diversity

MDPI

Article

New Insights into Plastid and Mitochondria Evolution in Wild Peas (*Pisum* L.)

Natalia V. Shatskaya †, Vera S. Bogdanova †, Oleg E. Kosterin * and Gennadiy V. Vasiliev

Institute of Cytology and Genetics of the Siberian Branch of Russian Academy of Sciences, Academician Lavrentyev Ave. 10, 630090 Novosibirsk, Russia

* Correspondence: kosterin@bionet.nsc.ru

† These authors contributed equally to this work.

Abstract: Plastids and mitochondria are organelles of plant cells with small genomes, which may exhibit discordant microevolution as we earlier revealed in pea crop wild relatives. We sequenced 22 plastid and mitochondrial genomes of *Pisum sativum* subsp. *elatius* and *Pisum fulvum* using Illumina platform, so that the updated sample comprised 64 accessions. Most wild peas from continental southern Europe and a single specimen from Morocco were found to share the same organellar genome constitution; four others, presumably hybrid constitutions, were revealed in Mediterranean islands and Athos Peninsula. A mitochondrial genome closely related to that of *Pisum abyssinicum*, from Yemen and Ethiopia, was unexpectedly found in an accession of *P. sativum* subsp. *elatius* from Israel, their plastid genomes being unrelated. Phylogenetic reconstructions based on plastid and mitochondrial genomes revealed different sets of wild peas to be most related to cultivated *P. sativum* subsp. *sativum*, making its wild progenitor and its origin area enigmatic. An accession of *P. fulvum* representing 'fulvum-b' branch, according to a nuclear marker (Weeden et al., 2021), appeared in the same branch as other *fulvum* accessions in organellar trees. The results stress the complicated evolution and structure of genetic diversity of pea crop wild relatives.

Keywords: mitochondrial genome; plastid genome; wild peas; *Pisum* L.; pea crop wild relatives; *Pisum sativum* L. subsp. *elatius* (Bieb.) Aschers. & Graebn. s.l.; wild peas; *Pisum fulvum* Sm.; *Pisum abyssinicum* A. Braun

Citation: Shatskaya, N.V.; Bogdanova, V.S.; Kosterin, O.E.; Vasiliev, G.V. New Insights into Plastid and Mitochondria Evolution in Wild Peas (*Pisum* L.). *Diversity* **2023**, *15*, 216. https://doi.org/10.3390/d15020216

Academic Editors: Kirill Azarin and Michael Wink

Received: 19 December 2022
Revised: 30 January 2023
Accepted: 30 January 2023
Published: 2 February 2023

1. Introduction

Plastids and mitochondria are plant cellular organelles with their own small genomes. Due to their predominantly uniparental, usually maternal inheritance [1], they have straightforward and usually coinciding evolutionary histories, not complicated and blurred by recombination as in case of nuclear genomes. Nevertheless, noncanonical biparental inheritance of plastids and/or mitochondria may occur and eventually result in a discordant pattern of their microevolution. Earlier we reported a case of discordant evolution of plastid and mitochondrial genomes in wild peas (*Pisum* L.) and considered it in phylogeographic context [2]. This case was of theoretical importance, demonstrating an option of discordant phylogenetic patterns of different cellular genomes in plant microevolution, with potential practical output in view of pea crop wild relatives being a potential source of genetic diversity for improvement [3,4] of this ancient [5] but still important crop. Hybrids of wild and cultivated peas often show variably reduced fertility and sometimes abnormalities of pigmentation and morphology due to conflict between the nucleus and plastids, which can be overcome due to leakage of paternal plastids [6,7]. Therefore, knowledge on the diversity of organellar genomes of wild peas is important for their use in breeding programs.

Wild peas are represented by a quite uniform species *Pisum fulvum* Sm. from the eastern Mediterranean and a morphologically and genetically diverse *Pisum sativum* L.,

the species to which the cultivated pea also belongs. In the Mediterranean and Near East, populations of genuine wild representatives of *P. sativum* still exist, which are inclusively classified as the wild subspecies *P. sativum* L. subsp. *elatius* Aschers and Graebn. s.l. [8]. According to the recently proposed alternative classification [9,10] more fitting the reconstructed phylogenetic pattern [11], this taxon is named *Lathyrus oleraceus* Lamarck subsp. *biflorus* (Rafin.) H. Schaefer, Coulout et Rabaute.

Our previous study on the phylogenetic relationships of pea organellar genomes [2] was based on 42 accessions of mostly wild peas originated from all over the natural geographical range of the genus. However, due to a limited number of accessions from some geographic regions, additional sampling from them was warranted.

First, one of our inferences [2] was that most of European wild peas resulted from an ancient hybridisation. In this respect, the small Athos Peninsula in Greece was remarkable, since it harboured populations with quite unrelated plastid and mitochondrial genomes. To clarify this issue and reveal predominant lineages of European wild peas and their distribution, we sequenced organellar genomes in eleven accessions from Spain, France, Italy and Greece.

Second, Israel has long been known for extraordinary diversity of wild peas, which appeared even more impressive with respect to organellar genomes [2]. For this reason, we increased the sample of Israeli wild peas and revealed several predominant variants of organellar genomes as well as unexpected new ones.

Third, certain peripheral regions of the wild pea range were badly underrepresented in our analysis. There was just one accession from North Africa [2], moreover, with doubtful attribution. The virtual absence of North African wild peas in germplasm collections was specially acknowledged by Hellwig et al. [12]. We updated this with one more accession collected in a natural population in Morocco. Just two accessions from Iran represented the eastern one third of the wild pea range. In the current analysis we sequenced organellar genomes from a wild pea accession from Turkmenistan, which represents the easternmost known locality of wild peas in the world.

Fourth, Weeden et al. [13] found a great diversity of *P. fulvum* with respect to the intron of the nuclear gene *cotyledon green* (syn. *staygreen*), which in some reconstructions comprised remote branches 'Fulvum A' and 'Fulvum B' thus making *P. fulvum* polyphyletic. The three accessions with so far sequenced organellar genomes [2] represented the 'Fulvum A' branch, so we now added a representative of the 'Fulvum B' branch by the cited authors.

Last, we proceeded with searching for wild peas most closely related to the cultivated subspecies *P. sativum* L. subsp. *sativum* and location of their current geographical distribution. For this, we sequenced one more accession from the presumed Core Area of the Near East plant domestication in south-eastern Turkey [14].

These updates comprised 22 *Pisum* accessions in which we sequenced plastid and mitochondrial genomes in the course of this study. Involving them, our updated samples include organellar genomes of 64 pea accessions, 59 of them representing wild peas. On this basis, we made more detailed phylogenetic reconstructions that provide further insights into the evolutionary history of pea crop wild relatives.

2. Materials and Methods

2.1. Plant Material

The phylogenetic analyses presented in this paper were based on the plastid and mitochondrial genomes of 64 pea accessions, mostly representing genuine wild peas (*P. sativum* subsp. *elatius* and *P. fulvum*), of which 42 accessions were included in our previous study [2], and in 22 accessions of wild peas, the organellar genomes were sequenced in the course of this study. These accessions were received from the Agricultural Research Service of the United States Department of Agriculture, courtesy of Clarice Coyne; the John Innes Centre, courtesy of Michael Ambrose; Palacky University of Olo-

mouc, courtesy of Petr Smykal; Vavilov All-Union Institute of Plant Breeding; or were directly collected from nature. Information on the origin of the 22 accessions, the organellar genomes of which were sequenced in this study, and GenBank IDs of the sequences obtained are provided in Table 1; for relevant information on accessions sequenced in the former study see Table 1 in [2] (that publication missed the accession number for the mitochondrial genome of accession WL_1446 (*Pisum abyssinicum* A. Braun); it was reconstructed as two 'chromosomes', which were ascribed the identifiers MW394515 and MW394516). All 22 newly studied accessions were proved to be genuine wild peas, as having spontaneously dehiscing pods (phenotype Dpo), by sowing and examination in the greenhouse at the SB RAS Artificial Plant Growing Facility. All accessions, except for Pe_6, had gritty seed testa (phenotype Gty); in accessoins PI_344539 and CE_24, the grittiness was weak. The seeds of VIR_6071, as any *P. fulvum*, had very thick yet smooth testa, but its F_1 hybrids with cultivated pea had gritty testa, hence evidencing for the presence of the *Gty* allele in the *P. fulvum* parent. For each accession, progenies of a single plant were analysed.

Table 1. Information on the pea accessions (in geographic order) from which the plastid and mitochondrial genomes were sequenced in this study, with accompanying information, including their origin (with coordinates in square brackets if reconstructed), their combination of three molecular markers, their constitution of organellar genomes with respect to main evolutionary branches according to [2], differences of the plastid *psbA-trnH* spacer from the consensus, and GenBank IDs of the genomes sequenced in this study.

Accession Designation Used in This Paper	Taxonomic Attributions and Other Known Designations	Origin; Date of Collection (Where Relevant/Available), Collector	Lati-Tude (N)	Longi-Tude (E)	Organellar Constitution, Allele Combination of Three Markers	Plastid Spacer *psbA-trnH*. (Difference from Consensus)	Gene Bank IDs for Plastid and Mictochondrial Genomes
Pisum fulvum, wild							
VIR_6071	*Pisum fulvum* var. *striatum* Makasheva	Palestine, foothills approx. 30 km south-west of Jerusalem. Collected in 1960.	[31.6]	[35.0]	P1 M1, A	-	ON357683, ON186758,
Pisum sativum L. subsp. *elatius* (Bieb.) Aschers. et Graebn. s.l., wild							
CE_23		Morocco, Er-Rif Mts, Chefchaouen Province, Chefchaouen, Jbel Tissouka Mt western foot, a femce made of stones and dry branches between a tourist circuit trial and a small abandoned garden with sparse trees. Collected on 19 May 2021 by O. Kosterin and N. Solovyeva	35.16837	−5.25504	P4 M3, C	-	OP928222, OQ078748
PIS_2844		España, Salamanca, 2 km de Puerto de Béjar, Camino de la Plata, 740 m a.s.l.	[40.35]	[−5.84]	P4 M3, C	-	MZ648183, MZ707507
CE_12 (-D^w, -d)	JI_3558, SE1, W6_56891 (D^w), W6_56893 (d),	España, Cataluña, comarca de Conca de Barberà, Muntanyes de Prades, Vall de Monestir de Poblet, Barranc de Castellfollit	41.3517	01.0614	P4 M3, C	-	MZ677459, ON165401

Table 1. *Cont.*

Accession Designation Used in This Paper	Taxonomic Attributions and Other Known Designations	Origin; Date of Collection (Where Relevant/Available), Collector	Lati-Tude (N)	Longi-Tude (E)	Organellar Constitution, Allele Combination of Three Markers	Plastid Spacer *psbA-trnH*. (Difference from Consensus)	Gene Bank IDs for Plastid and Mictochondrial Genomes
CE_13	JI_3553; W6_56891; FE1	France, Région Sud Provence-Alpes-Côte d'Azur, département du Var, canton de Brignoles, commune de Rougiers, Massif de la Sainte-Baume (ca 50 km from Marseille), open stand of (*Quercus pubescens* Willd.). Collected by Michel Papazyan	43.3839	05.8567	P3 M3, C	75: T→G	MZ677460, ON165402
PIS_2850		Italy, [Liguria,] Commune de Camogli, Mortola	[44.33]	[9.16]	P4 M3, C	-	MZ648184, MZ707508
JI_2055		Italy, [Campania], Mt. Alburni	40.55	15.30	P4 M3, C	-	OP919340, OQ078752
PI_344539		Italy, Sicily, Palermo [Prov.], Piana degli Albanesi. Collected by A. Di Martino before 1969	[38.0]	[13.3]	P4 M4, C	-	ON259091, ON186755,
JI_1092	PI_344006, W6_8706, 22618	Greece, Athos Peninsula, Xeropotamou Monastery, moist mountain slopes, rocky or well littered soil, 200 m a.s.l. Collected in June 1969 by H.S. Gentry	[40.23]	[24.22]	P4 M4, C	-	ON243975, ON165398
JI_1093	PI_344010, W6_8707, 22732, introgressed	Greece, Athos Peninsula, below Karyes, high macchia vegetation, 270 m a.s.l. Collected in June 1969 by H.S. Gentry	[40.26]	[24.25]	P4 M4, C	75: T→G	ON243976, ON165399
JI_1095	PI_344012, W6_8709, 22734	Greece, Athos Peninsula, above Ivyron Monastery, 180 m a.s.l. Collected in June 1969 by H.S. Gentry	[40.24]	[24.28]	P3 M4, C	-	ON243977, ON165400
PI_344008	W6_8710, 22735	Greece, Athos Peninsula, 1 km S of Daphne. Collected in June 1969 by H.S. Gentry	[40.20]	[24.22]	P3 M4, C	75: T→G	ON259089, ON186753,
PI_344009	22729	Greece, Athos Peninsula, Panteleimonos Monestry, scrub oak macchia. Collected in June 1969 by H.S. Gentry	[40.28]	[24.20]	P3 M4, unusual (+—S) [1]	-	ON259090, ON186754,
PI_344001	22701	Turkey, [Mersin Il], 17 km north of Mersin on road to Gonze, limestone rocks among macchia, 360 m a..l. Collected in May 1969 by H.S. Gentry	[37.0]	[34.6]	P2 M2 (no ins.), A	-	ON259088, ON186752,
Pe_6	PI_639960, W6_2639	Turkey, Mardin Il, 12 km on the road to Bozova from the main Shanlyurfa-Diyarbakyr road, edge of pistachio grove. Collected before 2005 by S. Abbo.	[37.29]	[38.67]	P5 M4, B	-	OP928224, OQ078750

