* Schrank’, the plant from the Madrid Botanical Garden is also identical to this form as well.

Discussion

Evidence for major dichotomy within *Pisum sativum*

We studied three dimorphic markers each representing one of the three cellular genomes and found that each of the morphs had comparable frequencies in the available wild pea germplasm. A tight co-occurrence of the variants of the functionally unrelated markers with different types of inheritance was completely unexpected. The concerted variability of unrelated genes from different cellular genomes clearly indicates the common evolutionary fate of their carriers. Although three dimorphic markers are too few for a phylogenetic reconstruction, this striking coincidence points to a major divergence event in the history of the genus *Pisum* which concerned all the three cellular genomes and resulted in two main lineages. We conventionally call them lineage A (with the above mentioned combination A: SCA^F *cox1*+ *rbcL*+) and lineage B (combination B: SCA^S *cox1*– *rbcL*–).

Presently it is widely accepted that the genus *Pisum* contains a clear-cut and rather homogenous wild species *Pisum fulvum* Sibth et Smith., a small clear-cut, mostly cultivated species *Pisum abyssinicum* A. Br. (Govorov 1937; Ellis et al. 1998), and a large and variable aggregate of forms, wild and cultivated, which could be considered as species *Pisum sativum* L. in a broad sense (see Ben-Ze’ev and Zohary 1973). *P. abyssinicum* is morphologically much closer to *P. sativum*, so that some authors consider them conspecific (Makasheva 1979), but it was domesticated independently of *P. sativum* (Govorov 1937; Ellis et al. 1998). Both *P. fulvum* (Ben-Ze’ev and Zohary 1973) and *P. abyssinicum* (Rosen 1944; Lamprecht 1963) differ from *P. sativum* by a number of chromosome rearrangements which made them almost reproductively isolated, but the barrier is more substantial between *P. fulvum* and *P. sativum* than between *P. abyssinicum* and *P. sativum*. However, wild representatives of *P. sativum* also contain translocations and roughly fall into two karyotypic classes (Ben-Ze’ev and Zohary 1973). On the morphological basis, one can suppose that the most ancient divergence event within the genus had separated *P. fulvum*, next divergence separated *P. abyssinicum*, while the remaining lineage of *P. sativum* irradiated to provide a variety of forms (Fig. 4).

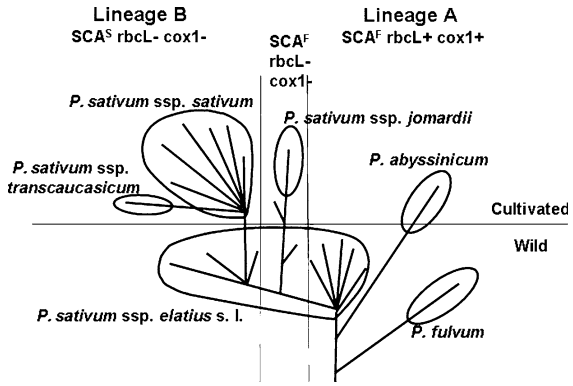

Fig. 4 Hypothetical scheme of phylogeny of the genus *Pisum*

We found that representatives of the both conventional lineages A and B are almost equally frequent within wild representatives of *Pisum sativum*. At the same time, the examined accessions of *P. fulvum* and *P. abyssinicum* belong entirely to the lineage A while almost all cultivated forms of *P. sativum* subsp. *sativum* L. belong to the lineage B. So we should conclude that the combination A is a plesiomorphic state and the variants SCA^F, *cox1+*, *rbcL+* should have existed in the common ancestor of the genus *Pisum* and inherited by *P. fulvum* and *P. abyssinicum*. Since wild forms of *P. sativum* represent both conventionally isolated lineages, we should assume that the allele variants SCA^S, *cox1–*, *rbcL–* as well as their steady combination in the lineage B, originated within this species. Most probably, this happened after the separation of *P. fulvum* and *P. abyssinicum* from the lineage leading to *P. sativum*. The opposite is highly improbable but not excluded. For example, the ancestors of *P. fulvum* and *P. abyssinicum* could inherit a part of pre-existing variation for the markers studied and then undergo rapid morphologic evolution.

