

Spatial patterns and intraspecific diversity of the glacial relict legume species *Vavilovia formosa* (Stev.) Fed. in Eurasia

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Abstract *Vavilovia formosa* is one of five genera in tribe Fabeae, (Fabaceae, Leguminosae) with close phylogenetic relationships to *Pisum*. It grows in subalpine and alpine levels in Armenia, Azerbaijan, Georgia, Iran, Iraq, Lebanon, Russia and Turkey and is recognized as an endangered and protected plant. This study was conducted to reveal its intraspecific variability, as well as to predict the past, extant and future species distribution range. We analysed 51 accessions with common phylogenetic markers (*trnF-trnL*, *trnS-trnG*, *matK*, *rbcL*, *psbA-trnH* and ITS). These represent in total up to 2551 bp of chloroplast and 664 bp of nuclear sequences per sample. Two populations from Turkey and Armenia were analysed for genetic diversity by AFLP. Leaf morphometry was conducted on 1457 leaflets

from 43 specimens. Extracted bioclimatic parameters were used for niche-modelling approach. Analysis of cpDNA revealed two haplotypes, 12 samples from Armenia, Daghestan, Nakhichevan and Iran belonged to H1 group, while 39 samples of all Turkish and part of Armenian were in H2 group. The mean intrapopulation diversity based on AFLP was low ($H_E = 0.088$) indicating limited outcrossing rate. A significantly positive correlation between geographical latitude and leaf area ($\rho = 0.527$, $p < 0.05$) was found. Niche modelling has shown temporal variation of predicted occurrence across the projected time periods. *Vavilovia formosa* has suffered a range reduction following climate warming after last glacial maximum, which classify this species as cold-adapted among the Fabeae species as well as a glacial relict.

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Introduction

Tribe Fabeae contains five genera, some of which are among the most important crop legumes: *Lathyrus* L. (grass pea) (about 160 species); *Lens* Mill. (lentil) (4 species); *Pisum* L. (pea) (2–3 species); and *Vicia* L. (vetch) (about 140 species) and monotypic genus *Vavilovia formosa* (Stev.) Fed. (Smýkal et al. 2015). Several species are endangered, with many not yet IUCN assessed, and there are some species suspected to be recent extinctions (Schaefer et al. 2012). Tribe Fabeae has a pantemperate distribution (excluding Australasia), with the centre of diversity in the Mediterranean and the adjacent Caucasus and Irano-Turanian floristic regions. *Lens*, *Pisum* and many

species of both *Lathyrus* and *Vicia* are native to these areas. The latter two genera share remarkably similar distributions and species numbers (Kupicha 1981, 1983) with both extending through temperate Eurasia and into the New World. Both *Lathyrus* and *Vicia* are found in temperate South America, with 20–30 species in each with most species in the temperate southern cone, and five or six species in each genus in the tropical Andean uplands (Schaefer et al. 2012). Humans have introduced at least six Mediterranean species now found naturalized in the New World and Australia.

Vavilovia formosa (Stev.) Fed. grows in screes at altitudes from 1500 up to 3500 m; its heartland is the Central and Eastern Caucasus, Taurus and Zagros mountains. It is distributed in Armenia, Azerbaijan, Georgia, Iran, Iraq, Lebanon, Russia and Turkey (Mikič et al. 2013) and in most of these countries, is recognized as endangered and receives protection (Mikič et al. 2014). *Vavilovia formosa* has the typical intricate taxonomic history of Eurasian tribe Fabeae, having been placed in various genera: first as *Orobus* L. (today, largely a synonym of *Lathyrus*) and described as *Orobus formosus* Stev. (Steven 1812), Boissier reported the specimen identified as *Lathyrus frigidus* Schott & Kotschy distributed in exsiccate series Iter Cilicum 1853 (see Boissier 1856). In most regional floras, this monotypic genus was included in *Pisum* L., as *Pisum formosum* (Stev.) Alef., (Alef 1861; Boissier 1872; Townsend and Guest 1974; Reshiger 1979; Makasheva 1983; Lehman and Blixt 1984; Maxted and Ambrose 2001), but it was again treated as a separated genus in other works (Davis 1970a, b; Kupicha 1981; Lock and Maxted 2005). More than a century after its discovery, it gained the status of a separate genus in tribe Fabeae Rchb. as the monospecific *Vavilovia* Fed. (Fedorov 1939). Segregate taxa have been established based on leaf morphology; Govorov (1937) separated two intraspecific subspecies: *Pisum formosum* subsp. *formosum* Gov. with larger 7–17 mm leaves with entire margins, and subsp. *microphyllum* Ser. with smaller 5–7 mm leaves and serrate stipules. Fedorov, in the second edition of Flora of the Caucasus, distinguished two species: *V. formosa* and *V. aucheri* with existing transitory forms (Fedorov 1952). There are reports on variability of flower colour and leaf pubescence (resulting in separation of *P. formosum* var. *pubescens* C.C.Townsend). Recently Sinjushin and Belyakova (2010) have made morphometric analysis of 412 leaves from 22 specimens. Although differences were found between samples from different origins, with Armenian samples morphologically most diverged, no distinct intraspecific separation was observed.

Vavilovia formosa is perennial plant, and in contrast to the tendrils in *Pisum*, the leaf rachis terminates in an arista and has supervolute ptyxis (conduplicate in *Pisum*), a trait

reported as one of high significance by Kupicha (1981). Actually, *Vavilovia* leaves combine some features which can be found in juvenile forms of *Pisum* species (Sinjushin and Akopian 2011). *Vavilovia* leaves are compound, but having only one pair of leaflets. The leaflets are broad, of a cuneate-obovate to suborbicular shape, thick, with glabrous surface and with entire margins. Some populations have leaflets with a keel-like base (Sinjushin et al. 2009). Stipules are small, semi-sagittate, foliaceous and free from the petiole.

Tribal phylogeny was analysed by morphological, chemotaxonomic and lately also by molecular approaches (Schaefer et al. 2012; reviewed in Smýkal et al. 2015). The tribe is morphologically characterized by paripinnate, often tendrillous, leaves and a pubescent style, the ‘pollen brush’ (Lavin and Alfonso Delgado 1990). Stylar shapes and hair patterns are one of the principal diagnostic characters between some genera of Fabeae (Gunn and Kluge 1976; Kupicha 1981; Endo et al. 2008). Steele and Wojciechowski (2003) suggested for the first time a close relationship between *Vavilovia* and *Pisum* using chloroplast gene *matK*. Moreover, Lock and Maxted (2005), without analysing any DNA sequences for *Vavilovia*, postulated that the genera are sister taxa, reflecting *Vavilovia*’s earlier classification in *Pisum*. Due to its rarity and unclear status, several recent studies have addressed its phylogenetic status (Kenicer et al. 2009; Sinjushin et al. 2009; Oskoueian et al. 2010; Schaefer et al. 2012; Mikič et al. 2013). Intraspecific diversity was extensively studied in economically important legume crops such as pea (Jing et al. 2010, 2012; Smýkal et al. 2011, 2015) using various molecular markers, predominantly based on repetitive sequences, such as microsatellites and retrotransposons. However, in all of these studies domesticated material dominated with only some portion of wild collected representatives. Comparably fewer studies are published on *Lathyrus* and *Vicia* diversity, being focused only on a few representatives of cultivated species (*Lathyrus sativus*, *Lens* spp., the *Vicia sativa* complex and *V. faba*) (van de Wouw et al. 2001; Potokina et al. 2002; Tomooka et al. 2002). Some limited studies have been made on wild-growing species of these genera such as *Vicia cracca* (Eliášová et al. 2014) or *Lathyrus pannonicus* (Schlee et al. 2011).