Table 1. *Cont.*

Accession Designation Used in This Paper	Taxonomic Attributions and Other Known Designations	Origin; Date of Collection (Where Relevant/Available), Collector	Lati-Tude (N)	Longi-Tude (E)	Organellar Constitution, Allele Combination of Three Markers	Plastid Spacer *psbA-trnH*. (Difference from Consensus)	Gene Bank IDs for Plastid and Mictochondrial Genomes
W6_2107	Psh 008, 120689-0302	Turkey, Siirt Il, 6.3 km north of Batman (across from the airport), frequent as weed in lentil field on fine soil; 630 m a.s.l. Collected on 12 June 1989 by W.J. Kaiser, F.J. Muehlbauer, C.V. Sperling	37.92	41.13	P5 (no inv.) M2, unusual (- + S) [2]	-	ON357684, ON186759,
CE_15	Phs 10–Phs 12, Phs 98–Phs 99, 'southern *humile*'	Israel, eastern Lower Galilee, 2.4 km north-east of Ginosar Kibbutz, near Khirbat al-Minya ruins and Atar Safir Pump Station, 420 m from Lake Tiberias north-western bank, in ruderal vegetation (including *Lathyrus hierosolymnitanus* and *Vicia* sp.) at a wheat field margin, 202 m below sea level. Collected on 15 April 2019 by S. Abbo and O. Kosterin	32.86824	35.53594	P4 M2, A	-	MZ677461, ON165403
CE_16	Pe 25–Pe 26, Pe 41–Pe 43, Pe 50–Pe 54, Pe 139–Pe 140	Israel, Northern District, eastern Lower Galilee, 666 m north-east of Livnim Settlement, Vadi Amud, shrubbery, 141 m below sea levell. Collected on 15 April 2019 by S. Abbo and O. Kosterin	32.86801	35.50259	P4 M3, C	-	ON310561, ON186757,
711	JI_3272, PI_560068, L_99, 'southern *humile*'	Israel, 2 km west of Jerusalem, Jerusalem Forest, edges of the abandoned terrace field [31.0]	[~31.8]	[35.2]	P4 M1, A	-	ON310560, ON186756,
714	JI_3275, PI_560071, L_102, 'southern *humile*'	Israel, between Bet Shemesh and Bet Gurvin, field edges and roadsides [31.0]	[~31.7]	[~34.9]	P4 M1, A	-	OP919341, OQ078753
CE_24		Russia, Republic of Dagestan, Magaramkent District, Samur Forest, 2 km west-north-west of Primorskiy village, sparse oak stand in oak/hornbeam forest, 6 m below sea level. Collectedon 25 June 2021 by O.E. Kosterin	41.85736	48.55370	P5 M3/M4, B	142–149 deleted	OP928223, OQ078749
YD-1		Turkmenistan, Kopet-Dagh, Yol-Dere Valley. Collected on 1 June 2018 by S. Abbo	38.50	56.38	P5 M4, B	142–149 deleted	OP919339, OQ078751

[1] Unusual marker combination: The relevant restriction sites present in *rbcL*, absent in *cox1*, slow variant of SCA. [2] Unusual marker combination: The relevant restriction sites absent in *rbcL*, present in *cox1*, slow variant of SCA.

2.2. Organellar DNA Extraction, High Throughput Sequencing, Assembly and Alignment of Organellar Genomes

Organellar DNA was extracted from ca 40–50 etiolated seedlings (3–5 g of leaf tissue) grown from seeds obtained from a single plant of each accession, following the protocol by Jansen et al. [15] with modifications by Bogdanova et al. [2,7].

While in our previous studies [2,7] we used the Ion Torrent platform for high throughput sequencing, in this study we used the Illumina platform at the IC&G Joint Center for Genome Studies.

Genome libraries were prepared using the Roche KAPA Hyper Prep Kit and KAPA UD indexed adapters according to the manufacturer's manual with size selection modification. DNA was fragmented on a Covaris M220 device with parameters optimized for a maximum of fragments in the range of 350. One hundred nanograms of fragmented DNA per sample was used as input material; amplification of libraries was carried out in 7 cycles. The quality and molarity of the resulting libraries were determined using a Bioanalyzer BA2100. Libraries were pooled and sequenced on a NextSeq550 sequencer using the NextSeq 550 High Output v2 Kit 300 cycles (Illumina, San Diego, CA, USA).

To assemble mitochondrial genomes, we followed the procedure described in [2] using MIRA4 [16]; Tablet 1.13.07.31 [17] software was used to visualise genomic assemblies. Plastid genomes were assembled as described in [7] using MIRA4 software. Genome assembly and phylogenetic reconstruction was performed at the Computational Facility of the Siberian Supercomputer Center SB RAS and Computational Facility of Novosibirsk State University.

The organellar genomes were aligned using the ClustalW program [18] in Mega 6 package [19] and manually adjusted. Since structural rearrangements are widespread in mitochondrial genomes, for their alignment, the sequence was broken into 5 to 13 parts, which were manually ordered and oriented according to the reference sequence, which was represented by mitochondrial DNA of accession WL_1238 (*P. sativum* subsp. *sativum*). The newly sequenced plastid and mitochondrial genomes were compared to those of WL_1238 and the differences are summarized in Tables S1 and S2, respectively.

2.3. Phylogenetic Analysis

Phylogenetic reconstructions were made using the Bayesian and Maximum Likelihood methods. Bayesian MCMC (Markov Chain Monte Carlo) analysis was made with the use of the BEAST 2.4.3. software [20]; jModelTest 2.1.10 [21,22] was used to choose the GTR substitution model. The proportion of invariant sites was set to 0.5 and the number of Gamma categories was set to 4; other parameters were set to default values. An uncorrelated lognormal relaxed clock model and Yule process of speciation were applied. One MCMC analysis was run for 200 million generations. The Effective Sample Size (ESS) value for the likelihood parameter estimated with Tracer v. 1.6 [20] reached 7000 and higher. To generate target trees, TreeAnnotator v.2.4.3 [20] was used with the burnin percentage 10. Trees were visualized in FigTree 1.4.3 (http://tree.bio.ed.ac.uk/software/figtree/ (accessed on 26 June 2017) by A. Rambaut). Phylogenetic reconstructions by the Maximum Likelihood method were carried out using the GTR model by means of the IQ-tree software [23] with branch support estimated by ultrafast bootstrap [24] with 1000-times sampling.

The phylogenetic trees were rooted with the outgroup consisting of *Vavilovia formosa* (Stev.) A. Fed. (MK604478 for the plastid genome and MK48602 and MK48603 for the mitochondrial genome) and *Vicia faba* L. (MT120813 and KC189947 for the plastid and mitochondrial genomes, respectively). Their organellar genomes differ from those of peas by a number of rearrangements, which for the purpose of phylogenetic reconstructions were manually adjusted by splitting and repositioning of fragments, as in case of structurally different pea mitochondrial genomes.

Computations were performed at the Computational Facility of the Siberian Supercomputer Center SB RAS and the Computational Facility of Novosibirsk State University.

3. Results

3.1. Mitochondrial and Plastid Genome Structure

The size of newly sequenced mitochondrial genomes ranged from 346,345 to 385,514 bp, corresponding well to the range 346,959–385,511 bp reported by [2], although a bit wider. Twenty of 22 newly sequenced mitogenomes fell into the most common size range of approximately 363–364 Kbp; one accession (711) possessed a long mitogenome of about 385 Kbp, typical of the most ancient branch of pea mitochondrial DNA, and one accession (CE_15) possessed a short mitogenome of about 346 Kbp. Earlier [2], we recognised six structural types of the pea mitochondrial genomes with respect to mtDNA rearrangements based on the compatibility of possible assemblies with the complements of the original reads. Of the 22 newly sequenced mitogenomes, only that of CE_15 had the order of mtDNA regions different from any of the six previously reported structural types.

The mitogenome of CE_15 appeared to be unusual among those of *P. sativum* in two respects: the unique order of mtDNA regions and the shortest length (346,345 bp). Remarkably, both of these features are found in the studied accessions of *P. abyssinicum*, an enigmatic pea species or subspecies cultivated (along with and less frequently than the common pea) in Ethiopia and Yemen [25–27]. Accordingly, CE_15 mitogenome shared a large deletion of approximately 9 Kbp with those of WL_1446 and VIR_2759 belonging to *P. abyssinicum*. Additionally, both WL_1446 and VIR_2759 possessed unique structural types of mitogenome differing from each other and CE_15.

The newly sequenced plastid genomes did not present structural novelties and fell into one of the two described types differing by an approximately 3.5 Kbp inversion, reported by Palmer et al. [28]. Plastid genomes of W6_2107 and Pe_6 possessed this inversion as compared to the most common type. Variation in the *psbA-trnH* intergenic spacer is worth mentioning: among the newly sequenced accessions, an 8-bp deletion typical of cultivated peas [29] was found in accessions CE_24 from Dagestan and YD-1 from Turkmenistan (Table 1).

Some accessions appeared to have the mitochondrial genomes identical in terms of nucleotide substitutions. Some of them differed in usually small indels, a large portion of which concerned length of homopolymers. Presumably, these indels resulted from sequencing errors [30]. These nearly identical accessions include: (i) a cultivar Cameor and a testerline WL_1072 (*P. sativum* subsp. *sativum*); (ii) two accessions of *P. abyssinicum*, VIR_2759 and WL_1446; (iii) four (of the seven studied) accessions of *P. sativum* subsp. *elatius* from the Athos Peninsula, PI_344008, PI_344009, JI_1093 and JI_1095; (iv) accessions JI_2724 and PI_343974 of *P. sativum* subsp. *elatius* from remote localities, such as Menorca Island and Turkey (Selimiye environs), respectively; and (v) accessions CE_16 and PI_639955 *P. sativum* subsp. *elatius* from Galilee, Israel. Remarkably, mitochondrial genomes of the accession group (ii) differed largely by structural rearrangements and long indels, but this did not concern nucleotide variability. The mitochondrial genomes of accessions PI_344008, PI_344009 and JI_1093 from group (iii) were completely identical, not differing even in small indels. The mitochondrial genomes of the two accessions of group (v) differed by 17 small indels and an ambiguous tract tttaaaaa vs. ttaaaaaa.

In the cases (iii) and (v), the plastid genomes also differed by small indels but not nucleotide substitutions. In case (iii), accessions PI_344008, PI_344009 and JI_1095 but not JI_1093 shared plastid genomes with no nucleotide substitutions. The pairwise p-distances between the plastid genomes and those between mitochondrial genomes of the accessions studied are presented in Tables S3 and S4, respectively.

3.2. Phylogenetic Reconstructions Based on the Plastid and Mitochondrial Genomes

We reconstructed phylogenetic trees for plastid (Figure 1) and mitochondrial (Figure 2) genomes in the updated set of 64 accessions via the Bayesian MCMC method. For the reconstruction of the plastid genome tree, the outgroup was also updated: in addition to *V. formosa*, *V. faba* was also included, the plastid genome of which was not yet available at the time of the previous study. In general, the topologies of the reconstructed phylogenetic

trees were similar to those obtained in [2]: the plastid genome tree revealed five main branches, designated by Bogdanova et al. [2] as P1-P5 (Figure 1), and the mitochondrial genome tree revealed four main branches M1-M4 (Figure 2). However, the mitochondrial genome of CE_24 branched off before separation of M3 and M4 (Figure 2). Expectedly, the reconstructed phylogenies of plastid and mitochondrial genomes had discordant topologies, as revealed earlier [2]. Phylogenetic positions of some newly sequenced accessions are worth mentioning. First of all, this concerns the position of accession CE_15 (*P. sativum* subsp. *elatius* var. *pumilio*, 'southern *humile*' according to [31]) from Israel, Southern Galilee, Lake Tiberias vicinity, on the mitochondrial genome tree. Unexpectedly, it clustered very closely to two accessions (VIR_2759 and WL_1446) of *P. abyssinicum*, a cultivated taxon endemic to Yemen and Ethiopia. The mitochondrial genome of CE_15 resided (together with those of Abyssinian peas) on a branch inside M2, in contrast to three other sequenced representatives of the so-called 'southern humile' (711, 712 and 714) residing in branch M1 (Figure 2). The *p*-distance between the aligned mitochondrial genomes of CE_15 and those of the two sequenced accessions of *P. abyssinicum*, VIR_2759 and WL_1446, comprised just 1.51×10^{-4} and 1.28×10^{-4}, respectively (Table S4), as compared to the overall mean of 11.65×10^{-4}. Remarkably and expectedly, all the four above mentioned accessions of 'southern *humile*' tightly cluster together in the plastid genome tree in branch P4 (Figure 1). In terms by Bogdanova et al. [2], three accessions of 'southern humile' had the organellar genome constitution P4 M1, while CE_15 had the constitution P4 M2.