Wild representatives of *Pisum sativum* were traditionally subdivided into two large groups (Govorov 1937; Ben-Ze'ev and Zohary 1973; Makasheva 1979): tall mesophylic plants of maquis (dense thickets of shrubs and low trees) of the Mediterranean were associated with the name '*elatius*' while rather small xerophylic plants growing in steppe-like communities of the Near East were associated with the names '*humile*' or '*syriacum*' (see 'Taxonomic implications' below). According to Ben-Ze'ev and Zohary (1973), the latter group differs from the former by a lower height, shorter internodes, peduncles and pods and smaller flowers. Makasheva (1979) characterised these differences numerically: plant height 80–250 vs 20–130 cm, 6 vs 4 sympodial bundles in the upper part of a plant, flowers 2–3.5 vs 1.3–1.8 cm, pods 5.8–8 vs less than 5 cm long. All these differences are quantitative and concern adaptations to two types of habitats. The subspecies characteristics given by Lehmann and Blixt (1984) are too strict and do not cover the real diversity of these wild forms. Cytogenetic subdivision by Ben-Ze'ev and Zohary (1973) did not coincide with morphological identification: the authors defined 3 '*elatius*' accessions and 3 '*humile*' accessions as referring to one karyotypic group, while 2 accessions of '*humile*', from Ankara and Golan Heights comprised another group, with the karyotype identical to that of cultivated peas. One representative of the second karyotypic group defined by Ben-Ze'ev and Zohary's as 'northern *humile*', 716 (=JI1794) belongs to the lineage B, that is in accordance with its karyotypic similarity to the cultivated peas, which also belong to the lineage B. Hence, the traditional subdivision into *elatius* versus *syriacum* = *humile* looks artificial. Townsend (1968) and Davis (1970) considered all wild forms of *Pisum sativum* as belonging to the same subspecies *P. s.* subsp. *elatius* s. l.

Our results show that both traditional groups of wild *Pisum sativum*, '*elatius*' and '*humile*'='*syriacum*', contain representatives of both conventional lineages, A and B, with some prevalence of the lineage B among '*elatius*' and lineage A among '*humile*' = '*syriacum*', as well as 10 (that is 28%) different 'recombinants'. In spite of the 'recombinant' combinations, each of which being rare, the A and B lineages may represent two main lineages of microevolution of *Pisum sativum* and hence two main natural (monophyletic) contemporary intraspecies taxa within it, with some intermediate forms existing ('recombinants').

Relative scarceness of 'recombinants' suggests that mutations leading to *cox1–*, *rbcL–*, and SCA^S occurred in some lineage of wild *P. sativum* for quite a short period of evolutionary time. Otherwise we would meet robust evolutionary lineages which diverged in the intervals between these mutational events. Most probably, the corresponding mutations were fixed by chance in some small population which gave rise to the lineage B.

Noteworthy, wild descendants of the lineage B then spread over a wider geographic range than that presently occupied by representatives of the ancestral lineage A. If we consider wild pea forms (plus cultivated *P. abyssinicum*), we see that Ethiopia, Yemen and a part of Palestine (the historical name implying the whole area regardless of the modern state borders) to the south of the Tiberiad Lake are occupied solely by the lineage A. The accession JI1794 (=716) from Golan Heights ('northern *humile*' by Ben-Ze'ev and Zohary (1973)) belongs to the lineage B. Turkey presents a mixture of lineages A and B with the latter predominant: of 10 wild accessions with the provenance available, 8 represent the lineage B. Two representatives of lineage A originate from central part (JI261 from Antalya vilayet and P015 from Tokat vilayet), and 'recombinant' P008 was collected in the east (Siirt vilayet). In Transcaucasia and Crimea, only lineage B is found. An accession from Greece and an accession from Sicily are 'recombinants' while that from Sardinia belongs to the lineage A. Unfortunately, we have no information on wild peas from western Mediterranean.