This study seeks to better understand the morphological and genetic diversity, the biogeography and possible climatic challenges faced by this important perennial crop wild relative. We analysed available herbarium samples by sequencing of genomic regions widely used in phylogenetic studies (cpDNA and ITS). In addition, we have addressed population diversity with two sampled populations from Turkey and Armenia. To assess the morphological differentiation of the genus proposed earlier (Govorov 1937; Grossheim 1949), we studied leaf features in specimens collected throughout the area of habitat.

Bioclimatic parameters assessed from distribution data were also used for niche-modelling approach to predict past, extant and future species distribution range.

Materials and methods

Herbarium specimens sampling, DNA extraction and sequencing analysis

Original herbarium vouchers were sampled with one to two leaves per specimen (Online Resource 1). Samples were homogenized in 2-ml Eppendorf tubes with glass beads using Retsch MM10 homogenizer. For the population study, leaf samples were collected in the field and dried in silica gel and stored at room temperature until use. Total genomic DNA was isolated from about 100 mg dry leaf material with commercial plant DNA extraction kit (DNeasy, Qiagen), following the manufacturers' manual. For the polymerase chain reaction (PCR), we used standard protocols using MyTaq RED polymerase (Bioline) with 40 cycles at 94 °C 30-s denaturation, primer annealing at 50 °C for *psbA-trnH*, *trnL-trnF*, *trnS-trnG*, or 55 °C for *rbcl*, *matK*, *ITS1-5.8S-ITS2* (ITS) regions (Kenicer et al. 2005; Schaefer et al. 2012) and 72 °C extension for 1–2 min. Most of primers used for *matK* were legume-specific and designed by Hu (2000), except for *matK10*, from Kato et al. (1998) (designed for Betulaceae). *Maturase K* was amplified in several pieces: as 960 bp of *trnK* 5'intron (using *tnK1L* and *matK10* primers, of which 901 bp was sequenced and aligned), 1247 bp of *matK* coding region (using *trnK685F* and *matK1932R*, of which 619 bp was sequenced and aligned) and 670 bp of *trnK* 3'intron (using *matK1777L* and *trnK2R* primers, of which 648/655 bp was sequenced and aligned). PCR product was purified using the GeneClean III kit (Bio101, Carlsbad, California, USA) or using Exonuclease-Alkaline Phosphatase (Thermo Scientific) protocol, and cycle sequencing was performed with BigDye Terminator v3.1 cycle sequencing kits on an ABI 3730 sequencer (Applied Biosystems, Foster City, CA, USA) at University of Edinburgh's Genepool sequencing service or at Laboratory of DNA sequencing, Faculty of Sciences, Charles University, Prague, CZ. Sequences were edited and aligned using Geneious v.7.1 programme.

Population sampling

Representative sampling of individual plants collected at 5–10 m distances in horizontal transect, and altitude gradient was taken at north slope of Mt. Kizlar Sivrisi, Elmali, eastern Taurus, Turkey (between 36°36'13.61"N, 30°5'39.03"E, 2055 m a.s.l.; 36°36'15.17"N, 30°5'43.85"E, 2086 m a.s.l. and

36°36'16.97"N, 30°5'51.44"E, 2145 m a.s.l.) sampled in early August 2009 (Deniz and Sümbül 2004) and Mt. Ughtasar, Sisian, Armenia (between 39°41'11.37"N, 46°03'36.01"E, 3330 m a.s.l. and 39°41'18.01"N, 46°03'31.85"E, 3401 m a.s.l. to 39°41'10.20"N, 46°03'23.19"E, 3311 m a.s.l. and 39°41'19.42"N, 46°03'22.85"E, 3394 m a.s.l.) sampled on early July 2013 shortly after snow melted (Akopian and Gabrielyan 2008). There were 30 samples from Armenia and 17 from the Turkish site. Plant samples were labelled and stored in silica gel until use.

AFLP analysis

AFLP analysis was carried out according to the procedure of Vos et al. (1995) using the protocol described in Kitzner et al. (2008) using preamplification primers *EcoRI* (5'gactgcgtaccaattca 3') and *MseI* (5'gatgagtctgagtaac 3'). In total, four selective primer combinations (*EcoRI*–GC/*MseI*–TG, *EcoRI*–GC/*MseI*–GAT, *EcoRI*–GG/*MseI*–TG, *EcoRI*–GG/*MseI*–GAT) were chosen to generate the AFLP profiles. The products of amplification were separated on a 6%, 0.4-mm-thick denaturing polyacrylamide gel (0.5 × TBE buffer) using the T-REX sequencing gel electrophoresis apparatus (Thermo Scientific Owl Separation Systems, Rochester, NY, USA). Silver staining was used for the visualization of AFLP patterns. To check the reliability of the AFLP analysis, the amplification for each primer combination with the whole sample set and, additionally, amplification of randomly chosen samples (from two to three samples from each population) were repeated. The gels were scored independently. The AFLP profiles were visually checked and coded as a binary matrix. In the last step, both results of scoring were compared and checked for the number of markers, intensity of markers and relative position of markers. In the final binary matrix, only verified markers (present in the original and repeated amplification) were used. The error rate was calculated as the difference in the total number and the number of fragments used in the final matrix (Bonin et al. 2004).

AFLP data analysis

Basic population statistic indices such as the mean number of bands per given population (N_{BP}) and the number of polymorphic bands (N_{POB}) at the 5% level (Lynch and Milligan 1994), number of private markers (N_{PrB} ; restricted to a given population), were calculated in FAMD (Schlüter and Harris 2006). Polymorphism (P), number of different genotypes (NG) and genotype diversity (GD) were calculated using the R-script of AFLPdat (Ehrich 2006). Total gene diversity (H_T) and Nei's gene diversity (i.e. expected heterozygosity, H_E), Analysis of molecular variance (AMOVA) and fixation index (F_{st}) was computed in

Arlequin ver. 3.5.1.2 (Excoffier and Lischer 2010). To explore the relationship within and among populations, Principal Coordinate Analysis (PCoA) was performed in NTSYSpC (Rohlf 1997) (Jaccard similarity matrix) and inspected on a 3D plot. To trace potential admixture of various genotypes within the two populations, a Bayesian clustering using Structure 2.3.4. (Falush et al. 2007) was performed (the recessive allele model; the admixture model with correlated allele frequencies). The K was set to 1–10 with 10 replicate runs for each K using the 1,000,000 MCMC iterations following the period of 100,000 burn-in iterations. Structure Harvester (Earl and von Holdt 2012) was used to summarize the output files, and the graphical output was generated in Distruct 1.1 (Rosenberg 2004). Evanno et al. (2005) statistics was used to identify the correct number of clusters (K).