Accession W6_2107, a wild pea with rather low habit from Siirt Il of Turkey, was taken into the analysis because of an unusual combination of three molecular markers from three cellular genomes, thereby not fitting classification by Kosterin et al. [32]: the relevant restriction site was absent in the plastid gene *rbcL*, the restriction site in the mitochondrial gene *cox1* was absent, and the SCA albumin had the slow variant (Table 1). It appeared in branch M2 in the mitochondrial phylogenetic reconstruction, forming there a sub-branch of its own (Figure 2). In the plastid genome tree (Figure 1), it occurred in the branch P3, very closely to accession Psh_004 from Mardin Il of Turkey. The resulting organellar genome constitution P4 M2 is also unusual, not yet found in Turkey.

The position of CE_24 on the phylogenetic tree of mitochondrial genomes (Figure 2) was rather unexpected. This accession diverged from the common stem of the M3 and M4 clade before their divergence from each other.

Accession 711 tightly clustered with 712 in both trees (Figures 1 and 2). Both originated from Israel and were morphologically similar. In the mitochondrial genome tree, their sub-branch represents the most ancient divergence and is quite separated from other representatives of the M1 branch (Figure 2). Thus, we have now supported this sub-branch represented by the sole accession 712 in our previous study [2].

Accessions CE_24 and YD-1 had the 8-bp deletion in the plastid *psbA-trnH* spacer typical of the cultivated pea [29] and, expectedly, were tightly clustered in the trees based on the plastid genomes (Figure 1) with other accessions having this deletion, including all accessions of the cultivated subspecies *P. sativum* subsp. *sativum*.

Accessions W6_2107 and Pe_6, with the 3.5 Kb long inversion in the plastid genome, clustered in the phylogenetic tree with other accessions having it and forming a large subgroup inside P5, which included about a half of this branch (Figure 1).

Accession VIR_6071 (Jerusalem environs) of *P. fulvum* clustered with other representatives of this species on both phylogenetic reconstructions (Figures 1 and 2).

Phylogenetic reconstructions via the Maximum Likelihood methods based on the plastid and mitochondrial genomes are presented in Figures 3 and 4, respectively. They revealed the same well supported topology of representatives of the genus *Pisum*, but the outgroup branches of *Vicia faba* and *Vavilovia formosa*, especially the former, appeared fairly long as compared to the short *Pisum* crown.

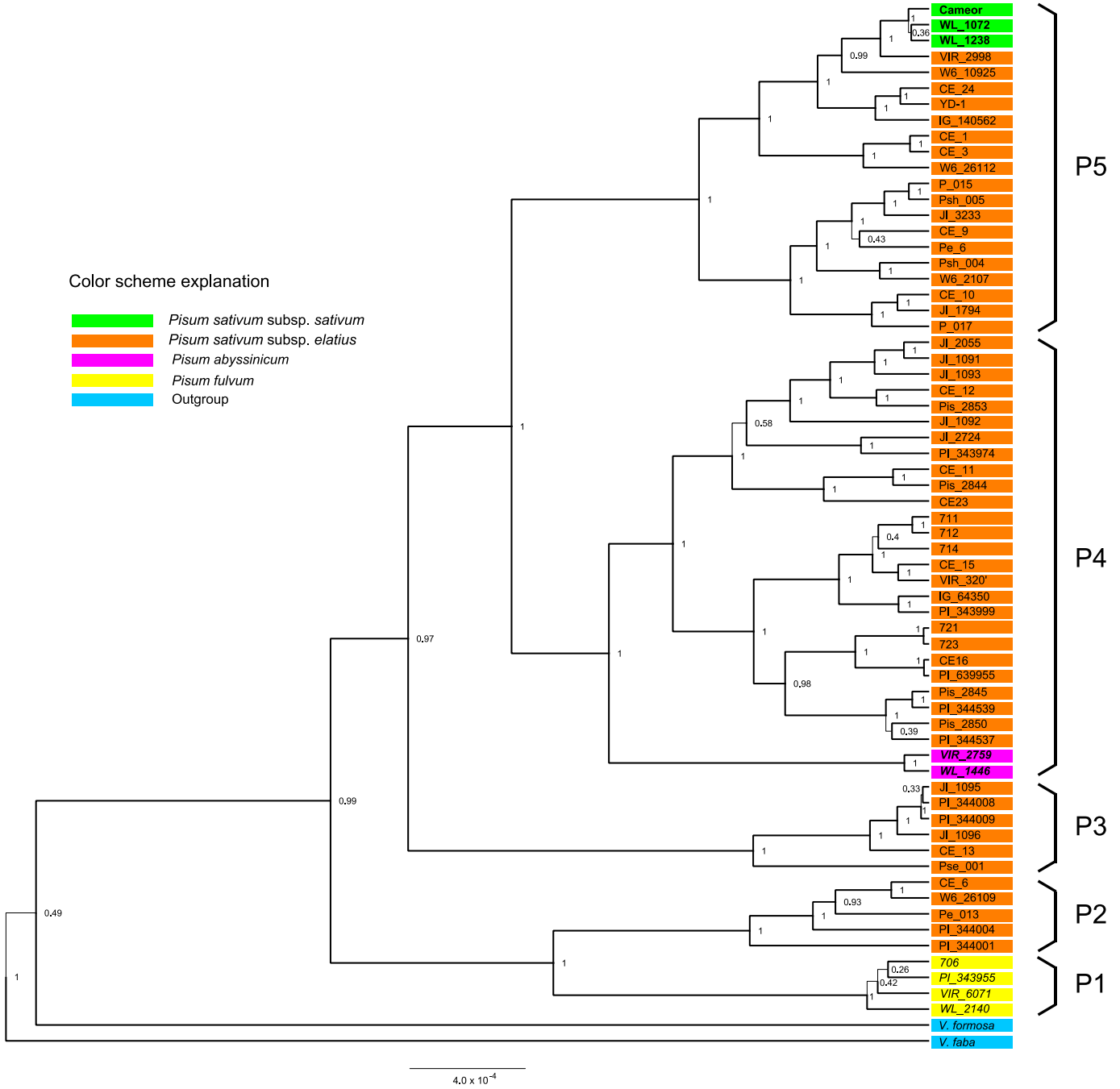


Figure 1. Reconstruction of the pea phylogeny of on the basis of the plastid genomes obtained with Bayesian MCMC method. Posterior probabilities of the nodes are indicated. Scale bar corresponds to the expected number of nucleotide substitutions per site. Main phylogenetic branches are denoted with P1-P5. Accessions of *Pisum fulvum* are italicised, *P. abyssinicum* are bold italicised, *P. sativum* subsp. *elatius* s.l. are regular Roman and the cultivated subspecies *P. sativum* subsp. *sativum* are boldfaced. *Vicia faba* and *Vavilovia formosa* comprise the outgroup.

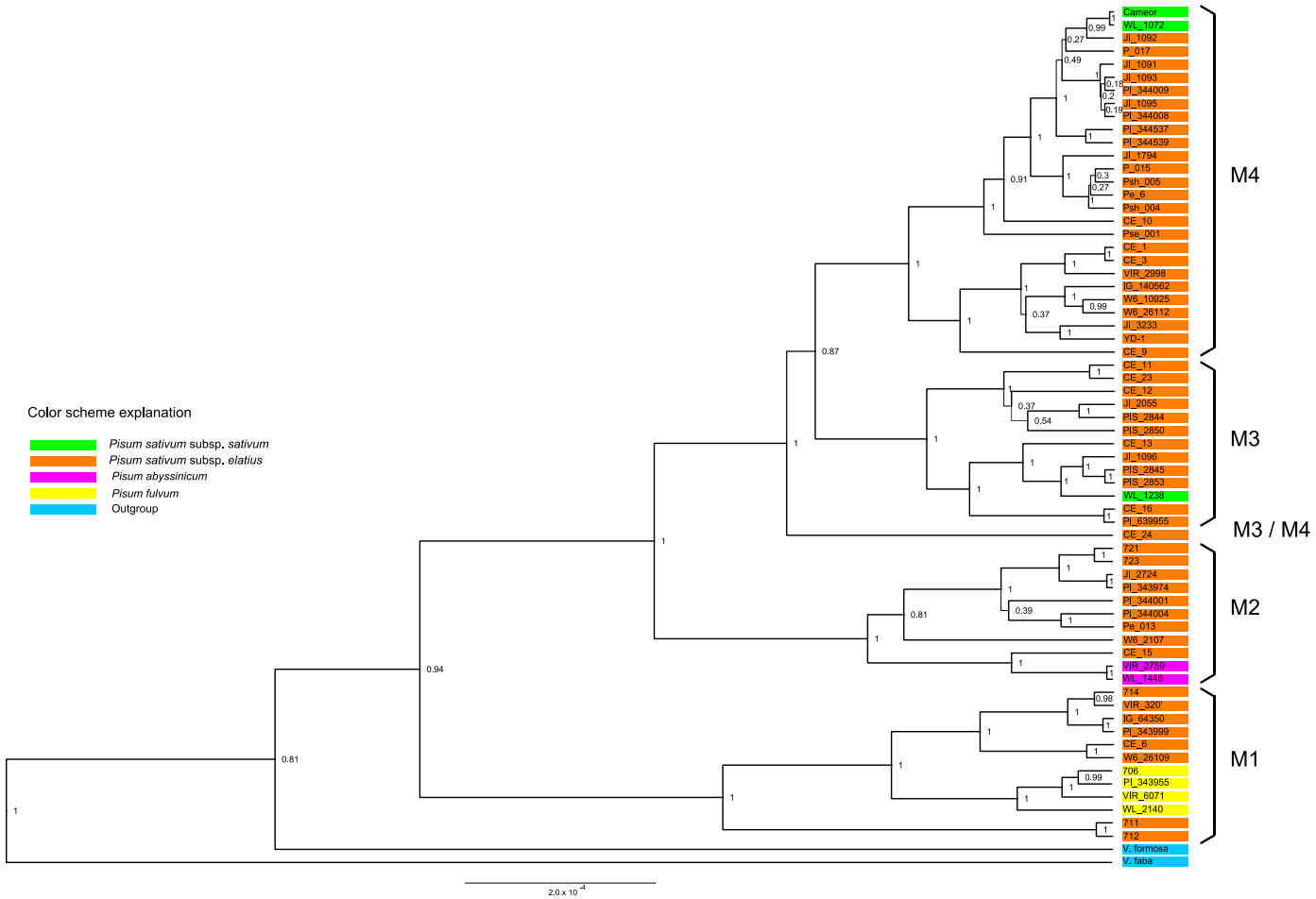


Figure 2. Reconstruction of phylogeny of pea accessions with Bayesian MCMC method on the basis of the mitochondrial genomes. Main phylogenetic branches are denoted as M1–M4. Designation of taxonomic attribution and the outgroup as in Figure 1.

Curiously, the node uniting the genera *Pisum* and *Vavilovia* Fed. got no support (the posterior probability value being 0.4) in the Bayesian phylogenetic reconstruction based on the plastid genomes (Figure 1) and a weak support (with the value of 0.81) in that based on the mitochondrial genomes (Figure 2). It also has no support in the Maximum Likelihood reconstructions (Figures 3 and 4). That means that the phylogenetic relationships of *Pisum*, *Vavilovia* and *Vicia* L. are not well resolvable based on organellar genomes.

3.3. A Mitogenome Region with Unusual Variation in Accessions of M2 Branch

The M1 clade of mitochondrial genomes represents the most ancient divergence in the phylogenetic tree. Naturally, it has accumulated the largest number of differences, nucleotide substitutions and indels, as compared to the reference genome of WL_1238. The total number of differences specific to M1 (that is met in all representatives of M1 and not met in any other accession) on the sample of 64 pea accessions comprised 1031, corresponding to an average of 28.3 per 10 Kb. The distribution of the number of such differences in intervals of 10 Kb along the mitochondrial genome is given in Figure 5a. This distribution is more or less even with two notable exceptions where the number of M1-specific differences falls to zero. First, this is the interval at positions 120,000–130,000 of the reference mitogenome, which harboured a M1-specific deletion of about 12 Kb. The second interval at positions 318,017–332,840 harboured zero M1-specific differences, rather the majority of differences from the reference mitogenome in this region was shared with M2, disregarding mitochondrial genomes of *P. abyssinicum* and the related

CE_15 in which the region in question was almost entirely deleted. Both accessions of *P. abyssinicum*, VIR_2759 and WL_1446 contained a deletion of 9928 bp corresponding to 321,908–331,835 positions, and CE_15 contained a deletion of 8849 bp corresponding to 322,971–331,819 positions of the reference mitogenome.