Most probably, existence of the supposed small founder population, which gave rise to lineage B, and its following wide expansion were associated with one of the climatic cycles of the Pleistocene. During these cycles, not only climate was changed and vegetational zones were shifted to and fro latitudinally, but the sea level also changed substantially, that led to periodical merging of islands into a continuous land and vice versa in the eastern Mediterranean, thus providing conditions for isolations and expansions.

Most of the 'recombinants' do not seem to represent old lineages of microevolution and might originate from spontaneous intercrosses between 'regular' pea forms which happened in nature. Although being generally a self-pollinator, pea is able of cross-hybridization. Cases of successful cross-pollination by insects do occur (Loenning 1984; Bogdanova and Berdnikov 2000). Unrelated forms of *P. sativum* hardly hybridise in nature since they are as a rule separated by some distance of mountainous relief, but they may easily cross-pollinate while reproduction in germplasm collections. The possibility that the 'recombinants' resulted from occasional crosses during reproduction in old germplasm collections looks especially plausible for two highly heterogeneous accessions, VIR320+VIR320* and WL2123. In this respect the relatively recent collections by Ben-Ze'ev and Zohary (1973) and Muehlbauer et al. (1990) are indicative. Neither of the samples from the collection of Ben-Ze'ev and Zohary available to us turned out to be a 'recombinant'. The material of F. Muehlbauer and colleagues was collected in Turkey in 1985 and 1989, and appeared at our disposal soon after propagation. Of 7 accessions tested, one is 'recombinant', one of the rest belongs to lineage A and 5 - to lineage B. So we found only one 'recombinant' sample in recently collected accessions.

One can wonder how the markers of the plastid and mitochondrial genomes may recombine with each other in all possible combinations, either in initial radiation of lineages or by secondary crosses, if both organelle genomes are maternally inherited. But this is not a strict rule. We have shown that in crosses of the VIR320 as a maternal parent with most of other accessions of *P. sativum*, maternal plastids are unable of proper functioning in the cytoplasm of F1 hybrids, however, green sectors can be seen on the otherwise chlorophyll-deficient background on leaflets and stipulae which acquire the paternal plastids, thus demonstrating deviation from the strictly maternal inheritance of the plastids, and discordancy with the inheritance of mitochondria (Bogdanova and Kosterin 2006; Bogdanova 2007). We succeeded to obtain a green viable F1 hybrid in a cross *P. fulvum* (WL2140) × *P. sativum* subsp. *sativum* (Sprint-1), in the direction supposed to be incompatible, and this hybrid possessed only paternal plastid DNA marker *rbcL*, while the mitochondrial marker *cox1* was maternally inherited (unpublished). These facts show that, in wild peas, post-zygotic barriers exist based on the nuclear-cytoplasmatic conflict and they may be overcome by uncanonical inheritance of organelles.

So, pea lineages co-existing in nature too hardly hybridise to provide an united population with free combination of characters, yet they are quite easily hybridise to produce some new lineages with combination of characters different from those of the parental ones, that is to be apt to reticulate mode of microevolution. Nevertheless, the extent of this reticulation is very small, since 'recombinants' are rare, especially in germplasm freshly collected in nature.