Environmental data

The environmental data that were used in niche modelling included altitudinal, tree cover and soil characteristics of the study area, as well as bioclimatic data. For altitude, we used the digital elevation model of the Shuttle Radar Topography Mission (SRTM), obtained from <http://srtm.csi.cgiar.org/>. Landsat tree cover thematic layer obtained from Global Land Cover Facility (www.landcover.org) was used as an estimation of the tree cover of the study area. Data concerning soil type were acquired from Harmonized World Soil Database (<http://web.archive.iiasa.ac.at/Research/LUC/External-World-soil-database/HTML/>). The bioclimatic data were downloaded at a 2.5-arc-minute resolution from the official site of WorldClim (www.worldclim.org). Apart from present climatic conditions, data about past and future conditions were also retrieved from the site. For the past, downscaled paleoclimatic data concerning the Last Glacial Maximum (about 22,000 years ago) and Last Interglacial (approximately 130,000 years ago) periods were used. For the future, we used climate projections for year 2050 under two different Representative Concentration Pathways (RCPs), namely RCP2.6 and RCP8.5. The two selected RCPs are the ones corresponding to the least and most dramatic changes in climate, predicting a radiative forcing of +2.5 and +8.5 W/m² in the year 2100 relative to pre-industrial times, respectively. To account for model variability in past and future climatic projections, we used data from three different general circulation models (GCMs) for the Last Glacial Maximum period as well as the future. Specifically, for last glacial maximum we used CCSM4 (<http://www.cesm.ucar.edu/models/ccsm4.0/>), MIROC-ESM (Watanabe et al. 2011) and MPI-ESM-P (<http://www.verc.enes.org/models/>

[earthsystem-models/mpi-m/mpi-esm](http://www.earthsystem-models/mpi-m/mpi-esm)), while for the year 2050, we used BCC-CSM1-1 (<http://forecast.bccsm.ncc-cma.net/web/channel-34.htm>), GISS-E2-R (<http://data.giss.nasa.gov/modelE/ar5/>) and MIROC5 (<http://www.icesfoundation.org/Pages/ScienceItemDetails.aspx?siid=181>). For Last Interglacial, we only used the one model that was available at the official site of WorldClim (Otto-Bliesner et al. 2006). All data layers were resampled to match the spatial extent and resolution of the present bioclimatic data.

Niche modelling

All the georeferenced sites of herbarium specimens have been tested for accuracy using as validation criterion the shortest distance between the given GPS point and the site position according to the description of the label of the herbarium specimen. A threshold value of 50 km has been used as the maximum accepted distance, and the validation process took place using free available scripts (<http://www.movable-type.co.uk/scripts/latlong.html>). All the rejected sites have been omitted from the analyses. Analogous validation test has been implemented by Alsos et al. (2009). The distribution patterns of *V. formosa* in current, past and future conditions were predicted with the use of environmental niche modelling and the Maximum Entropy (MaxEnt) algorithm (Phillips et al. 2006) in particular. This technique estimates the probability distribution of a species' occurrence based on the environmental constraints and its current occurrence data. The software MaxEnt 3.3.3 k, which was specifically created for this purpose, was used in this study (Phillips et al. 2006). Two separate models were run; the first one included all environmental variables as predictors and was used to estimate the relative contribution of each variable in the prediction of the present distribution of *V. formosa*, while the second only included bioclimatic variables and was used to predict the distribution of *V. formosa* in present, past and future conditions. Each of these models was evaluated via a leave-one-out procedure, using the area under the receiver operating characteristic curve (AUC) as a measure of performance. MaxEnt also provides a number of ways to measure variable importance, which can be useful in determining the relative contribution of environmental variables as range determinants. The permutation importance of each variable, which was recorded for the first model, measures the decrease in model AUC as each variable is randomly permuted. Another way MaxEnt can measure variable importance is by excluding each variable, or keeping only that variable, and measuring the decrease in AUC of the resulting model, a method that was also employed in the first model. These techniques allowed us

to assess the relative loss in accuracy resulting from removing all non-climatic variables, which was important since bioclimatic variables are the only ones available for the past conditions. Since the output of MaxEnt models is a continuous value between 0 and 1, we used threshold values to convert this output to predicted binary distributions. Following Bystrakova et al. (2014), we used thresholds which maximized training sensitivity and specificity values (MTSS), which were averaged over ten separate runs for each model. In order to further examine the niche differences between the two haplotypes, we employed a niche equivalency test (Warren et al. 2008) using the software ENMTools (Warren et al. 2010). This process tests whether the niches of two entities (here, the two haplotypes) are identical, by pooling their occurrence points and then randomly splitting them into two subsets, each subset equal in count to the original number of points for each species. This process is repeated 100 times, and the resulting indices of niche overlap (D , Schoener 1968 and I , Warren et al. 2008) are compared to the observed ones that are calculated from the actual predicted distributions of the two entities.

Leaf phenotype analysis

Measurements of leaflet length (l) and maximum width (w) were carried out on digital images of herbarium specimens using MeasureTM 2.0 (C Thing Software). On every specimen, all measurable leaflets were scored for quantitative features (length and width). If any leaf was composed of two leaflets, the larger one was appreciated. Totally 1457 leaflets were measured on 43 herbarium specimens. Measurements of leaflet area (S) were performed with ImageJ 1.49 software package (NIH, USA). Statistic treatment of results was carried out with usage of Statistica 8 (StatSoft Inc.). To avoid procedure of image processing prior to area measurements, we compared directly measured leaflet area and this value calculated as $S = 0.5lw$. Relation between these two values appeared linear ($S = 0.8568lw - 0.03$, $p = 0.99$), so we used calculated value of leaflet area instead of directly measured one. Analysis with usage of method of geometric morphometrics was carried out applying TPS software (Rohlf 2010). For shape description, 12 characteristic points were chosen on leaflet contour (base, tip, maximum width, and two equidistant points on intervals between first four points). To study shape differences between different localities, the following algorithm was applied. We obtained images of individual leaflets (minimum 15 from a locality) and reconstructed a consensus shapes for every locality. After this, the resulting consensus images were taken for relative warp analysis.

Results

Intraspecific genetic diversity

In total, 51 *V. formosa* samples from various herbarium collections (Online Resource 1) were analysed for ITS and cpDNA (*trnL-trnF*, *trnS-trnG*, *matK*, *rbcL* and *psbA-trnH*) sequences. All target sequences were successfully amplified from all samples despite some dating back to 1860. These represent in total up to 2551 bp of chloroplast and 664 bp of nuclear sequences per sample. These were complemented with published sequences from 5 samples (Sinjushin et al. 2009; Oskoueiyan et al. 2010; Schaefer et al. 2012) along with samples of the two populations we analysed in detail. These represent in total 17 samples from Turkey, 6 from Iran, 17 from Armenia, 1 from Lebanon and 11 from Caucasus (Georgia, Russia and Daghestan) (Fig. 1). Sequencing of *trnL-trnF* (609 bp), *trnS-trnG* (588 bp), *rbcL* (539 bp), *psbA-trnH* (332 bp) and ITS (664 bp) fragments did not show any polymorphism across samples, although the quality of *trnS-trnG* allowed us to align of only 478 bp of the sequence. The only polymorphism found was in the *trnK* 3'intron with a 7 bp indel (GATTGGT). This has separated two haplotypes, 12 samples with this insertion (H1 haplotype) (Online Resource 1, 2) originating from Armenia, Daghestan, Nakhichevan and Iran, while the remaining 39 samples (H2 haplotype) had deletion (all Turkish and part of Armenian).