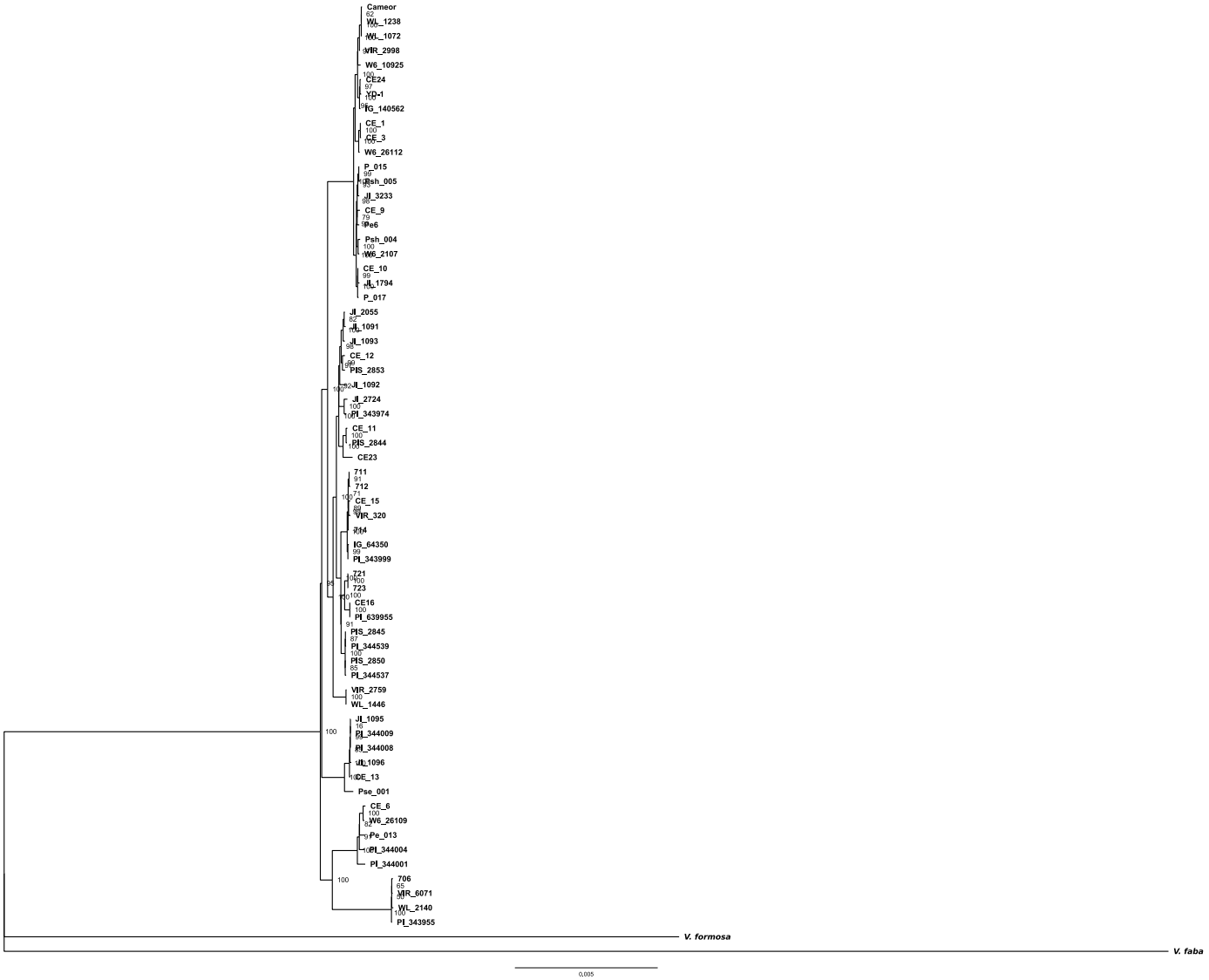


Figure 3. Phylogenetic tree of the studied pea accessions reconstructed on the basis of the plastid genomes using Maximum Likelihood method. Bootstrap support values of the nodes are indicated. Scale bar corresponds to the number of nucleotide substitutions per site.

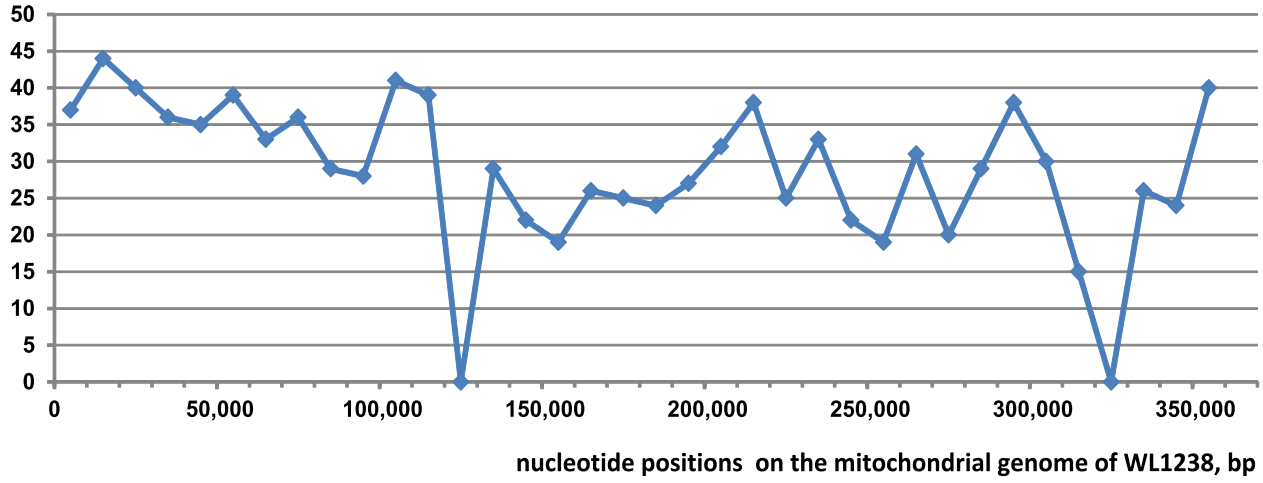
The number of differences from the reference genome met in all accessions of the M2 clade (except for the three accessions with *abyssinicum*-related mitogenomes where a large portion of the considered region was deleted), without respect to their occurrence in representatives of other clades, was 274 in total, corresponding to a mean value of 7.5 per 10 Kb. However, the region corresponding to positions 318,383–331,684 of the reference mitogenome concentrated an unexpectedly large number of such differences—38.3 per 10 Kb (Figure 5b), the diversity level characteristic of a much more diverged M1 clade. As mentioned above, the differences present in M2 clade scored in this interval were as well met in all or almost all representatives of the M1 clade, reflecting disappearance of M1-specific differences (Figure 5a).

Figure 4. Phylogenetic tree of the studied pea accessions reconstructed on the basis of the mitochondrial genomes using Maximum Likelihood method. Designations as in Figure 3.

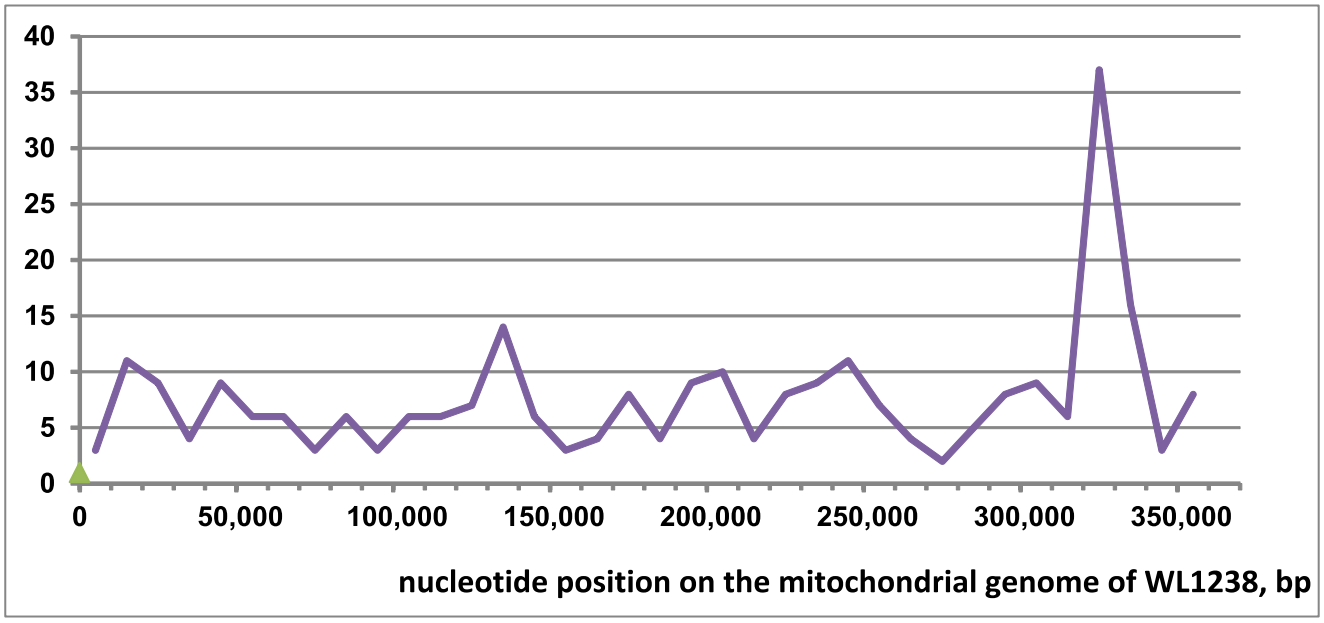
3.4. Phylogeography of European Wild Peas with Respect to Organellar Genomes

The sample of wild pea accessions from Southern Europe, with sequenced organellar genomes, was updated with 11 additional ones sequenced in this study to comprise 20 accessions in total plus one more accession 723 from Sardinia, which had nondehiscing pods, but both its organellar genomes were 'wild', most probably inherited from its local wild progenitor [2]. Among the newly sequenced accessions, four had the organellar genome constitution P4 M3, three P4 M4, three P3 M4 and one P3 M3 (Table 1), of which P3 M4 was not observed in our previous study [2]. The total sample contained seven accessions with P4 M3, five P4 M4, three P3 M4, three P4 M2, two P3 M3, and one P5 M4. Of them, the constitution P4 M2 was found only in islands, Menorca and Sicily, and the constitution P5 M4 in one accession from Bulgaria. Therefore, most of the continental Europe with the only exception of the mentioned accession from Bulgaria is occupied by wild peas with plastids belonging to the branches P3 and P4 and mitochondria belonging to the

branches M3 and M4 in all possible combinations, but the organellar genome constitution P4 M3 predominates. A striking diversity was observed in a sample of seven accessions collected from the Athos Peninsula in Chalkidiki, Greece, of which three represented the organellar genomic constitution P4 M4, three constitution P3 M4 and one constitution P3 M3. However, the combination P4 M3 predominating further west in the continental Europe was not found in the Athos Peninsula.



(**a**)



(**b**)

Figure 5. (**a**) Distribution along intervals of 10 Kb of the number of differences specific to the mitochondrial genomes of representatives of the M1 clade from the reference mitochondrial genome of accession WL_1238. (**b**) Distribution along intervals of 10 Kb of the number of differences from the reference mitogenome met in all representatives of the M2 clade except for the accessions with *abyssinicum*-related mitogenomes.

Figure 6 shows geographic distribution of particular organellar genome constitutions in the complete sample of the organellar genomes sequenced in *P. sativum* subsp. *elatius* s.l. in this and the previous [2] study, with accession IG_64350 from the ICARDA collection removed since its geographic origin from Algeria is suspected to be incorrect. Phenotypically and genetically, this accession looks like a typical 'southern humile' from Israel.

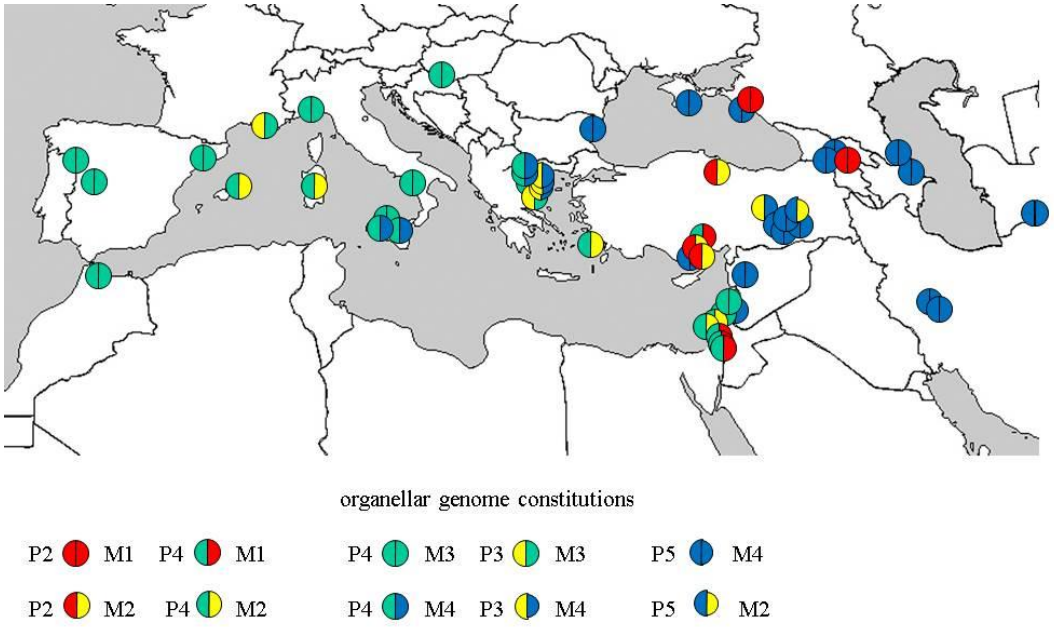


Figure 6. Geographic distribution of organellar genome constitutions of the *P. sativum* subsp. *elatius* accessions sequenced in this and the previous [2] study. The left half of a circle indicates plastid clades P2, P3, P4 and P5 coloured in red, yellow, green or blue, respectively; the right half indicates mitochondrial clade M1, M2, M3 and M4 coloured in the same sequence.