However, there is a class of 'recombinants', namely SCA^F *cox1*– *rbcL*–, which looks as representing a subtle lineage intermediate between A and B which might have sprout before formation of the latter, that is, after the mutational transitions of *cox1*+ to *cox1*– and *rbcL*+ to *rbcL*–. This class is represented by three accessions of the so-called '*Pisum jomardii* Schrank' from Egypt, two accessions originally designated as *P. sativum* ssp. *syriacum* from Turkey, an accession from the Madrid Botanical Garden, and an accession from the Pamirs. '*P. jomardii*' is characterised by smooth (*gty*) evenly olivaceous-green seeds without any spots and with light hilum (*pl*), very narrow leaflets, and small pale-coloured flowers with a narrowed standard. According to our unpublished data, it has an unique variant 2* of the histone H1 subtype 3, its histone H1 electrophoretic pattern according to the nomenclature introduced in Kosterin et al. (1994) being (2,2*121,2). The accession VIR3171 from the Madrid Botanical Garden exhibits the same phenotype, including the histone H1 electrophoretic pattern. No doubt, it represents '*P. jomardii*' as well. The accession VIR7335 from the Pamirs looks somewhat similar, has spotless seeds but with a black hilum (*Pl*), wider diamond-shaped leaflets, and different histone H1 pattern, represented by two variants: (1,1121,2) and (1, 1120,2) (unpublished). Anyway, it looks quite unusual for the Pamirian peas, a great collection of which we studied. The accessions VIR7327 and VIR7328 from Turkey, identified in VIR as *P. s.* subsp. *syriacum*, generally resemble VIR7335 in appearance and have similar H1 patterns (2, 1121, 2) and (1, 1121, 2), respectively. This group of accessions characterised by an unusual combination of the three markers studied may represents an old 'jomardii'-branch of the *Pisum sativum* evolution, or, alternatively, to be just of a hybrid origin.

The cultivated forms of *P. sativum* most probably represent a sublineage being just one of descendants of the lineage B within wild gene pool of this species, which was domesticated and then propagated, spread around the world and acquired a great variability of phenotypes as a result of unconscious and conscious selection. In fact, all the diversity of cultivated forms of *P. sativum* differ from *P. sativum* subsp. *elatius* s. l. only by characters associated with cultivation: first of all, in most (but not all) cases a greater vigour, biomass and seed productivity; non-dehiscing pods, non-tuberculate seed testa (*gty*). This character occurs in wild forms *P. sativum* as well as the tuberculate testa, *Gty*, without correlation with subspecific identification or variants of the three markers studied, but *Gty* is more frequent. The non-dehiscing pods, which are crucial for harvesting, is the only apomorphic character of cultivated forms.

We tested 37 accessions of the cultivated *P. sativum* for the three markers, and found only one unusual combination SCA^S *cox1*– *rbcL*+ in VIR1975 from Afghanistan (Table 1). This accession is highly heterogenous; e.g. contains 4 different haplotypes of the histone H1 gene cluster *His(2–6)* (not publishsed), so that contamination during reproduction cannot be excluded. Earlier we analysed about 850 accessions from the VIR collection of *P. sativum* for allelic variants of the SCA marker (Smirnova et al. 1992. and found them all to have SCA^S, with the only exception of SCA^F, found together with SCA^S in the accession VIR3658 (Xinjiang, Aksu, China), which is now lost. This accession looked as a normal cultivated pea and its heterogeneity for SCA, with the presence of SCA^F, might result from contamination during reproduction.

Comparison with results on other molecular characters

From the data on synonymous substitutions in the molecule of histone H1 subtype 1 (Trusov et al. 2004), the time of separation of *P. fulvum* and *P. sativum* may be estimated, although with a great uncertainty, as 560 thousand years ago, that is about a border of the Lower and Middle Pleistocene. The divergence within available accessions of *P. abyssinicum* domesticated independently of *P. sativum*, was dated by Ellis et al. (1998) as about 4,000 years ago but this does not inform us about the time of separation of *P. abyssinicum* from *P. sativum*.