Genetic variability and population genetic structure

Two population samples were studied in details to infer about population structure and pollination system. One population represented by 30 samples originated from Mt. Ughtasar, Sisian, Armenia being divided into two sub-populations each from different side of the mountain. The second population represented 17 samples from north site of Mt. Kizlar Sivrisi, Elmali, Turkey. These two populations differed in *trnK* 3'intron haplotypes (Fig. 1). A total of 143 unambiguous and easily discernible AFLP fragments were obtained from the set of 47 *V. formosa* samples analysed in this study. Replication of the analysis revealed the high reliability of AFLP, with an error rate of 2.1% (it represents three markers). The total gene diversity was high ($H_T = 0.252$) and reflected the differences between the two populations each from one site of the geographical range. The mean intrapopulation gene diversity was low ($H_E = 0.088$) with lower H_E value observed for Turkish (0.077) than in Armenian (0.098) population. The hierarchical AMOVA analysis revealed that most of the observed molecular variance (78.96%) is present among the two

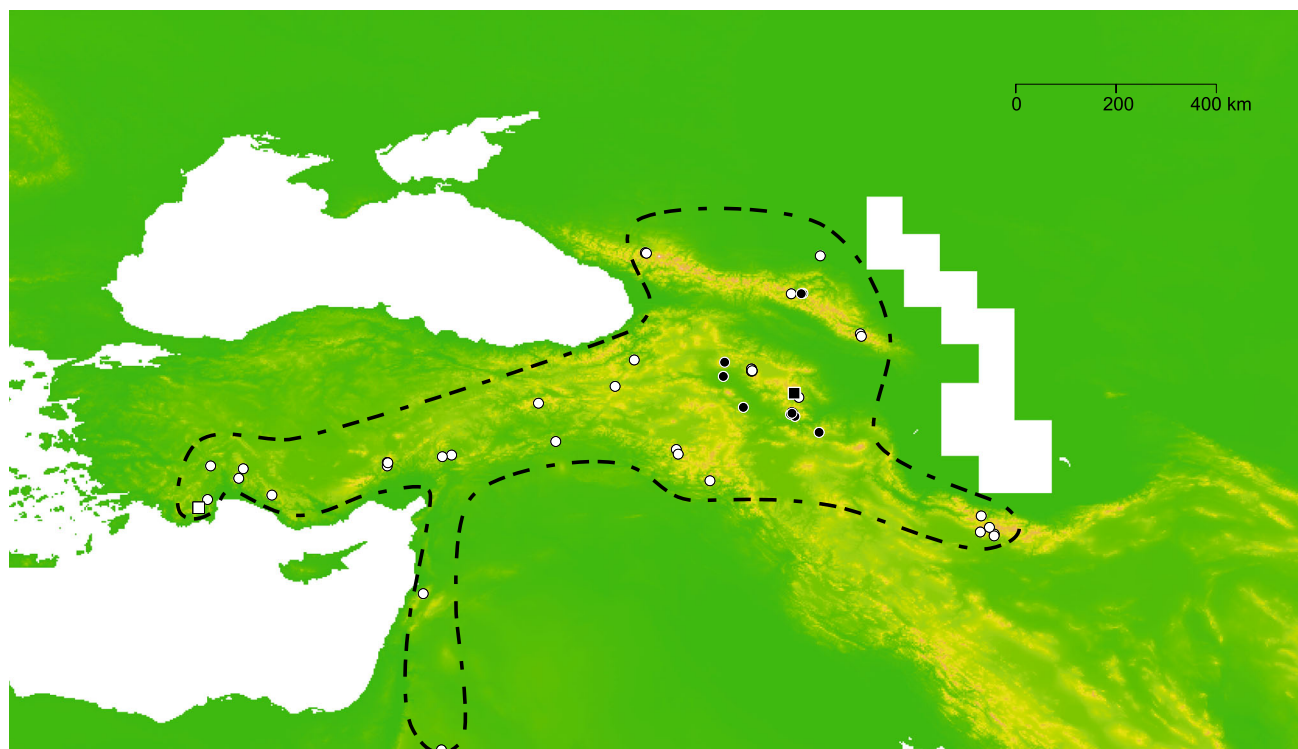


Fig. 1 Specimen distribution map (see Online Resource 1 for details) with relief and indication of two *matK* detected haplotypes, H1 (black) and H2 (white). In squares are labelled studied populations in

Turkey (white with black outline) and Armenia (black with white outline). White space corresponds to sea water areas. Dashed line shapes the recorded distribution area of the species

populations, while a minor part (21.04%) represents variation observed within populations. Both clustering methods (PCoA and Bayesian clustering, not shown) clearly divided the analysed *V. formosa* samples into two major groups according their population origin without admixture. Genetic differentiation between the two populations was observed, with the genetic differentiation measure, $F_{st} = 0.78$, being significantly different from zero.

Niche modelling

Both models gave very high values of AUC (0.952 ± 0.147 for the first model and 0.933 ± 0.191 for the second), indicating good predictive accuracy. Additionally, the relatively low difference in AUC output between the full and bioclimatic-only model indicates robustness in the ability of bioclimatic variables to predict the species' distribution. Based on permutation importance values (Table 1), altitude had the highest contribution in the first model (64.6), followed by variable bio8 (Mean Temperature of Wettest Quarter) (7.5). The projection of the distribution of the species according to the bioclimatic data (Fig. 2) during the Last Glacial Maximum ($\sim 22,000$ ybp) was broader compared to the current potential distribution as well as to the Last Interglacial Period

($\sim 130,000$ ybp), while its future distribution seems to be slightly narrower under both RCP scenarios.

Based on the polymorphism found from the genetic diversity analysis, the two distinct haplotypes were modelled using the same bioclimatic data used for the whole species. This was carried out using the sampling sites corresponding to each haplotype as presence points, in order to check for niche differences. The developed models both of which gave good accuracy results (AUC: 0.954 ± 0.114 and 0.874 ± 0.266 for H1, H2 haplotypes, respectively) were then projected onto past and future climatic conditions in the same way as the original models (Fig. 3). This procedure revealed some visible differences in the two haplotypes' distribution patterns across time. The H1 haplotype was predicted to have had a very extensive distribution during the Last Glacial Maximum, which was notably narrower in all other time periods. Conversely, H2 haplotype was predicted to fluctuate much less, although the general patterns remain.

The results of the niche equivalency test showed no statistically significant niche difference for the two haplotypes (p value = 0.2178 for D and p value = 0.0792 for I), indicating that the differences in their predicted distributions might not reflect differences in their environmental responses.

Table 1 Environmental predictors used for the full MaxEnt model, along with the corresponding percent contribution and permutation importance in the model

Variable	Description	Percent contribution	Permutation importance
alt	Altitude	72.1	64.6
bio8	Mean temperature of wettest quarter	9	7.5
T_OC	Organic Carbon	0.2	6.7
bio9	Mean temperature of driest quarter	1	6.3
bio18	Precipitation of warmest quarter	2.6	3.9
bio17	Precipitation of driest quarter	0.1	3.6
T_TEB	Total exchangeable bases	1.5	1.8
bio11	Mean temperature of coldest quarter	0	1.4
bio5	Max. temperature of warmest month	0.2	0.8
tree	Percent tree cover	0.1	0.8
bio16	Precipitation of wettest quarter	0	0.8
bio19	Precipitation of coldest quarter	1.4	0.7
T_CEC_CLAY	Cation exchange capacity of clay	0.1	0.6
T_GRAVEL	Volume percentage gravel	0.8	0.4
T_SAND	Percentage sand in topsoil	0.1	0.2
bio4	Temperature Seasonality (standard deviation \times 100)	0	0.1
T_BS	Base saturation in topsoil	4.4	0
bio1	Annual mean temperature	2.9	0
bio6	Min temperature of coldest month	1.8	0
bio2	Mean diurnal range (mean of monthly (max. temp–min. temp))	0.9	0
bio15	Precipitation seasonality (coefficient of variation)	0.4	0
T_CLAY	Percentage clay in topsoil	0.3	0
T_CASO4	Calcium sulphate (gypsum) content	0.1	0
T_ESP	Exchangeable sodium percentage	0	0
bio7	Temperature annual range (BIO5–BIO6)	0	0
bio12	Annual precipitation	0	0
bio10	Mean temperature of warmest quarter	0	0
bio13	Precipitation of wettest month	0	0
bio14	Precipitation of driest month	0	0
T_SILT	Percentage silt in topsoil	0	0
T_REF_BULK_DENSITY	Bulk density of topsoil	0	0
bio3	Isothermality (BIO2/BIO7) (\times 100)	0	0
T_CACO3	Calcium carbonate content	0	0