Our analysis involved accession CE_23 collected from a natural wild pea population of the Mediterranean area of Morocco (Er Rif Mountains, Chefchaouen environs, the Tissouka Mt. W foot), which is close to the Iberian Peninsula. It appeared to have the organellar genome constitution P4 M3, which, according to our data, is most frequent in South Europe; besides, it is the only constitutoin so far found in Iberian Peninsula.

3.5. Organellar Genome Constitutions of Israeli Wild Representatives of Pisum Sativum

The Israeli wild peas currently considered in *P. sativum* subsp. *elatius* s.l. were roughly classified by Ben-Ze'ev & Zohary [31] into three types, '*elatius*', 'southern *humile*' and 'northern *humile*'. In this study we updated the sample of Israeli wild peas with sequenced organellar genomes from four in [2] to eight. In this sample (Table 1 here and Table 1 in [2]), representatives of the '*elatius*' type possess organellar genome constitutions P4 M2 (accession 721) and P4 M3 (accessions PI_639955, CE_16); representatives of 'southern *humile*'—constitutions P4 M1 (accessions 711, 712 and 714) and P4 M2 (accession CE_15) and the only representative of 'northern humile' (accession JI_1794=716) has constitution P5 M4. The accession of 'southern *humile*' with P4 M2 is the above mentioned CE_15, the mitochondrial genome of which is closely related to those of *P. abyssinicum*. Curiously, the northernmost representative of 'southern *humile*', CE_15 from Lower Galilee, had the same genome constitution P4 M2 as accession 721 being '*elatius*', both situating at similar latitudes of 32.59° N and 32.87° N, respectively. At the same time, three other representatives of 'southern *humile*' originated from lower latitudes of 31.1–31.8° N and had the organellar genome constitution P4 M1 (Table 1; [2], Table 2 therein).

4. Discussion

4.1. Phylogenetic Reconstructions Based on the Updated Set of Organellar Genomes

The previous study of pea organellar genomes was based on a representative sample of 42 accessions of mostly wild peas belonging to *P. sativum* subsp. *elatius* s.l. [2]. The phylogenetic relationships revealed in this study based on the updated sample of sequenced accessions did not change substantially (Figures 1 and 2, compare to [2] (Figures 2 and 4 therein)), well supporting the discordance of the plastid and mitochondrial phylogenies

revealed in [2]. We use the designations P1-P5 of five main clades of the plastid genomes and M1-M4 of the four main clades of the mitochondrial genomes of peas introduced by Bogdanova et al. [2]. However, being just a representation of a more complex natural variability, this scheme could be challenged by expanding the sample studied. Indeed, in the phylogenetic tree of mitochondrial genomes, accession CE_24 from the Samur Delta Forest at the Caspian coast in southern Dagestan appeared to diverge from the common stem of the clades M3 and M4 before their divergence (Figure 2). Thus we formally could not classify the organellar constitution of this accession, and ascribed it the conventional constitution P5 M3/M4 in Table 1.

We cannot offer plausible interpretation of the striking identity of the mitochondrial genomes of accessions JI_2724 from Menorca and PI_343974 from Turkey while their plastid genomes, although belonging to the same sub- branch inside P4, are diverged at the level of 1.90×10^{-4} that excludes label confusion at the sequencing step. These accessions were obtained by us from the germplasm collections of John Innes Centre, Norwich, UK and Plant Introduction and Testing Research, Pacific West Area, Pullman, WA, USA, respectively, so introgression during reproduction can almost be excluded. However, label confusion at sample adoption by some collections cannot be excluded, so these two accessions could in fact originate from geographically close populations e.g., in Turkey.

A poor (if any) resolution of phylogenetic relationships of the genera *Pisum, Vavilovia* and *Vicia* L. is natural to interprete so that their radiation took place for rather short period of time. However, the lineage of *Vavilovia* appeared ca twice as long, and the lineage of *Vicia* ca four times as long as the lineage leading to *Pisum* in the Maximum Likelihood reconstruction based on the plastid genomes (Figure 3). In the ML reconstruction based on the mitochondrial genomes, the *Vicia* lineage was ca four times as long as both lineages of *Pisum* and *Vavilovia* (Figure 4). Although uncertainty of the reconstruction of their divergence is to be borne in mind, this could mean that rates of organellar genome evolution could have varied among genera. At the same time, the *Pisum* crown is very short in the Maximum Likelihood reconstructions as compared to lineages leading to genera, suggesting its rather recent radiation. Based on sequences of a number of genes encoded in plastids and the nuclear ITS spacer between rRNA genes, Schaefer et al. [11] estimated the *Pisum* crown age as 2.3–0.8 Ma, the time of divergence between *Pisum* and *Vavilovia* as 9.4–4.8 Ma and the crown age of Fabeae (which may also be taken as the time of divergence between *Pisum* and *Vicia*) as 23–16 Mya. Our Maximum Likelihood reconstructions (Figures 3 and 4) in general corresponded to these estimates.

4.2. A Mitochondrial Wild Relative of Pisum abyssinicum Found in Israel

Pisum abyssinicum [33], *P. sativum* L. subsp. *abyssinicum* [27,34] or *Lathyrus schaefferi* Kosterin [25] (for English translation see [26]) is an enigmatic cultivated pea, morphologically very similar to *P. sativum* s.str. but with a low crossing compatibility at least with *P. sativum* subsp. *sativum* [35]. The Abyssinian pea is endemic to Yemen and Ethiopia where it is cultivated along with local forms of *P. sativum* subsp. *sativum* [3,33,34]. It exhibits primary characters of the domesticated syndrome such as nondehiscing pods, absence of seed dormancy [25,36] and nongritty testa (phenotype gty) [25–27] and has never been reported from the wild [25,26], although sometimes unreasonably considered among pea wild relatives (e.g., [3]). Based on phylogenetic analyses of various nuclear markers, it was repeatedly shown to originate from an unknown wild relative, which was domesticated independently from the common cultivated subspecies [37–40] or, more likely, entered cultivation through the hitchhiking effect, as an admixture to the common cultivated pea *P. sativum* subsp. *sativum* [25,26,40] and then supplanted the latter in arid conditions because of a very fast development and short life cycle [25,26]. This was supposed to take place in Yemen [25,26] or southern Levant [40]. The independent origin of *P. abyssinicum* is supported by the fact that such useful domesticated traits as indehiscing pods and probably nondormant seeds, have different genetic basis in these two taxa of cultivated peas [27], and selection signatures traced in their nuclear genome did not overlap [40].

Vershinin et al. [37] and Jing et al. [38] supposed that *P. abyssinicum* was a hybrid between *P. sativum* subsp. *elatius* and *P. fulvum*, occurred somewhere in the Fertile Crescent and later domesticated independently from the common pea, since it shared alleles of molecular markers of both. Much earlier, the same hypothesis had been put forward by L.I. Govorov [33], who based on few morphological characters of doubtful importance [25,26]. However, Weeden [27], Trněný et al. [39] and Hellwig et al. [40] did not find evidence for such origin of *P. abyssinicum*. This controversy can be interpreted so that Vershinin et al. [37] underestimated genetic diversity of *P. sativum* subsp. *elatius* and considered some alleles common between this subspecies and *P. fulvum* (or very similar) as belonging to the latter. As a result, Weeden [27] supposed that *P. abyssinicum* (considered as *P. sativum* subsp. *abyssinicum*) either resulted from a hybrid between *P. sativum* subsp. *elatius* and *P. sativum* subsp. *sativum,* or the latter did not participate in its origin, so that it could be a hybrid between different evolutionary lineages of *P. sativum* subsp. *elatius*; such a hybridisation would have occurred still in the wild state.

Our finding of an unusual mitochondrial genome for *P. sativum* subsp. *elatius* resembling that of *P. abyssinicum*, in accession CE_15 from Lower Galilee points to a wild relative of the Abyssinian pea. This accession represents a wild population (the so-called 'southern *humile*' sensu Ben-Ze'ev & Zohary [31]) where the plants grow at a wheat field margins in a weedy manner (Table 1). While its plastid genome clusters tightly with those of other sequenced representatives of 'southern *humile*', its mitochondrial genome is unrelated to theirs but appears tightly related to those of *P. abyssinicum*. Thus, we face a case of hybridisation involving a relative of *P. abyssinicum*. This case can hardly be interpreted through hybridisation with the present-day *P. abyssinicum* itself because of the geographical remoteness of its range (Yemen and Ethiopia) from Lower Galilee. Therefore, we have to suppose that CE_15 descended from an ancient spontaneous hybrid between the 'southern *humile*', which provided the plastid genome, and some unknown wild relative of *P. abyssinicum,* which served as donor of mitochondrial genome. Actually, the latter progenitor could be the genuine wild ancestor of the Abyssinian pea, which most probably is extinct at present.

This interpretation does not invoke any hybridisation in the prehistory of *P. abyssinicum,* neither as proposed by Vershinin et al. [37] and Jing et al. [38] nor by Weeden [27]. At the same time, CE_15 obviously resulted from hybridisation, which no doubt took place in the wild and most probably in remote past. So, our finding of the unexpected combination of organellar genomes of CE_15 provided indirect evidence that the otherwise unknown 'genuine wild *P. abyssinicum*' did exist somewhere in Levant.

Indeed, based on analysis of restriction site associated DNA sequencing data, Hellwig et al. [40] supposed *P. abyssinicum* to originate from the 'southern *humile*' in Levant. So far, our data correspond to this supposition only in part as we have found among the four accessions of the latter a very close relative of the former with respect to mitochondria but still not to plastids. As follows from Figures 1 and 2, the plastid genomes of *P. abyssinicum* (see accessions VIR_2759 and WL_1446) belong to the evolutionary branch P4 and its mitochondrial genomes to the branch M2 but are quite unrelated to other representatives of those branches, that is *P. abyssinicum* looks like a quite well diverged lineage of *P. sativum* subsp. *elatius* s.l., similar to phylogenetic reconstructions by all other authors [2,13,27,36–42].

It is noteworthy that CE_15 resembles *P. abyssinicum* in its habit having serrate leaflets, relatively small flowers with a pinkish standard, absence of the anthocyanin ring at axils (phenotype d) and a rather low plant height. At the same time, CE_15 differs from *P. abyssinicum* by such characters, common in *P. sativum* subsp. *elatius*, as dehiscing pods (phenotype Dpo) and perfectly round (not appressed or smoothly squarish) seeds with a gritty testa (phenoptype Gty) carrying a pale fork at the radicle (phenotype Fu) and altogether three types of maculation: brownish marble pattern (phenotype M), fine violet dots (phenotype Fs) and large violet strokes (phenotype U^{st}). It would be of a great interest to investigate the crossing compatibility between CE_15 and *P. abyssinicum*.

It should also be added that the above considerations are further strong arguments to consider the Abyssnian pea as a subspecies *P. sativum* subsp. *abyssinicum*, as suggested by Weeden [27], rather than an independent species.

4.3. A Probable Case of Ancestral Recombination between Mitogenomes of M1 and M2 Clades

Mitochondrial genomes of the two accessions of *P. abyssinicum* and that of accession CE_15 shared a large deletion in the genome region corresponding to the positions around 320,000–330,000 of the reference mitogenome of WL_1238, which evidences for the common origin of these genomes. In *P. abyssinicum*, the deletion is ca 9.9 kb and in CE_15 is ca 8.8 kb long. According to our phylogenetic reconstruction (Figure 2), these accessions form a tight cluster inside the M2 clade. Curiously, the corresponding region of the mitogenome has a striking peculiarity with respect to diversity distribution. While the M1 clade was first to diverge and accumulated the largest number of differences from the reference genome, in the M2 clade the number of differences was much less: 1316 vs. 274, respectively (here, the count refers to the differences, which are common to all representatives of a clade, whether these differences occur in other clades or not). These differences were distributed along the mitogenome rather evenly with a notable exception of the region in question. Here, the number of differences common to the representatives of M2 (without the accessions where a large portion of the region was deleted) increased to 38.3 per 10 Kb as compared to the average of 7.5 per 10 Kb over the entire genome. Notably, all differences in these regions present in representatives of M2 were also present in all (or almost all) representatives of M1, while M1-specific (Figure 5a) and M2-specific differences disappeared. This observation can be interpreted as a consequence of an ancient recombination between the mitogenome of some of the ancestors of the M2 lineage and that of a representative of the M1 lineage, so that about 10 Kb from an M1 mitogenome replaced the homologous region of the M2 mitogenome giving rise to the mitogenomes of the present day M2 clade. The recombination event could have been accompanied by deletion in some descendants, which could have given rise to the *abyssinicum*-related mitogenomes. Alternatively, the region in question can, for some reason, represent a hotspot of mitogenome rearrangements and could be lost later in evolution.