Hoey et al. (1996) undertook an analysis of phylogenetic relationships of the set of lines of Ben-Ze'ev and Zohary (1973), added with some cultivars, using morphological, allozyme and RAPD characters. According to Hoey et al. (1996) *P. fulvum* is the most distant branch, the next branching is represented by 'southern *P. humile*', while the cultivated peas form a clade sprouting among different '*P. elatius*'. Position of the 'northern *P. humile*'

(line 716, =JI1794) depended on the method of tree construction and varied from being a sister group to cultivated peas and '*P. elatius*' to a separate subbranch within the branch of cultivated peas, the latter case corresponding the treatment of the 'northern *humile*' by Ben-Ze'ev and Zohary (1973). The same research team (Saar and Polans 2000) examined the set of Ben-Ze'ev and Zohary's lines for the sequence of internal transcribed spacers between the genes coding for nuclear ribosomal RNA, ITS1 and ITS2. The UPGMA analysis of their data revealed that '*P. elatius*' was the closest relative to the cultivated pea, followed by the 'northern *humile*', while the 'southern *humile*' was most distinct from the cultivars within *P. sativum*. Naturally, *P. fulvum* appeared to be the earliest branch of the tree.

Our notion of the divergence between the conventional lineages A and B within *Pisum* does not contradict the phylogenetic relationships of pea forms reconstructed by Ellis et al. (1998) by two methods: the amplified fragment length polymorphism approach (AFLP) and the sequence specific amplification polymorphism approach, with specific primers to the polypurine tract of *PDR1*, a *Ty1-copia* group retrotransposon (*PDR1* SSAP); the latter method used the polymorphism for the retrotransposon insertion sites. Both approaches resulted in phylogenetic trees in which *P. fulvum* and *P. abyssinicum* formed neighbouring but separate branches, a variety of cultivated *Pisum sativum* (including JI250 '*P. jomardii*') formed another large branch (in which highland Asiatic cultivated forms were grouped together). On the AFLP tree, the 11 branches leading to different accessions of '*P. elatius*' (the cited authors preserved original taxonomic identifications of accessions) occupied the space between the branches of *fulvum+abyssinicum* and cultivated *P. sativum*. On the *PDR1* SSAP tree, five of the *elatius*-lines (JI64, JI254, JI261, JI262, JI1074) were clustered with *P. fulvum*, one (JI199) occurred inside the cultivated *P. sativum*, and only five (JI1092, JI1093, JI1096, JI2055, JI2201) retained their position between *fulvum+abyssinicum* and cultivated *sativum*. Of the three accessions identified as '*P. humile*', one (JI1794) occurred among '*P. elatius*' and two (JI241 and JI1854) among cultivated *P. sativum*. (We should note that we examined plants of JI241, identified as '*P. humile*' but they did not exhibit characters of wild pea.). The authors concluded that there are three main groups of *Pisum*: *P. abyssinicum*, *P. fulvum* and all other *Pisum* (that is considered here as *Pisum sativum*). Unfortunately, our data are not strictly comparable with those by Ellis et al. (1998) since we had only two accessions in common, JI261 and JI1794. Earlier, an attempt of the same team (Lu et al. 1996) to reconstruct phylogenetic relationships within *Pisum* using AFLP-approach, which involved fewer accessions, resulted in a similar tree, with *P. abyssinicum*, *P. fulvum* and most of cultivated *P. sativum* plus JI241 ('*P. humile*') forming three compact branches, while the branches leading to 2 accessions of '*P. elatius*' (JI64, JI261) and one of '*P. humile*' (JI1794) branched close to *P. abyssinicum*, and two more accessions of '*P. sativum*' and one of '*P. jomardii*' (JI250) comprised a branch of their own.

Our unpublished data show that the set of alleles of different histone H1 subtypes in cultivated forms of *P. sativum* looks like an impoverished sample of those found in its wild forms, but updated with some alleles originated *de novo*. This is in line with the view of the cultivated *P. sativum* as a sublineage within the lineage B.