Rows are sorted by decreasing order of permutation importance

Morphological differences between localities

In early descriptions, leaf size and shape were reported as sufficient for subdivision of single genus into two variations (Govorov 1937) or even species (Grossheim 1949). We made an effort to characterize these two features among studied specimens. All observed plants are remarkable for their heterophylly, a phenomenon reflected across Fabeae tribe. Every shoot begins with very small leaves having obovate leaflets with keeled base (Fig. 3b, c). Flower stalks are usually subtended by larger leaves with leaflets having population-specific shape. In most specimens, the coefficient of variation (CV) for leaflet area

exceeded 50%. Distribution of this character in most cases was lognormal rather than normal, so for correlation studies, we used median instead of average value. These parameter meanings range between populations but distribution throughout natural habitat is gradual, without any extremes, so one cannot reveal clear separation of specimens into distinct groups according to leaf sizes. Some geographical regularity was found in distribution of leaf sizes. The significant positive correlation between geographical latitude and maximum leaf area (Spearman's test; $\rho = 0.5269$, $p = 0.0033$) may reflect ecological significance of this feature: plants of northern populations (Kuban, Daghestan) have larger leaf area. When moving

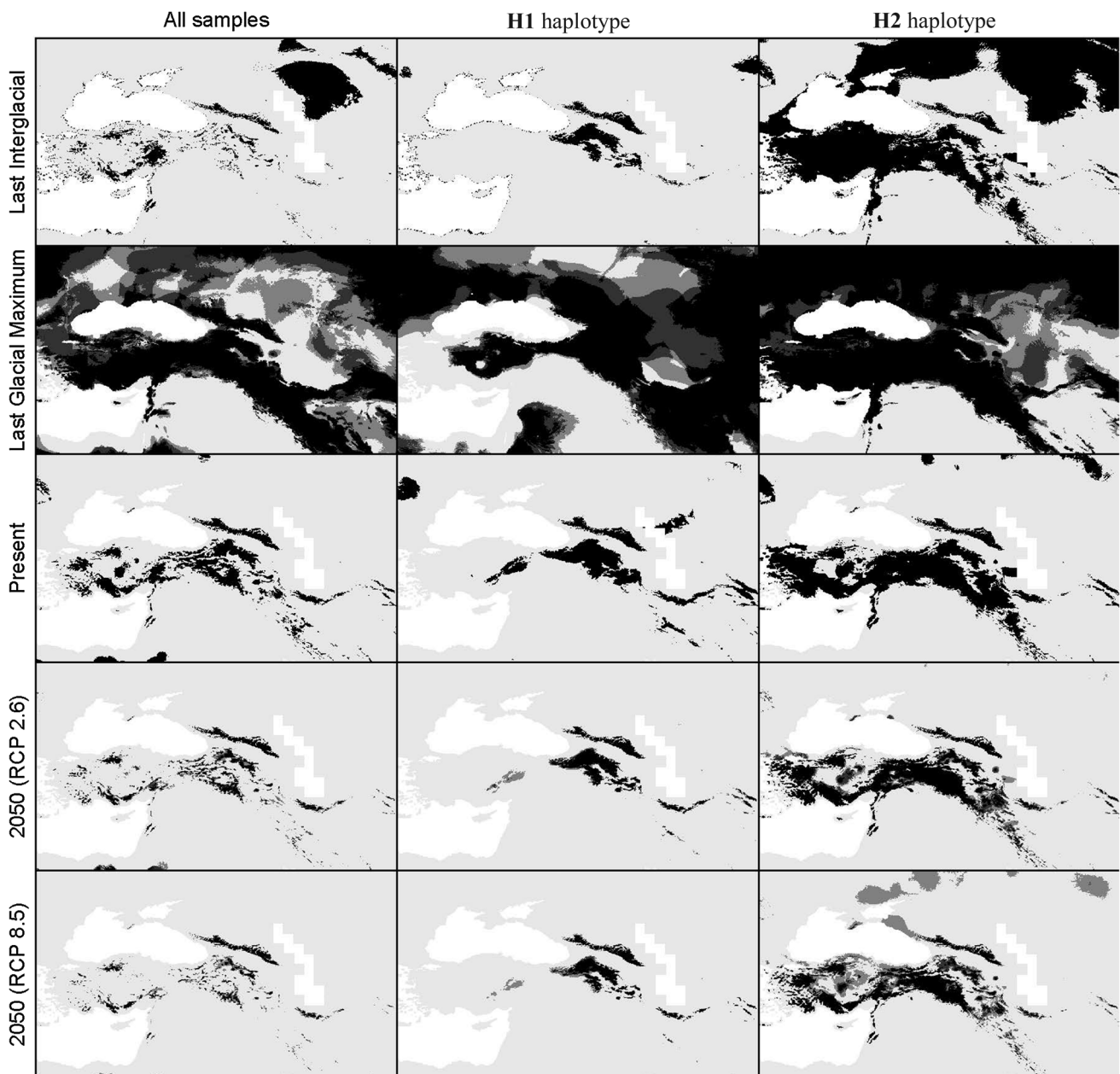


Fig. 2 Predicted distribution of *Vavilovia formosa* and its two revealed haplotypes in past (last interglacial and last glacial maximum), present and future (based on RCP 2.6 and RCP 8.5 climate change scenarios) climatic conditions. Predictions resulted from applying thresholds on the outputs of MaxEnt models

from northern populations (Kuban, Daghestan) to south (Turkey, Lebanon), leaf area slightly decreases. In upland habitats, many ecological characteristics are strongly influenced with altitude (Pescador et al. 2015). No significant correlation was found between leaf area and altitude (Spearman's test; $\rho = -0.1335$, $p = 0.4983$). Correlation between position of locality from west to east (longitude) and leaf size also appeared significant (Spearman's test; $\rho = 0.5326$, $p = 0.0029$). Similar level of ontogenetic

predictions. For last glacial maximum and future predictions, three separate global climate models were used, and *darker* areas represent predicted presence for a higher number of the used models. For present and last interglacial maps, *black areas* represent predicted presence, while *light grey* areas represent predicted absence

variation was observed for leaflet shape (Fig. 3b). To minimize ontogenetic variations in leaflet shapes when characterizing them in different populations, we chose only upper flower-subtending leaves (those exhibiting maximum specificity between populations) and categorized them on qualitative level as either ovate (1) or elliptic (2), or obovate (3). To test whether distribution of different leaf phenotypes is latitude-independent, we attributed latitudes as high ($>40^{\circ}\text{N}$) and low ($<40^{\circ}\text{N}$) and applied Chi-square