4.4. Organellar Diversity of Israeli Wild Peas

Israel is a small country with diverse wild peas belonging to two species, *P. fulvum* and *P. sativum*. Ben-Ze'ev & Zohary [31] have subdivided the Israeli wild peas currently attributed to *P. sativum* into three groups, which they designated as '*P. elatius*' (tall climbers with very large flowers), 'northern *Pisum humile*' and 'southern *Pisum humile*', both with a low habitus but the former growing in natural grassland of Golan Heights, while the latter grows in secondary habitats like cereal field margins, roadsides and abandoned plantations across Israel. In Israel, these groups indeed are three distinct phenotypic classes, but the border between them blurs with extending geographical scale, so currently all of them are considered within *P. sativum* subsp. *elatius* s.l. [8]. Ben-Ze'ev & Zohary [31] showed that 'northern *humile*' had the same karyotype as the cultivated pea (*P. sativum* subsp. *sativum*), while '*P. elatius*' and 'southern *humile*' shared a common translocation. This inference was later corroborated by the complete nuclear genome analysis [36]. Molecular phylogeny based on histone H1 genes also revealed relatedness of 'northern *humile*' to the cultivated pea and of '*P. elatius*' and 'southern *humile*' to each other, the former group belonging to evolutionary 'lineage B', while the two latter to 'lineage AC' [41,42]. Recently, Hellwig et al. [12,40], based on restriction site associated DNA sequencing, claimed 'southern *humile*' to be a genetically distinct and rather homogeneous group.

We have sequenced the organellar genomes in eight Israeli accessions of *P. sativum* subsp. *elatius* s.l., five of which represented lines analysed by Ben-Ze'ev & Zohary [31] (Table 1; [2] (Table 1 therein)): one of the 'northern *humile*' type; four of the 'southern *humile*' type and three of the '*P. elatius*' type. The only representative of 'northern *humile*' expectedly had the organellar genome constitution P5 M4, typical of the cultivated

peas [2]. Interestingly, in both 'southern *humile*' and '*P. elatius*' we found substantial organellar diversity.

The 'southern *humile*' appeared heterogeneous with respect to mitochondria because of the above discussed accession CE_15 with a *P. abyssinicum*-related mitochondrial genome. This accession has the organellar constitution P4 M2, while the three other accessions of 'southern *humile*' have P4 M1 (Table 1; [2]). This heterogeneity is contrasted to the homogeneity of 'southern *humile*' found by Hellwig et al. [12] with respect to the nuclear genomes.

Earlier [2], accession 712 was found to represent a peculiar ancient branch first to diverge in the M1 clade of the mitochondrial phylogenetic tree. Accession 711 added in this study expectedly occurred tightly related to it, since their original location in Negev Desert and the foothill plain of the Judean Mountains, respectively [31], are situated just some 60–70 km apart.

4.5. Organellar Monophyly of Pisum fulvum and Putative Introgressions

Weeden et al. [13] made phylogenetic reconstructions of mostly wild peas on the base of the intron in the nuclear gene *i* (synonym *SGR*), involved still in the work by Gregor Mendel, where *P. fulvum*, a morphologically very clear-cut and unmistakable species, unexpectedly appeared nonmonophyletic. In their Bayesian reconstruction, ten involved accessions of *P. fulvum* fell into two branches, 'fulvum A' (5 accessions, taking into account synonymy of JI_2205 and VIR_6070), which derived first from the rest of the genus *Pisum*, and '*fulvum* B' (4 accessions), which strikingly was a sister subbranch to 'group C' of *P. sativum*, represented mostly by accessions of the cultivated subspecies *P. sativum* subsp. *sativum*. In the phylogenetic tree reconstructed with the maximum parsimony method, accessions of *P. fulvum* occupied basal positions without a reliable resolved topology. The Bayesian reconstruction could be interpreted so that 4 of 10 analysed *P. fulvum* accessions showed evidence of some, most probably ancient introgression of some nuclear alleles from *P. sativum* to *P. fulvum*.

The study by Weeden et al. [13] and our earlier study [2] had only one *P. fulvum* accession in common, WL_2140 = JI_2204, which belonged to the 'fulvum A' group according to Weeden et al. [13]. To infer the relationship of organellar genomes of accessions belonging to groups A and B according to the cited authors, we included a representative of the '*fulvum* B' group, VIR_6071. In the phylogenetic reconstructions based on both plastid and mitochondrial genomes it fell into the tight cluster, with the maximum support of the posterior probability being unity, with three other sequenced accessions of *P. fulvum* (Figures 1 and 2), thus differing from the pattern revealed by Weeden et al. [13] based on a nuclear marker.

It is noteworthy that in the phylogenetic reconstructions based on both plastid (Figure 1) and mitochondrial (Figure 2) genomes, the two morphologically distinct species of *Pisum*, *P. sativum* and *P. fulvum*, did not form the first divergence of the *Pisum* cluster as expected. Instead, accessions of *P. fulvum* resided in one of the two first diverging branches of the *Pisum* cluster (P1 + P2 in the plastid tree and M1 in the mitochondrial tree) together with some accessions of *P. sativum* subsp. *elatius* s.l. In our previous analysis of a smaller sample of 42 accessions [2], the mitochondrial tree revealed the same pattern, while in the plastid tree the position of clade P1 of *P. fulvum* was not resolved with respect to the branches P2 and P3+P4+P5 of *P. sativum* (*P. abyssinicum* is very similar to *P. sativum* and appeared as a minor lineage of the latter in all phylogenetic reconstructions [2,13,27,36–42]). The pattern revealed may be interpreted if we suppose that also some cases of introgression of organelles between the *P. fulvum* evolutionary lineage and some representatives of the *P. sativum* lineages could take place after initial divergence of these lineages 2.3–0.8 mya [11] but still before divergence of the ancestors of the *P. fulvum* accession studied by us.

The discussed introgression of organelles could have occurred by ways other than hybridisation, via so-called horizontal gene transfer. The latter implies mechanic capture of foreign plastids [43] or mitochondria [44] upon physical contact, direct or mediated by

parasites etc. [45]. Although mechanisms of horizontal transfer are not yet fully uncovered, it deserves special attention since it appears to be widespread among plants [46] and can contribute to the discordant mode of organelle evolution.

4.6. Organellar Phylogeography of European Wild Peas

The genus *Pisum* L. is thought to have its origin in the eastern Mediterranean area, where both its species occur and to which one of them, *P. fulvum*, is endemic [31]. Figure 6 shows geographic distribution of the organellar genome constitutions of accessions of *P. sativum* subsp. *elatius* s.l. sequenced in this and the previous [2] study, with one accession from Algeria removed (see below). Actually, this is the updated version of Figure 6 in [2] (note a *lapsus calami* in its caption: *P. sativum* subsp. *sativum* should be read as *P. sativum* subsp. *elatius*). As seen from Figure 6, the eastern Mediterranean area (including the Black Sea coastal area) is mostly inhabited by wild peas possessing plastids and mitochondria from either the basal clades (that is diverged earlier during the genus evolution), designated in Figure 6 with red colour, or terminal clades designated with blue. The combination P5 M4 composed of most terminal clades of both organellar genomes (all blue circles) extends to the east to be met in Iran and Turkmenistan. At the same time, the western part of the genus' range, that is, southern Europe (including islands) and Morocco (represented by a single accession) are inhabited by peas with mostly intermediate clades (designated with yellow and green).

A similar picture based on less informative three diallelic molecular markers from the three cellular genomes was observed earlier [32] and led us to sketch an evolutionary scenario where European peas represented an 'intermediate stage' of the *P. sativum* subsp. *elatius* s.l. microevolution. The so-called 'lineage B', corresponding to organellar genome constitution P5 M4 with organellar genomes from terminal clades of corresponding phylogenetic trees, originated from that 'intermediate stage' pea and 'returned' to the Eastern Mediterranean [32]. However, later analysis of phylogenetic relationships of the organellar genomes [2] ruled out that supposition. Instead, we claimed that wild peas from most of Southern Europe were of hybrid origin, based on substantial diversity of their organellar constitutions. The pattern of Figure 6 suggests that this is true for the Mediterranean islands and Greece, where this diversity is striking, indeed. At the same time, the continental south of Europe from Hungary to Portugal is occupied by wild peas with the P4 M3 constitution (6 accessions in total), with the only exception of the constitution P3 M3 found in Provence. We may suppose that these were peas with the constitution P4 M3 composed of both organellar genomes from 'intermediate clades', which colonised Europe during the species' expansion to the west, although we have no basis to consider it of hybrid origin or not.

The western direction of that expansion is supposed from the overwhelming predominance of the genomic constitution P4 M3 in continental Europe (Figure 6), while its supposed parental evolutionary lineages occur in the Near East and in view of the overall much greater diversity of organellar genomes in the latter region. Curiously, formal computer analysis of data of restriction site associated (nuclear) DNA sequencing carried out by Hellwig et al. [12] revealed signs of the opposite, eastward expansion of some genotypes of *P. sativum* subsp. *elatius*: from their Genetic Cluster 3, occupying continental Europe from Spain to Greece, to Genetic Cluster 1, distributed in the southern Asia Minor and Genetic Cluster 6, broadly occurring in Anatolia, Black Sea Region and the Caucasus. At least in part, this contradiction could be explained by different, scarcely overlapping samples of, respectively, 81 and 56 accessions of *P. sativum* subsp. *elatius* involved in their [12] and our analyses, which were still insufficient for reliable inferences. At the same time, it would be logical to suppose that organellar genomes evolve discordantly not only from each other [2] but also from the nuclear genome. For instance, Hellwig et al. [12] putatively associated the eastward expansion they reconstructed with the climate amelioration after the last Pleistocene cooling, while the organellar genomes could retain signs of the earlier expansion of peas from their initial origin centre in the Near East. It would be most interesting to test

these hypotheses by simultaneous phylogeographic analysis of the nuclear and organellar genomes in the same large wild pea sample, which hopefully could be a matter of future.

A greater diversity of organelle genome constitutions in the islands and Balkan Peninsula as compared to the main continental Europe may result from occasional nature of island colonisation by plants. Alternatively, it may reflect island natural history being complicated by changes of the Mediterranean Sea level in the past, e.g., be the remnant of genetic diversity of wild peas once existing in presently inundated lands. Presence of such different combinations of organellar genomes in these areas is to be interpreted through past hybridisation events between unrelated pea lineages indeed [2].

Especially impressive appeared the genetic diversity of wild peas collected in 1969 by Howard Scott Gentry [47] in the monastery area sized some 9 × 6 km in the small Athos Peninsula in Chalkidiki, Greece, of which we have now sequenced seven of the eight accessions available in germplasm collections (accession JI_1094 is not yet sequenced). The seven sequenced accessions revealed three organellar genome constitutions, P3 M3, P3 M4 and P4 M4. Curiously, the most common European constitution P4 M3 was not found in the Athos Peninsula.

Hellwig et al. [12] (p. 8) made the following note, "The number of available genebank accessions from north Africa is very limited, often with questionable passport data. Future expeditions to this area may enable researchers to shed more light on the role of this area in wild pea evolution". We met a probable case of 'questionable passport data' in two *P. sativum* subsp. *elatius* s.l. accessions, IG_64350 and IG_108291 from Algeria and Tunisia, respectively; both originated from the ICARDA collection where label confusions are said to occur (P. Smýkal, pers. comm.). Although attributed to close geographic locations, they were strongly dissimilar to each other. The former was almost identical with respect to its morphology and organellar genomes (P4 M1) to the Israeli 'southern *humile*' [2]; the latter resembled 'nothern *humile*' and had markers of 'combination B' [29] indicating at the P5 M4 genome constitution distributed in the eastern Mediterranean and the Caspian area (Figure 6). Both organellar constitutions are highly improbable to be met in Western Mediterranean, so we excluded these accessions from our consideration. Instead, in May 2021, one of us (O.K.) undertook an expedition to Morocco and collected a wild pea in the Er Rif Mountains situated on the Tanger Peninsula approaching the Iberian Peninsula. There was no surprise that it appeared to have the genome constitution P4 M3, the only one so far found in wild peas of the Iberian Peninsula.

4.7. A Problem of Pea Crop Closest Wild Relatives

Zaytseva et al. [29] revealed a phylogenetic marker shared by the overwhelming majority (one exception found in Afghanistan) of the cultivated peas attributed to the subspecies *P. sativum* subsp. *sativum*, but very few wild peas belonging to *P. sativum* subsp. *elatius*, an 8-bp deletion in the plastid *psbA-trnH* spacer. In fact, the deleted sequence TTAGAAGA is represented by two tandemly repeated copies in both wild pea species, *P. fulvum* and *P. sativum* subsp. *elatius*. One of the copies is missing in *P. sativum* subsp. *sativum*. The exceptional wild pea (*P. sativum* subsp. *elatius*) accessions with this deletion found by Zaytseva et al. [29] were W6_10925 and VIR_2998. Bogdanova et al. [2] updated this set with accession IG_140562 and this study with CE_24 and YD-1. All these five accessions represent the evolutionary lineage in *P. sativum* subsp. *elatius* to which the cultivated subspecies also belongs, which was designated as the lineage B by Kosterin et al. [32] and Zaytseva et al. [41,42]. Four of them have the organellar genomic constitution P5 M4 according to Bogdanova et al. [2], while accession CE_24 revealed a conventional constitution P5 M3/M4, as discussed above.