Comparison of the phylogenetic reconstructions by Meyer (1980), Palmer et al. (1985), Hoey et al. 1996 and Saar and Polans 2000, Lu et al. (1996) and Ellis et al. (1998) is scarcely possible due to different sets of accessions involved. Some of them could be identical but this remains hidden in different designation systems. Accumulation from different sources of as many accessions of wild peas as possible is necessary to form a standard set for comparative studies via different approaches, along with information on the history of each accession, including presence and designation in other collections and the history of reproduction. Our Table 1 is a step towards this goal.

Taxonomic implications

A plant genus represented by both wild and cultivated forms is always a difficult case for botanical taxonomy since there exists a great variety of cultivated forms with some characters often extending beyond the very definition of the genus. If the plant is a self-pollinator, the case is especially difficult, for the biological definition of species does not work for a set of clones. In peas, there exists a limited gene

exchange by crossing, nevertheless, this genus is one of the champions for confusing systematics. A lot of taxa have been proposed at a species level (for review see Makasheva 1979). Some quite recent fundamental botanical compendiums, e.g. the Flora of the European part of the USSR (Chefranova 1987), still includes both *Pisum sativum* and *P. arvense* L. as two distinct species differing by white versus purple flowers. There is still a notion among botanists that each distinct combination of characters must receive a botanical name at least below the species level. Lehmann (1954) and Makasheva (1979) attempted to provide such names for all the pea diversity as classified into subspecies, convariations, variations and subvariations. (Later Lehmann's system was reproduced in Lehmann and Blixt (1984), without consideration of Makasheva's taxa). For a geneticist, this work seems to be useless, at least for cultivated forms. At the same time, main natural gene pools with detectable differences should acquire formal botanical names for convenience of not only researchers of natural floras but also for geneticists and selectionists. There are two main problems in this respect: isolation of taxa according to biological notions and their valid names according to the formal rules of botanical nomenclature.

Isolation of *Pisum fulvum* as a species has not been doubted by all recent authors. As for *Pisum abyssinicum*, there is a controversy if to consider it either as a species (Braun 1841; Govorov 1937; Ellis et al. 1998) or subspecies, *Pisum sativum* subsp. *abyssinicum* (A. Br.) Berger (Berger 1928; Makasheva 1979). *Pisum abyssinicum*, cultivated in S. Arabia and Ethiopia and rarely occurring wild in the mountains (Govorov 1937; Makasheva 1979), was domesticated independently of the common cultivated pea (Ellis et al. 1998). A serious karyologic barrier for crossing with *Pisum sativum* and a clear-cut phenotype, although rather similar to *Pisum sativum*, make us to agree with its species status.

The rest of the genus is a fuzzy aggregation of forms which does not exhibit any internal distinctions attaining a species level, and most of the authors reasonably consider them as a single species *Pisum sativum* L. (e.g. Ben-Ze'ev and Zohary 1973; Makasheva 1979). This species embraces diverse wild peas widely distributed in the Mediterranean and all the variety of cultivated peas except for those belonging to *P. abyssinicum*.

Many names were proposed for wild representatives of *Pisum sativum*, but only three of those used to be widely used to denote recongisable taxa of subspecies or species rank: *elatius* Bieb., *humile* Boiss. et Noë and *syriacum* Boiss. et Noë. The history of their taxonomy has been reviewed by R. Kh. Makasheva (1979). The first of the mentioned names was introduced as a species *Pisum elatius* by Bieberstein (1808), its rank was first downgraded to subspecies by Schmalhausen (1895) who denoted it as "subspecies *γ-elatius*" of *Pisum sativum*, so the correct name of the subspecies is *Pisum sativum* ssp. *elatius* (Bieb.) Schmalh., although many authors ascribe the authorship of subsp. *elatius* to Ascherson et Graebner (1910). In 1856 Boissier and Noë described *Pisum humile* Boiss. et Noë (Boissier 1856), however, it was a junior homonym for *Pisum humile* Miller, proposed in 1768 for a form of cultivated pea. Nevertheless, the invalid name *P. humile* Boiss. et Noë is in use until now. Berger (1928) downgraded the rank of this taxon from species to subspecies and simultaneously gave it a new name: *P. sativum* subsp. *syriacum* (Boiss. et Noë) Berger. Note, that this was just a replacement of the name for the same taxon, based on the same type. Its status was again raised to a species *Pisum syriacum* (Berger) Lehm. by Lehmann (1954), but this was not supported.