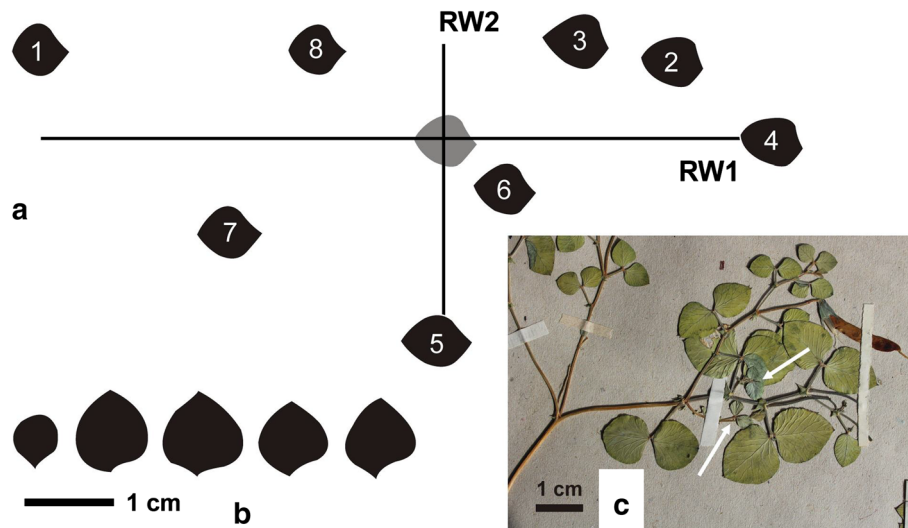


Fig. 3 Variations in leaflet shape in *Vavilovia* between different localities (a) and during ontogeny of the same shoot (b). Relative warp (RW) analysis of leaflet shape in different sites. 1 Armenia, 2 Daghestan, 3, 8 Kuban, 4 Chechnya, 5 Iran, 6 Azerbaijan, 7 Turkey (A). Size differences on (a) are not reflected. Grey contour—

consensus shape of leaflet in studied specimens. Ontogenetic variations of leaflets shape and size on subsequent nodes of shoot from herbarium specimen from Kuban (b), given as scheme (b) and photo of herbarium specimen (c). Note variations in size and shape between lower (arrows) and upper leaves

test to this distribution which appeared non-random ($p < 0.01$). Plants from the northern part of the natural range are mostly characterized by large ovate leaflets while ones from southern collection points usually bear smaller obovate (sometimes almost roundish) leaflets (Fig. 3a).

Population diversity and habitat

We have analysed two available populations. One corresponded to study of Akopian et al. (2010) from Mt. Ughtasar, Armenia from locality between 3305–3453 m a.s.l. in area of about 75,000 m² on the south-western slope. These habitats are well sunlit and sun-warmed, and due to volcanic nature of rocks, it retains heat during the night. There is clear altitudinal optimum, with upper limit likely dictated by temperature, e.g. length of vegetation period, while the lower seems to be related to competing plant species once they stabilize the volcanic gravel. We have not confirmed the statement of Akopian et al. (2010) that plants reproduce mainly clonally. Plants 1 m apart are already genetically distinct, as revealed by AFLP. The rough estimate of population size is between 1000 and 3000 individuals, i.e. one plant per 25–75 m². There is, however, no homogenous distribution within the area, but plants are in patches of 6–20 m². They seem to be distributed in vertical lines corresponding to edges of moving gravel. In Armenia, *V. formosa* grows in alpine belts and occurs on south facing slopes of the upper Quaternary volcanic slag cones and moving screes, with slag particle size varying from several mm up to

10 cm and more. It occurs in Geghama (mountain peaks Sevsar, Ziaret) and Zangezur (mountain peaks Kaputdjugh, Parakan, Mets Ishkhanasar, Ukhtasar) floristic regions. Area of distribution is fragmented. The extent of occurrence is less than 5000 km², the area of occupancy is less than 500 km², and the number of locations is 5 (Tamanyan et al. 2010). *Vavilovia* is accompanied by other species of alpine plants: *Campanula aucheri*, *C. tridentata*, *Coluteocarpus vesicaria*, *Corydalis alpestris*, *Delphinium foetidum*, *Draba bruniifolia*, *Gentiana pontica*, *Pseudovesicaria digitata*, *Taraxacum stevenii* and others (Online Resource 3). In Mountain of Sevsar (3258.5 m high), the *V. formosa* population area is about 700 m long and 100 m wide. On the western, south-western and southern slopes, it starts at an altitude of 2995 m a.s.l. and continues up to 3203 m. Besides *Vavilovia*, in this area other rare species under protection occur: *Eunomia rotundifolia*, *Potentilla porphyrantha*, *P. seidlitziana*, *Scilla rosenii*, and other alpine plants such as *Chaerophyllum aureum*, *Campanula aucheri*, *Merendera raddeana*, *Ornithogalum transcaucasicum* and *Puschkinia scilloides* Adams (Baloyan 2004). The flowering of *V. formosa* is observed from July to end of August (beginning of September), fruiting from August to September.

The second population was located on north-western slopes of Mt. Kizlar Sivrisi, western Taurus, Turkey at an altitude between 2000 and 2150 m in limestone scree. This is by our knowledge the most western distribution range of *Vavilovia*. As described in (Deniz and Sümbül 2004), the climate in the area varies between the continental climate of

Inner Anatolia and the Mediterranean climate. The typical climate of the Mediterranean predominates, characterized by hot and dry summers and cold and rainy winters. The mean annual temperature is 7.5 °C, and precipitation is 616 mm. The rainy period extends from November to February, while the dry period extends from the beginning of May until the end of September. The geological structure of the research area mostly consists of aged calcareous rocks which precipitate on Mesozoic old basic rocks. Vegetation accompanying *Vavilovia* is sparse and consists of 59 species (Deniz and Sümbül 2004) (Online Resource 3). There is a nearby occurrence of *Vavilovia* at Bakirli Dagi (Eren et al. 2004) located some 40 km (36°49'30"N, 30°20'00"E close to Antalya) in the same mountain range. Geologically it is also Mesozoic sedimentary rocks predominantly limestone. *Vavilovia* is reported at altitudes between 1800 to 2500 m above Saklikent ski resort on screes. The scree vegetation of the Western Taurus belongs to a particular *Lamietalia cymbalariifolii* order within the Heldreichietea class. Both in floristic and vegetational respects, the study area clearly belongs phytogeographically to the Lycian Sector of the Tauric Subprovince and S Anatolian Province (Eren et al. 2004). Anatolia's dissected mountainous landscape has been crucial for the long-term survival of *V. formosa*. There was no major Pleistocene ice-shield, and only the higher mountain peaks above 2200 m were glaciated (Erinç 1978; Atalay 1996), providing opportunities for local population survival. Furthermore, the mountain systems provided moist conditions at higher elevations, offsetting the drier climate that prevailed during the glacial periods (Webb and Bartlein 1992). The mountains of the 'Anatolian diagonal' in eastern Anatolia are substantially different (2000 m), providing extensive areas for species preferring cool moist climates to grow at higher elevations. Indeed, the diagonal is characterized by numerous plant and insect endemics (Davis 1970c; Ekim and Güner 1986; Çiplak 2003), which may have persisted locally or nearby during recent glaciations (Gündüz et al. 2007). Importantly, the diagonal mountains have a north-east/south-west orientation, whereas most Anatolian mountain systems have east-west orientations, and this provides a continuous mountain chain connection between the Mediterranean and Caspian seas. This links the southern Taurus diversity hotspot (Medail and Diadema 2009) to the nearby Caucasus and Near East mountain systems.