In the phylogenetic reconstruction based on the plastid genomes (Figure 1), the mentioned five accessions with the discussed deletion in the *psbA-trnH* spacer expectedly tightly cluster with cultivated peas and may be interpreted as their closest wild relatives; more precisely, their plastid genomes had the latest last common ancestor with those of *P. sativum* subsp. *sativum* quite recently. Other possibilities cannot, however, be excluded, such as

occasional introgression of plastids from cultivated to wild peas in some cases [29] or an independent origin of this deletion due to slippage mispairing resulting in the loss of a copy of the twice tandemly repeated sequence.

The provenances of the five accessions with the deletion in the plastid *psbA-trnH* spacer are as follows: Koped-Dagh Mts in Turkmenistan (YD-1), the Caspian coast in southern Dagestan (CE_24), Azerbaijan (IG_140562), Georgia (VIR_2998, no locality information) and the Black Sea coast of Bulgaria (W6_10925). It worth mentioning that accession YD-1 represents the easternmost population of *P. sativum* subsp. *elatius* known in the world.

However, looking for the mitochondrial genome most closely related to that of the cultivated *P. sativum* subsp. *sativum*, we have to nominate other wild pea accessions. First, we have to make a reservation that WL_1238 may not represent the cultivated subspecies since it has mitochondria not so closely related to the two other accessions of *P. sativum* subsp. *sativum* in our analysis. This is an experimental testerline with a complicated pedigree, as discussed in [2]. Cameor is a commercial pea cultivar, while accession WL_1072 has the mitochondrial genome identical to it, so we assume this genome to represent the genuine *P. sativum* subsp. *sativum*—at least its European stock. The accessions most close to it on the phylogenetic tree of the mitochondrial genomes (Figure 2) are P_017 from Mersin Il of Turkey and JI_1092 from Athos Peninsula in Greece. Somewhat less related are other accessions from this peninsula, except for the unrelated JI_1096.

Thus, the closest 'plastid relatives' and the closest 'mitochondrial relatives' of the cultivated pea are two different sets of accessions originating from very broad, but not overlapping, regions. This situation somewhat resembles the above considered case (see Section 4.2) of finding of 'a mitochondrial relative' but no 'plastid relatives' of another cultivated pea taxon, *P. abyssinicum*.

The nuclear genome analysis by Hellwig et al. [40] also revealed a very broad origin area of accessions *P. sativum* subsp. *elatius*, which they considered as closely related to *P. sativum* subsp. *sativum* (see Figure S3 in the cited reference). The four closest relatives originated from Antalya, Hatay and Kilis Ils of Turkey (the Mediterranean coast). The 12 less closely related accessions originated from whole Turkey, Black Sea coast and the Caucasus. Four of them (CE_1, CE_2, CE_3 and W6_26112), from Crimea and the Caucasus were also involved in our analysis. According to our data, they were indeed related to *P. sativum* subsp. *sativum* as having the same organellar constitution P5 M4, but as seen from above, did not belong to the closest relatives with respect to both organellar genomes.

The five closest wild 'plastid relatives' of the cultivated pea originated from the Black Sea/Transcaucasian/Caspian area while the closest 'mitochondrial relatives' (as well as the closest 'nuclear relatives' according to [40]) were from the eastern Mediterranean coast (although from quite remote Chalkidiki and southern Anatolia). The former area is situated to the northwest, north and north-east and the latter area to the west of the so-called Core Area of domestication of the founder crops, including pea, of Near East some 10 thousand years ago, which gave rise to the so-called agrarian Neolithic Revolution [14,48]. The Core Area is reconstructed roughly in the Mardin and Diyarbakir Ils in south-eastern Turkey. Neither the above considered 'plastid relatives' nor 'mitochondrial relatives' of the cultivated peas originated exactly from the presumed Core Area.

At the same time, earlier [2] we sequenced plastid genomes in two wild pea accessions from Mardin Il and one from Diyarbakir Il, and in this study we added one more accession (Pe_6) from Mardin Il, four accessions in total. They are all low habitus peas informally classified as 'northern *humile*' type, which used to be considered as a progenitor of the cultivated pea [31]. Accession Pe_6 is remarkable as it originated from an area just 24 km west northwest of the famous epipaleolithic archaeological site Göbekli Tepe, which was the oldest monumental temple complex on the planet but was constructed by a society still in the hunter-gatherer stage of lifestyle development [49,50]. The earlier sequenced accession P_015 originated from the Karaçadag shield volcano, which is considered to be the center of origin of all cultivated einkorn wheat based on molecular evidence [51]. Although these four accessions belong to the same evolutionary 'lineage B' according to [41,42] and have

the same organellar genome constitution P5 M4 according to [2] as the cultivated peas, they are not the closest relatives of the latter with respect to either plastid (Figure 1) or mitochondrial (Figure 2) phylogenetic reconstructions.

If we assume that the cultivated subspecies *P. sativum* subsp. *sativum* had a singular origin, we have to suppose that its wild progenitor had both its organellar genomes most closely related to those of the cultivated pea, e.g., of cultivar Cameor. Curiously, so far we did not find such wild pea accession, either in the Core Area or elsewhere. This circumstance may have different interpretation but evidently points at incompleteness of our current knowledge.

First, our results showed that Near East, alike the above considered Europe, was also an arena of 'free recombination' of plastid and mitochondrial lineages via occasional hybridisation of wild peas in the past, which could take place, still in the wild state, both before and after Neolithic Revolution. It may happen that the once domesticated wild pea lineage, with 'proper' plastids and mitochondria, has been extinct from the wild, or even was domesticated entirely, as it was supposed for the 'missing ancestor' of the broad beans [52]. Also, it may have not yet been found among the extremely genetically diverse wild peas of Turkey. Smýkal et al. [53] and Hellwig et al. [12,40] revealed considerable genetic variation in wild peas of south-eastern Turkey but information on the organellar genomes was missing.

It should also be taken into account that for the ten thousand years passed since pea domestication, the distribution of wild pea lineages could shift because of climatic changes or other factors so that the closest wild relatives of the cultivated pea could still be found beyond the Core Area. This can be elucidated by niche modelling of the flora of Anterior Asia at the time of the onset of agriculture; alike it was performed for wild peas for the time of the last glaciation maximum [54].

Alternatively, we may suppose that different local wild pea gene pools were involved into formation of the domesticated pea via their introgression into the cultivated pea while spreading its cultivation from the Core Area or even by independent attempts of domestication beyond that area, as suggested by the so-called Protracted Domestication Model [55–58]. What we now assume to be the 'proper' cultivated pea genomes may refer to the contemporary West European cultivated gene pool, while local traditional pea landraces from ancient agriculture areas, like those analysed by Berdnikov et al. for histone H1 variation [59], may harbour more organellar genome lineages.

Regardless which alternative is true, both demand further increasing of the sample: the version of singular origin of the domesticated pea demands more wild peas analysed from Anterior Asia and the version of recruitment of multiple wild gene pools demands analysis of more traditional cultivated peas.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/d15020216/s1, Table S1: Differences of the sequenced plastid genomes as compared to that of accession WL_1238 (*Pisum sativum* subsp. *sativum*); Table S2: Differences of the sequenced mitochondrial genomes as compared to that of accession WL_1238 (*Pisum sativum* subsp. *sativum*); Table S3: Pairwise p-distances between plastid genomes; and Table S4: Pairwise p-distances between mitochondrial genomes.

Author Contributions: Conceptualization, N.V.S., V.S.B. and O.E.K.; methodology, N.V.S., V.S.B. and G.V.V.; software, V.S.B.; validation, G.V.V. and V.S.B.; formal analysis, V.S.B.; investigation, V.S.B. and N.V.S.; resources, G.V.V.; data curation, G.V.V.; writing—original draft preparation, O.E.K.; writing—review and editing, N.V.S., V.S.B., O.E.K. and G.V.V.; visualization, V.S.B.; supervision, O.E.K. and V.S.B.; project administration, O.E.K. and V.S.B.; funding acquisition, G.V.V. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by Ministry of Science and High Education, project FWNR-2022-0016.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The organellar genome sequences obtained in this study are available in Gene Bank; their identification numbers are provided in Table 1.

Acknowledgments: We are grateful to Clarice Coyne, Michael Ambrose, Petr Smýkal, Shahal Abbo, Michele Papazyan, Juan Modolell and Josep Vigo for providing wild pea accessions and to Shahal Abbo, Nadeszhda Solovyeva and Elena Ilyina for crucial help in expeditions. We are also grateful to the two reviewers for their comments, which greatly improved the paper. High-throughput sequencing was performed at the IC&G Joint Center for Genome Studies. Genome assembly and phylogenetic reconstruction were carried out with the use of the Computational Facility of the Siberian Supercomputer Center SB RAS and Computational Facility of Novosibirsk State University. Plants were grown in the greenhouse at the SB RAS Artificial Plant Growing Facility, ICG SB RAS.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Camus, M.F.; Alexander-Lawrie, B.; Sharbrough, J.; Hurst, H.D.D. Inheritance through the cytoplasm. *Heredity* **2022**, *129*, 31–43. [CrossRef]
2. Bogdanova, V.S.; Shatskaya, N.V.; Mglinets, A.V.; Kosterin, O.E.; Vasiliev, G.V. Discordant evolution of organellar genomes in peas (*Pisum* L.). *Mol. Phylogenet. Evol.* **2021**, *160*, 107136. [CrossRef]
3. Maxted, N.; Kell, S.P. *Establishment of A Global Network for the In Situ Conservation of Crop Wild Relatives: Status and Needs*; FAO Commission on Genetic Resources for Food and Agriculture: Rome, Italy, 2009; p. 266.
4. Kosterin, O.E. Prospects of the use of wild relatives for pea breeding. *Russ. J. Genet. Appl. Res.* **2016**, *6*, 233–243. [CrossRef]
5. Zohary, D.; Hopf, M. *Domestication of Plants in the Old World: The Origin and Spread of Cultivated Plants in West Asia, Europe and the Nile Valley*, 3rd ed.; Clarendon Press: Oxford, MS, USA, 2000; p. 316.
6. Bogdanova, V.S.; Kosterin, O.E.; Yadrikhinskiy, A.K. Wild peas vary in their cross-compatibility with cultivated pea (*Pisum sativum* subsp. sativum L.) depending on alleles of a nuclear-cytoplasmic incompatibility locus. *Theor. Appl. Genet.* **2014**, *127*, 1163–1172. [CrossRef] [PubMed]
7. Bogdanova, V.S.; Zaytseva, O.O.; Mglinets, A.V.; Shatskaya, N.V.; Kosterin, O.E.; Vasiliev, G.V. Nuclear-cytoplasmic conflict in pea (*Pisum sativum* L.) is associated with nuclear and plastidic candidate genes encoding Acetyl-CoA carboxylase subunits. *PLoS ONE* **2015**, *10*, e0119835. [CrossRef]
8. Maxted, N.; Ambrose, M. Peas (*Pisum* L.). In *Plant Genetic Resources of Legumes in the Mediterranean. Current Plant Science and Biotechnology in Agriculture 39*; Maxted, N., Bennett, S.J., Eds.; Kluwer Academic Publishers: Dordrecht, Germany, 2001; Volume 3, pp. 181–190.
9. Coulot, P.; Rabaute, P. Monographie de Leguminosae de France. Tome 4. Tribus des Fabeae, des Cicereae et des Genisteae. *Bull. Société Bot. Cent.-Ouest* **2016**, *46*, 1–902.
10. Coulot, P.; Rabaute, P. Deuxièmes compléments à la Monographie des *Leguminosae* de France. *Monde Plantes* **2017**, *516*, 11–35.
11. Schaefer, H.; Hechenleitner, P.; Santos-Guerra, A.; Menezes de Sequeira, M.; Pennington, R.T.; Kenicer, G.; Carine, M.A. Systematics, biogeography, and character evolution of the legume tribe Fabeae with special focus on the middle-Atlantic island lineages. *BMC Evol. Biol.* **2012**, *12*, 250. [CrossRef] [PubMed]
12. Hellwig, T.; Abbo, S.; Ophir, R. Drivers of genetic differentiation and recent evolutionary history of an Eurasian wild pea. *J. Biogeogr.* **2021**, *49*, 794–808. [CrossRef]
13. Weeden, N.F.; Coyne, C.J.; Lavin, M.; McPhee, K. Distinguishing among *Pisum* accessions using a hypervariable intron within Mendel's green/yellow cotyledon gene. *Genet. Resour. Crop Evol.* **2021**, *68*, 2591–2609. [CrossRef]
14. Lev-Yadun, S.; Gopher, A.; Abbo, S. The cradle of agriculture. *Science* **2000**, *288*, 1602–1603. [CrossRef] [PubMed]
15. Jansen, R.K.; Raubeson, L.A.; Boore, J.L.; de Pamphilis, C.W.; Chumley, T.W.; Haberle, R.C.; Wyman, S.K.; Alverson, A.J.; Peery, R.; Herman, S.J.; et al. Methods for obtaining and analyzing whole chloroplast genome sequences. *Methods Enzym.* **2005**, *395*, 348–384. [CrossRef]
16. Chevreux, B.; Wetter, T.; Suhai, S. Genome sequence assembly using trace signals and additional sequence information. In *Computer Science and Biology, Proceedings of the German Conference on Bioinformatics (GCB), Hannover, Germany, 4–6 October 1999*; Hannover, Germany; pp. 45–56. Available online: https://dblp2.uni-trier.de/db/conf/gcb/gcb1999.html (accessed on 26 June 2017).
17. Milne, I.; Stephen, G.; Bayer, M.; Cock, P.J.A.; Pritchard, L.; Cardle, L.; Shaw, P.D.; Marshall, D. Using Tablet for visual exploration of second-generation sequence data. *Brief. Bioinform.* **2013**, *14*, 193–202. [CrossRef]
18. Larkin, M.A.; Blackshields, G.; Brown, N.P.; Chenna, R.; McGettigan, P.A.; McWilliam, H.; Valentin, F.; Wallace, I.M.; Wilm, A.; Lopez, R.; et al. Clustal W and Clustal X version 2.0. *Bioinformatics* **2007**, *23*, 2947–2948. [CrossRef] [PubMed]
19. Tamura, K.; Stecher, G.; Peterson, D.; Kumar, S. MEGA6: Molecular evolutionary genetics analysis version 6.0. *Mol. Biol. Evol.* **2013**, *30*, 2725–2729. [CrossRef] [PubMed]
20. Drummond, A.J.; Rambaut, A. BEAST: Bayesian evolutionary analysis by sampling trees. *BMC Evol. Biol.* **2007**, *7*, 214. [CrossRef]
21. Darriba, D.; Taboada, G.L.; Doallo, R.; Posada, D. jModelTest 2: More models, new heuristics and parallel computing. *Nat. Methods* **2012**, *9*, 772. [CrossRef]