Due to the vagueness of differences between the large and small wild peas, many modern researchers follow Townsend (1968) and Davis (1970) to compile all the diversity of wild representatives of *Pisum sativum* into one wild subspecies, which acquires the priority name *P. sativum* subsp. *elatius* (Bieb.) Schmalh. s. l. We agree that this is the best conventional solution for such a complicated set of forms, in spite of a great morphologic diversity and examples of scarce cross-compatibility, due to chromosomal rearrangements (Ben-Ze'ev and Zohary 1973) or nuclear-cytoplasm conflict (Bogdanova and Berdnikov 2001; Bogdanova and Kosterin 2006; Bogdanova 2007). Meikle (in Davis 1969) proposed to name the smaller forms within this subspecies as *P. sativum* subsp. *elatius* var. *pumilio* (Boiss. et Noe) Meikle, but *pumilio* is again just a new name in a new rank for the same biologic taxon which was described as *Pisum humile* by Boissier et Noë. Davis (1969) also described *P. sativum* subsp. *elatius* var. *brevipedunculata* Davis from Turkey and

Cyprus as a variation "largerly replacing var. *elatius* and var. *pumilio* in C. Anatolia and somewhat intermediate between them, though closer to the former." By doing this he in fact stressed a failure to divide wild forms of *Pisum sativum* into clear-cut subspecies.

The subspecies concept in botany is not well defined and lacks a strictly geographic sense, as in zoology. It rather implies more or less morphologically distinguishable forms which grow in different habitats over the same geographical range. *P. s.* subsp. *elatius* and *P. s.* subsp. *syriacum* would formally fit this subspecies concept, if they were more easily distinguishable. But we have shown that they are not monophyletic. While robustness and height of the plant and the correlated characters are hardly classifyable into two types, here studied markers referring to three cellular genomes evidence for the existence of two major microevolutionary lineages A and B, within the wild representatives of *Pisum sativum* and provide a system of exact identification of specimens. These lineages are most probably monophyletic and could be considered as separate subspecies, although unidentifiable by external characters. Representatives of these lineages coexist in Anterior Asia and provide a number of 'recombinants' which could not be classified as one of the two subspecies, but the subspecies concept allows existence of transitional forms.

The proper names for these supposed subspecies are, however, a problem which could be solved only if we were able to learn the lineage attribution of the types of *P. elatius* Bieb. and *P. humile* Boiss. et Noe and hence distinguish whether subspecies names *P. s.* subsp. *elatius* (Bieb.) Schmalh. and *P. s.* subsp. *syriacum* (Boiss. et Noe) Berger refer to different lineages or to the same one. In the latter case, a novel subspecies name will be required. The *locus typicus* of *P. elatius* was mentioned as follows: "Habitat in Iberia" (Bieberstein 1808). This of course meant Georgia not Spain, for Bieberstein's book was about the flora of the Caucasus and Crimea and 'Iberia' is an ancient name of Georgia; all three our accessions of wild peas from Transcaucasia belong to the lineage B, and were identified by their originators as '*elatius*'. So we may suppose that the type of *P. elatius* most probably represented lineage B. *P. humile* Boiss. and Noë was described from Bakker-Maaden, which we failed to trace exactly but of which it was indicated (Boissier, 1856) that it was in Turkish Armenia. Of four available accessions from East Turkey (P001, P008, P012 and P015) three belonged to the lineage B and one (P008) differed from combination B in having mitochondrial *cox1*+. So, with a less confidence, we may suppose that the type of *P. humile*/*P. syriacum* also belonged to the lineage B. If both considerations are true, the lineage A within *Pisum sativum* in fact misses any botanical name proposed in the subspecific rank and deserves description as a new subspecies. The types by Bieberstein are preserved in Botanical Institute, St. Petersburg, while those by Boissier in Geneva. In such old herbaria, DNA should have degraded but if there are seeds, even unripen, then the SCA protein can be isolated and electrophoresed. Unless this is done, we accept preliminarily that all wild representatives of *Pisum sativum* belong to *P. s.* ssp. *elatius* (Bieb.) Schmal. sensu lato.