Discussion

Intraspecific genetic diversity

The study of intraspecific diversity of *Vavilovia formosa* of its own and in comparison with closely related *Pisum* species focused on two basic issues, whether *V. formosa* is

a distinct species from pea and whether the species can be divided into variations or subspecies, while it was also open issue whether the genetic diversity of the species follows or not a clear geographical pattern. In this study, we have conducted molecular analysis of 51 samples by sequencing of several chloroplast and nuclear (ITS) regions. Previous molecular genetic analysis based on these sequences demonstrated that the genus *Vavilovia* belongs to the same clade as *Lathyrus* and *Pisum* within Fabae (Kenicer et al. 2009; Schaefer et al. 2012; Mikič et al. 2013). The results of other analyses, based on nuclear DNA (ITS) and plastid (*trnF-trnL*, *trnS-trnG*) DNA sequences, suggested that *V. formosa* is not distinct enough from pea and therefore, they considered it a pea species, *Pisum formosum* (Oskoueiyani et al. 2010). Later studies, based on four phylogenetically informative regions, have taken the alternative view that *V. formosa* is distinct based on its monophyly within tribe Fabae and sister relationship to (i.e. not nested inside) *Pisum* (Kenicer et al. 2009; Schaefer et al. 2012; Mikič et al. 2013, 2014). This confirmed the results of previous comparative anatomical, morphological and biochemical analyses suggesting it was a separate species of a separate genus within the tribe Fabae (Makasheva et al. 1973). Morphological analysis of leaf characteristics in the specimens of *V. formosa* of diverse geographical origin opened a possibility that this species may consist of two subspecies (Sinjushin and Belyakova 2010), or even two distinct species, *V. formosa* and *V. aucheri*, as recently accepted in work of Schaefer et al. (2012). In the presented study, we found two chloroplast haplotypes, based on the 7 bp insertion-deletion found at *trnK'3* intron of *matK* gene. One being predominant in the eastern range of the area (Iran, Daghestan, Armenia), while another was found in the western range (Turkey), with the area of overlap in the eastern Caucasus.

Having freshly collected material from two populations, we aimed to study the diversity pattern. Unfortunately, pea microsatellites (Loridon et al. 2005) showed to be of little use (data not shown) as only some *Vavilovia* samples (18%; 6 of 32 tested) provided limited amplification and were not able to detect differences between Turkish and Armenian populations. This is likely the consequence of greater phylogenetic distance as estimated to be 9–11 Mya from *Pisum* (Schaefer et al. 2012). Thus, we could not conduct codominant marker type analysis required to reveal heterozygosity. Consequently, we used the more universal and sensitive AFLP method which provided reproducible results, clearly showed population structure, differences between populations and even differences between plants within population. Although the mean intrapopulation gene diversity was low ($H_E = 0.088$), there was clear diversity within populations. This indicates that despite being a perennial species with rhizomes, the

individuals are sufficiently distinct from each other even when only few meters apart. This also supports probability of at least partial open pollination and accompanied gene flow. The question of reproductive strategy and possible pollinator is interesting in relation to observation of Akopian et al. (2010) frequent frost damage of flowers due to late flowering. The lower H_E value for Turkish than in Armenian population could be partially explained by imbalanced sampling due to time constraints at the locality.

Morphological features do not serve as a basis for clear intraspecific separation

In earlier work (Sinjushin and Belyakova 2010), we demonstrated that leaflet shape varies gradually between different populations of *V. formosa*. Although polar variants are easily distinguishable, shapes of leaflets from different localities overlap resulting in gradual variation. This gradient is consistent with geographical position of sites and leaflet size: northern populations (Kuban, Dagestan, Chechnya) are characterized by large, ovate leaflets while specimens from the southern part of natural habitat (Armenia, Turkey, Lebanon) produce smaller obovate leaflets. The morphological differences exhibit no correlation with either nuclear or plastid genotype. In upland habitats, many ecological characteristics are strongly influenced with altitude (e.g. Daubenmire 1943). No statistically significant correlation was found between leaf features and altitude in studied specimens of *Vavilovia*. As no crosses were ever made between different *Vavilovia* accessions, one can hardly conclude whether variations in leaf phenotype are heritable. In related model object, pea, numerous genes are known which affect leaflet number, size and shape (e.g. Härstedt 1950; Hofer et al. 1997). In *V. formosa*, variation in leaflet shape seems to be under certain selection, as smaller leaflets are more preferred in arid climatic conditions. This is in accordance with the theory of the role of leaf shape and size in leaf thermoregulation, whereas other non-mutually exclusive theories, such as that of the leaf shape variation in response to selection on flower form cannot be rejected (Nicotra et al. 2011). On the other side, heterophylly of *V. formosa*, which is a phenomenon reflected across much of tribe Fabeae, can arise either in response to different external environments or to ontogenetic signals some of which may be influenced by environment as well (Nicotra et al. 2011). Shape polymorphism (e.g. existence of few alleles controlling leaflet shape) may be transitive within the whole ancestral population. Inhabitants of remaining spots exhibit different manifestation of this trait. Interestingly, a garden pea exhibits similar mode of developmental variations in leaflet shape, from almost triangular obovate to ovate. It is more a matter of taste to attribute gradual variation in leaf features

lacking clear distinction to different intraspecific taxa (variations or subspecies), but it might be more informative to prove heritable (not conditioned) nature of this polymorphism. Usage of these morphological markers for microsystematics is complicated with wide range of ontogenetic and (possible) ecological plasticity (Franks et al. 2014).

Geological and paleobotanical aspects

At 10–12 Mya (in Upper Miocene), the Armenian upland (East and North-East part of Turkey and Armenia), the Caucasus and Iranian mountains (West and North-West part of Iran) had much lower altitudes than today. At that time, there were a small seas, lagoons, gulfs and islands. From this time, these territories began to uplift. Climate during the Upper Miocene in this area was more or less similar to today's Mediterranean climate—dry summers, wet and not cold winters. Following the Upper Miocene time—during the Early Pliocene (5–4 Mya)—the area was higher, well above sea level, with lakes and river systems among the mountains, and ranges were formed. Climate was wet and less warm than during Late Miocene time. Forests were more widespread. In the Late Pliocene and Early Pleistocene time (3–1 Mya), before ice ages the climate, vegetation and palaeoenvironment more or less looked similar as today—less forest and more open vegetation. Mountains were lower than today (200–300 m less) and climate comparably warm (Sayadyan 2006; Joannin et al. 2010). At that time (before ice ages), the area of today's alpine vegetation was likely more extensive and less disjunctive (fragmented) with the probability that the area of *Vavilovia formosa* and other high mountain species was connected among the Greater Caucasus to the Armenian Upland and Iranian mountains. In ice age times (from 900,000–800,000 up to 30,000–15,000 years ago), the climate, landscapes and vegetation changed many time and slowly went to similar as today's with continuing uplift processes. The western Caucasus and part of the coastal zone of the Black Sea have the most favourable moisture and temperature conditions in northern Eurasia for the growth of broadleaved evergreen/warm mixed forests today (Dolukhanov 1989; Tarkhnishvili et al. 2012), and Grichuk (1984) suggested that these areas were the most likely refuge for the warm flora during the coldest stage of the last glaciation. The reconstruction of LGM vegetation revealed the expansion of the cryoxerophilic vegetation (a combination of steppe and shrub tundra communities and associations of salty soils, with no-analogues in the modern pollen spectra from Europe and Siberia) across the northern mid-latitudes of Eurasia (Tarasov et al. 2000). The cold and dry conditions during the LGM favoured the extension of Eurasian steppe (Tarasov et al. 2000; Wu et al. 2007). The