22. Guindon, S.; Gascuel, O. A simple, fast and accurate method to estimate large phylogenies by maximum-likelihood. *Syst. Biol.* **2003**, *52*, 696–704. [CrossRef]
23. Nguyen, L.-T.; Schmidt, H.A.; von Haeseler, A.; Minh, B.Q. IQ-TREE: A fast and effective stochastic algorithm for estimating maximum likelihood phylogenies. *Mol. Biol. Evol.* **2015**, *32*, 268–274. [CrossRef]
24. Hoang, D.T.; Chernomor, O.; von Haeseler, A.; Minh, B.Q.; Vinh, L.S. UFBoot2: Improving the ultrafast bootstrap approximation. *Mol. Biol. Evol.* **2018**, *35*, 518–522. [CrossRef]
25. Kosterin, O.E. Abyssinian pea (*Lathyrus schaeferi* Kosterin nom. nov. pro *Pisum abyssinicum* A. Br.) is a problematic taxon. *Vavilovskii Zhurnal Genet. Sel. Vavilov J. Genet. Breed.* **2017**, *21*, 158–169, (In Russian, English Summary). [CrossRef]
26. Kosterin, O.E. Abyssinian pea (*Lathyrus schaeferi* Kosterin nom. nov. pro *Pisum abyssinicum* A. Br.) is a problematic taxon. *Acta Biol. Sib.* **2017**, *3*, 97–110. [CrossRef]
27. Weeden, N. Domestication of pea (*Pisum sativum* L.): The case of the Abyssinian Pea. *Front. Plant Sci.* **2018**, *9*, 515. [CrossRef] [PubMed]
28. Palmer, J.D.; Jorgensen, R.A.; Thompson, W.F. Chloroplast DNA variation and evolution in *Pisum*: Patterns of change and phylogenetic analysis. *Genetics* **1985**, *109*, 195–213. [CrossRef] [PubMed]
29. Zaytseva, O.O.; Bogdanova, V.S.; Mglinets, A.V.; Kosterin, O.E. Refinement of the collection of wild peas (*Pisum* L.) and search for the area of pea domestication with a deletion in the plastidic *psbA-trnH* spacer. *Genet. Resour. Crop Evol.* **2017**, *64*, 1427–1430. [CrossRef]
30. Ross, M.G.; Russ, C.; Costello, M.; Hollinger, A.; Lennon, N.J.; Hegarty, R.; Nusbaum, C.; Jaffe, D.B. Characterizing and measuring bias in sequence data. *Genome Biol.* **2013**, *14*, R51. [CrossRef] [PubMed]
31. Ben-Ze'ev, N.; Zohary, D. Species relationship in the genus *Pisum* L. *Israel J. Bot.* **1973**, *22*, 73–91.
32. Kosterin, O.E.; Zaytseva, O.O.; Bogdanova, V.S.; Ambrose, M. New data on three molecular markers from different cellular genomes in Mediterranean accessions reveal new insights into phylogeography of *Pisum sativum* L. subsp. *elatuis* (Beib.) Schmahl. *Genet. Resour. Crop Evol.* **2010**, *57*, 733–739. [CrossRef]
33. Govorov, L.I. [Pea]. In *Kul'turnaya Flora [Cultivated Flora of the USSR]*; Vulf, E.V., Ed.; Gosudarstvennoe Izdatelstvo Sovkhoznoi i Kolkhoznoi Literatury: Moscow, Russia, 1937; Volume 4, Grain Legumes; pp. 229–336. (In Russian)
34. Makasheva, R.K. Pea. In *Kul'turnaya Flora SSSR [Cultivated Flora of the USSR]*, 2nd ed.; Brezhnev, D.D., Ed.; Kolos: Leningrad, Russia, 1979; Volume 4, Grain Legumes, Part 1; pp. 7–322. (In Russian)
35. Kosterin, O.E.; Bogdanova, V.S. Reciprocal compatibility within the genus *Pisum* L. as studied in F_1 hybrids: 3. Crosses involving *P. abyssinicum* A. Br. *Genet. Resour. Crop Evol.* **2020**, *67*, 967–983. [CrossRef]
36. Kreplak, K.; Madoui, M.A.; Cápal, P.; Novák, P.; Labadie, K.; Aubert, G.; Bayer, P.E.; Gali, K.K.; Syme, R.A.; Main, D.; et al. A reference genome for pea provides insight into legume genome evolution. *Nat. Genet.* **2019**, *51*, 1141–1422. [CrossRef] [PubMed]
37. Vershinin, A.V.; Allnutt, T.R.; Knox, M.R.; Ambrose, M.J. Transposable elements reveal the impact of introgression, rather than transposition, in *Pisum* diversity, evolution, and domestication. *Mol. Biol. Evol.* **2003**, *20*, 2067–2075. [CrossRef] [PubMed]
38. Jing, R.; Vershinin, A.; Grzebota, J.; Shaw, P.; Smýkal, P.; Marshall, D.; Ambrose, M.J.; Ellis, T.H.N.; Flavell, A.J. The genetic diversity and evolution of field pea (*Pisum*) studied by high throughput retrotransposon based insertion polymorphism (RBIP) marker analysis. *BMC Evol. Biol.* **2010**, *10*, 44. [CrossRef] [PubMed]
39. Trněný, O.; Brus, J.; Hradilova, I.; Rathore, A.; Das, R.R.; Kopecký, P.; Coyne, C.J.; Reeves, P.; Richards, C.; Smýkal, P. Molecular evidence for two domesticated events in the pea crop. *Genes* **2018**, *19*, 535. [CrossRef] [PubMed]
40. Hellwing, T.; Abbo, S.; Ophir, R. Phylogeny and disparate selection signatures suggest two genetically independent domestication events in pea (*Pisum* L.). *Plant J.* **2022**, *110*, 419–439. [CrossRef] [PubMed]
41. Zaytseva, O.O.; Bogdanova, V.S.; Kosterin, O.E. Phylogenetic reconstruction at the species and intraspecies levels in the genus *Pisum* L. (peas) using a histone H1 gene. *Gene* **2012**, *504*, 192–202. [CrossRef]
42. Zaytseva, O.O.; Gunbin, K.V.; Mglinets, A.V.; Kosterin, O.E. Divergence and population traits in evolution of the genus *Pisum* L. as reconstructed using genes of two histone H1 subtypes showing different phylogenetic resolution. *Gene* **2015**, *556*, 235–244. [CrossRef]
43. Stegemann, S.; Keuthe, M.; Greiner, S.; Bock, R. Horizontal transfer of chloroplast genomes between plant species. *Proc. Natl. Acad. Sci. USA* **2012**, *109*, 2434–2438. [CrossRef]
44. Gurdon, C.; Svab, Z.; Feng, Y.; Kumar, D.; Maliga, P. Cell-to-cell movement of mitochondria in plants. *Proc. Natl. Acad. Sci. USA* **2016**, *113*, 3395–3400. [CrossRef]
45. Davis, C.C.; Wurdack, K.J. Host-to-parasite gene transfer in flowering plants: Phylogenetic evidence from Malpighiales. *Science* **2004**, *305*, 676–678. [CrossRef]
46. Bergthorsson, U.; Adams, K.L.; Thomason, B.; Palmer, J.D. Widespread horizontal transfer of mitochondrial genes in flowering plants. *Nature* **2003**, *424*, 197–201. [CrossRef]
47. Gentry, H.S. *Foreign Travel Report. Italy, Greece, Turkey, Germany, England. 8 April–28 June 1969*; New Crops Research Branch: Beltsville, MD, USA, 1969; pp. 1–4, *unpublished report*.
48. Abbo, S.; Gopher, A.; Bar-Gal, G.K. *Plant Domestication and the Origin of Agriculture in the Ancient Near East*; Cambridge University Press: Cambridge, UK, 2022; p. 288. [CrossRef]
49. Schmidt, K. *Sie Bauten Die Ersten Tempel. Das Rätselhafte Heiligtum der Steinzeitjäger*; C.H. Beck: Munich, Germany, 2006; p. 288.

50. Dietrich, O.; Dietrich, L.; Notroff, J. Cult as a driving force of human history: A view from Göbekli Tepe. *Expedition* **2017**, *59*, 657–663.
51. Heun, O.; Schäffer-Pregl, R.; Kalavan, D.; Castagna, R.; Accerbi, M.; Borghi, B.; Salamini, F. Site of einkorn wheat domestication identified byDNA fingerprinting. *Science* **1997**, *278*, 1312–1314. [CrossRef]
52. Kosterin, O.E. The lost ancestor or the broad bean (*Vicia faba* L.) and the origin of plant cultivation in the Near East. *Vavilovskii Zhurnal Genet. Sel. Vavilov J. Genet. Breed.* **2014**, *18*, 831–840. [CrossRef]
53. Smýkal, P.; Trněný, O.; Brus, J.; Hanáček, P.; Rathore, A.; Roma, R.D.; Pechanek, V.; Douchoslav, M.; Battacharrya, D.; Bariotakis, M.; et al. Genetic structure of wild pea (*Pisum sativum* subsp. *elatius*) populations in the northern part of the Fertile Crescent reflects moderate cross-pollination and strong effect of geographic but not environmental distance. *PLoS ONE* **2018**, *13*, e0194056. [CrossRef]
54. Smýkal, P.; Hradilová, I.; Trněný, O.; Brus, J.; Rathore, A.; Bariotakis, M.; Das, R.R.; Battacharrya, D.; Richards, C.; Coyne, C.J.; et al. Genomic diversity and macroecology of the crop wild relatives of domesticated pea. *Sci. Rep.* **2017**, *7*, 17384. [CrossRef]
55. Tanno, K.; Wilcox, G. How fast was wild wheat domesticated? *Science* **2005**, *311*, 1886. [CrossRef] [PubMed]
56. Fuller, D.Q.; Willcox, G.; Allaby, R.G. Cultivation and domestication had multiple origins: Arguments against the core area hypothesis for the origins of agriculture in the Near East. *World Archaeol.* **2011**, *43*, 628–658. [CrossRef]
57. Fuller, D.Q.; Willcox, G.; Allaby, R.G. Early agricultural pathways: Moving outside the 'core area' hypothesis in Southwest Asia. *J. Exp. Bot.* **2012**, *63*, 617–633. [CrossRef] [PubMed]
58. Asouti, E.; Fuller, D.Q. From foraging to farming in the southern Levant: The development of the Epipaleolithic and Pre-pottery Neolithic plant managing strategies. *Veg. Hist. Archeobot.* **2012**, *21*, 149–162. [CrossRef]
59. Berdnikov, V.A.; Bogdanova, V.S.; Rozov, S.M.; Kosterin, O.E. Geographic patterns of histne H1 allelic frequencies formed in the course of *Pisum sativum* L. (pea) cultivaion. *Heredity* **1993**, *71*, 199–209. [CrossRef]