Following the same logic, the above discussed group of accessions with the combination SCA^F *cox1*– *rbcL*– which includes '*Pisum jomardii*' and may represent a minor evolutionary lineage which diverged from the stem leading to the lineage B after acquisition of the restriction sites in *cox1* and *rbcL* but before the transition SCA^F-SCA^S, should be given a subspecies rank as well. *Pisum jomardii* Schrank was described as a species (Schrank 1818) but than was downgraded to *P. sativum* var. *jomardii* (Schrank) Alef. (Alefeld 1866). We prefer to consider *jomardii* as a subspecies, and since nobody seems to have ascribed this form a subspecies name, we do this formally here:

Pisum sativum L. subsp. ***jomardii*** (Schrank) Kosterin, **stat. n**. Syn.: *Pisum jomardii* Schrank, 1818, Fl. Monac. 4:309.—*P. sativum* var. *jomardii* (Schrank) Alefeld, 1866, Landwirtschaftliche Flora. 8:43.—*P. sativum* subsp. *asiaticum* Govorov prol. *aegypticum* var. *jomardii* (Schrank) Govorov 1937, Kul'turnaya flora SSSR: 384.—*P. sativum* subsp. *asiaticum* convar. *persicum* Govorov var. *jomardii* (Schrank) Alef.: Makasheva 1979, Fl. Cult. Plants USSR, IV(1):79.

The overwhelming majority of cultivated forms of *P. sativum* belong to the conventional lineage B. Most of them are considered within subspecies *P. sativum* subsp. *sativum*. L. Govorov isolated peas of Afghanistan and Tadjikistan into subsp. *asiaticum*, based on the presence of a flavonoid pigmentation in

the corolla and a combination of some characters present in other forms (Govorov 1937; Makasheva 1979). At the same time, Govorov claimed that Afghanistan harbored the greatest diversity among cultivated peas. Presently Govorov's characters of *asiaticum* are reasonably considered impermanent and insufficient to isolate a subspecies. However, in a comparative study of the insertion sites of *Ty1-copia* class retrotransposons (Ellis et al. 1998), the highland Asiatic forms formed a group of their own, but this group was narrower than *P. s.* subsp. *asiaticum* sensu Govorov. So we do not accept this susbpecies. A very peculiar vetch-like pea form ranges in Transcaucasia and the Caucasus. Most probably it is a product of unconscious selection which made a fodder crop from a weed. It was isolated as *P. sativum* subsp. *transcaucasicum* Govorov (Govorov 1937; Makasheva 1979) and this group more worth retaining as a subspecies than *asiaticum*. Ellis et al. (1998) found out that their four accessions of *P. s.* subsp. *transcaucasicum* formed a distinct group branching from the tree near the branching point of '*P. elatius*'.

A hypothetical scheme of phylogenetic relationships between different pea forms in view of the presented results is given in Fig. 4. Here *Pisum sativum* subsp. *elatius* s. l. is paraphyletic, as would be a subspecies with the same name used in a more narrow sense, representing only the lineage B among the wild forms of *Pisum sativum*. However a strict cladistic classification excluding paraphyletic taxa is not so helpful at intraspecies level.

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