Caucasus vegetation and the Eurasian hominid environment were constantly under arid continental climate during the Pleistocene. Aridity, however, increased through time, as demonstrated by disappearance of relict taxa (Joannin et al. 2010). In this scene, the results of the niche modelling support the hypothesis that *V. formosa* survived and expanded as a cryoxerophilic species in steppe and shrub tundra vegetation during LGM. The niche-modelling results indicated that *V. formosa* was favoured during the LGM (~22,000 ybp) compared to the Last Interglacial Period (~130,000 ybp) and to the current environmental conditions. These findings are in line with the view that oroxerophytic floristic elements of Caucasus are not only Boreal and Arcto-Alpine elements, which penetrated into this area during Pleistocene, but partly represent ancient autochthonous elements (Nakhutsrishvili 2013 and references therein). This view supports the hypothesis that *V. formosa* had been formed in the Miocene–Pliocene as a more thermophilous element, and after, in the Pliocene–Pleistocene it changed in the direction of cryo- and xerophytization. *V. formosa* has suffered a range reduction following climate warming after LGM, which testify and classify this species as cold-adapted among the Fabaceae species. This species is also classified as a Glacial relict following Hampe and Jump (2011), who define that Glacial relicts were more widely distributed during Quaternary cold stages and have experienced significant range contractions since the LGM. This finding is also supported by the results of Joannin et al. (2010), who report that in the regional Caucasus arboreal plants mainly developed during interglacials whilst herbaceous ones were dominant during glacials. Despite the evidence of *V. formosa* being cold-adapted species able to survive unusual warm periods in these refugia (sensu Hampe and Jump 2011) in the last ca 10,000 years (see also Ohlemüller et al. 2008), it is possible that other factors can act on local scale, such as rhizobia, known for their contribution in mineral nutrition of legumes, especially in severe and drought conditions (Zahran 1999). The first 16 rhizobia isolates from *V. formosa* were described in Safronova et al. (2014), and thereafter more strains were isolated from root nodules of the same species (Safronova et al. 2015). Cold-adapted rhizobia isolated from arctic or subarctic regions showed the capacity to improve symbiotic nitrogen fixation and yield of legumes under low temperature conditions (Prévost et al. 1999). Although no specific measurements have been carried out in this study, we hypothesize that beyond climate, the mutualistic relationship between rhizobia and *V. formosa* also sustains the species in the cold and dry environment of high elevation areas improving mineral nutrition. This assumption suggests that *V. formosa* would belong to the category of Biotically Sustained (BS) climate relicts (Hampe and Jump 2011), i.e. species that requires

for its persistence a mutualist which is also limited by climate. Although several Tertiary relict species have been suggested as Biotically Sustained (e.g. *Zelkova abelicea* see Bosque et al. 2014), to our knowledge *V. formosa*, which persisted range reduction since LGM is the first suggested Biotically Sustained Glacial relict species. Taking also into consideration that range reduction of a species is most likely to cause loss of intraspecific genetic diversity (Aguilar et al. 2008; Alsos et al. 2012), we can hypothesize that *V. formosa* has suffered a loss of intraspecific genetic diversity. Of course the loss of intraspecific genetic diversity due to range reduction is not a simple case, as many parameters are involved in this relationship, such as the long-distance dispersal. For example, it has been reported that species without adaptations to long-distance dispersal (and/or herbaceous species) are expected to lose genetic diversity at about twice the rate of species adapted to long-distance dispersal by animals or wind (Alsos et al. 2012). Moreover, the loss of genetic diversity is also dependent on the geographical distribution of genetic diversity within the species range. For species with a rather even level of genetic diversity throughout most of its distribution range, the effect of range reduction is rather independent on which part of the range is lost. For species such as those with high levels of genetic diversity in one part of their geographical range, the loss of genetic diversity is strongly depend on which part of the range is lost. In case of *V. formosa*, a clear geographical variation of intraspecific genetic diversity was revealed and both haplotypes of the species recorded follow the same cold-adapted pattern of the entire species, as both of them were favoured during the LGM. Assuming that the existing geographical variation of the two haplotypes also reflects part of a non-even pattern of genetic diversity in the range of the species during the LGM, we cannot exclude that the range reduction of *V. formosa* was likely related to loss of intraspecific genetic diversity strongly depended on which part of the range has been lost.

***Vavilovia formosa* and future climate change**

Future climate change is expected to alter the current geographical ranges and the genetic diversity patterns of many plant species; hence, predicting this response will be critical to managing the conservation of plant resources. According to the niche-modelling results, *V. formosa* is expected to suffer further range reduction in both RCP scenarios for 2050. The same is also expected for both haplotypes of the species. Although we cannot exclude that the range reduction of *V. formosa* since LGM was likely related to loss of genetic diversity, the future range reduction is not expected to result in high loss of intraspecific genetic diversity, as in both projections no

haplotype extinction is predicted. This seems to be consistent with the loss of genetic diversity in the case of northern plants due to climate change, where the relationship between loss of genetic diversity and loss of range is nonlinear (Alsos et al. 2012).

On the other side, it is important that the results of the niche equivalency test showed no statistically significant niche difference for the two haplotypes. It indicates that the differences in their predicted distributions might not reflect differences in their climatic responses. From a conservation perspective, this evidence is of high interest, especially in planning in situ conservation efforts of the species, where other factors could be taken into consideration.

Implications for conservation

From a conservation perspective, several issues should be taken into consideration in the conservation management of this species, based on our findings. Despite its restricted habitats, *V. formosa* has raised interest in legume community, as characteristics such as frost tolerance and perenniality are of great practical interest to breeders and geneticists (Akopian et al. 2010; Mikič et al. 2014). Ex situ conservation has already taken place in Armenia, but up to now, it is not sufficient in itself to secure long-term persistence (Akopian et al. 2010) of the species. Appropriate strategies for the conservation of *V. formosa* also require a sound understanding of its origin, current condition and future prospects. Based on our findings, we can hypothesize that more populations are expected to occur, and taking into consideration that the genetic diversity of the species is geographically differentiated, we cannot exclude that a new haplotype may result searching new populations. Niche modelling can greatly contribute in discovering new populations of the target species (Guisan et al. 2006). Current predicted distribution of the species could also be helpful to identify proper sites for ex situ conservation, where the climatic conditions follow the requirements of the species. Additionally, detailed population records in the broader area, as well as detailed measurements studying the population ecology of the species are needed. It is also important as a broader matrix in which adjacent high elevation refuge areas (possibly cryptic refugia) of cold-adapted and xerophytic species, which have been more widespread during past glacial periods, co-occur and which are crucial for the long-term persistence and dynamics of biodiversity (Hewitt 1999; Stewart et al. 2010; Tzedakis et al. 2013). Biotic interactions, which were not included in our methodology, such as competitive relationships within communities (Namba 1984) or fluctuations in the natural herbivore population abundance (Speed et al. 2012), and changes in soil nutrient dynamics (Soudzilovskaia et al. 2013) may also play a role.

Conclusions

Based on cpDNA sequences, two haplotypes were detected with geographical pattern. Population analysis by AFLP showed intraspecific diversity and limited outcrossing rate. The results of the niche modelling support the hypothesis that *V. formosa* survived and expanded as a cryoxerophilic species in steppe and shrub tundra vegetation during LGM. The niche-modelling results indicated that *V. formosa* was favoured during the LGM (22,000 ybp) compared to the Last Interglacial Period (~130,000 ybp) and to the current environmental conditions. *Vavilovia formosa* has suffered a range reduction following climate warming after Last Glacial Maximum, which classify this species as cold-adapted among the Fabaceae species as well as a glacial relict.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Information on Electronic Supplementary Material

Online Resource 1. List of samples used in the study, with indicated origin, collector, locality information, GenBank accession numbers and molecular ITS and cpDNA haplotypes.

Online Resource 2. Nucleotide alignment of sequenced part of *matK* gene.

Online Resource 3. List of species accompanying *Vavilovia formosa* at studied localities.

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