

## RELATIONSHIP BETWEEN GEOGRAPHICAL DISTRIBUTION RANGE AND LEVELS OF GENETIC VARIATIONS IN ASTRAGALUS (FABACEAE)

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**Abstract** Information on relationship between geographic distribution range and genetic variations among congeneric species are useful for understanding the ecological and evolutionary patterns of population geneticists. We studied this relationship in three *Astragalus* species of *A. sahendii* with narrow endemic, *A. tabrizianus* with relatively wider-endemic and *A. caspicus* with widespread geographical distribution range using Randomly Amplified Polymorphic DNAs (RAPDs) in a region where their distributions do overlap each other. The number and percentage of polymorphic RAPDs loci were 56 and 46.08% in *A. sahendii*, 48 and 44.12% in *A. tabrizianus*, and 70 and 63.73% in *A. caspicus*. The smallest (0.122) and greatest (0.200) Nei' diversity were detected in *A. tabrizianus* and *A. caspicus* populations, respectively. Higher genetic diversity detected in *A. sahendii* with very restricted narrow endemic distribution compared to variation of *A. tabrizianus* with relatively wider-endemic distribution may suggest that the other factors e.g. pollination mode play stronger role than geographical distribution on the genetic structure and diversity of plant population and species.

**Keywords:** geographical distribution; genetic variations; narrow endemic; widespread distribution; RAPDs

### INTRODUCTION

The plant species are dramatically different in terms of geographical distributional range, as it ranges from very narrow-restricted to world-wide distributions. Even at inter-specific level, some species have restricted-endemic distribution range while their congeneric species have widespread distributional range. This variation in geographical distribution has attracted the plant ecologists, bio-geographers and population geneticists [11, 20], and this question is still remains poorly resolved [11]. There could be many causes behind this interspecific variation in extent of geographical distribution range including life history traits, levels of genetic diversity, geographical barriers to dispersal, ecological requirements, and interactions with pollinator and competitors [5, 6, 20, 26].

Levels of genetic variation at species and population levels can impact on geographical distribution extent in plants through affecting on reproductive fitness and success [18, 33]. Relationship between levels of genetic variation and distributional range has been the subject of many studies in evolutionary biology [12, 14, 31]. Recently, the interest in this relationship has turned to urgency, since nowadays more species exposed to extinction, and there are attempts to prevent further loss of biotic diversity [12].

Reports on relationship between levels of genetic variation and distributional range are conflicting. Some comprehensive reviews have revealed a direct correlation between levels of genetic variability and extent of geographical distribution [e.g. 12, 14, 17] where species with endemic geographical distribution show very low levels of genetic variation both at the species and population levels, while those species of widespread distribution have higher amount of genetic diversity, meanwhile species with intermediate distribution range (regional) have intermediate levels

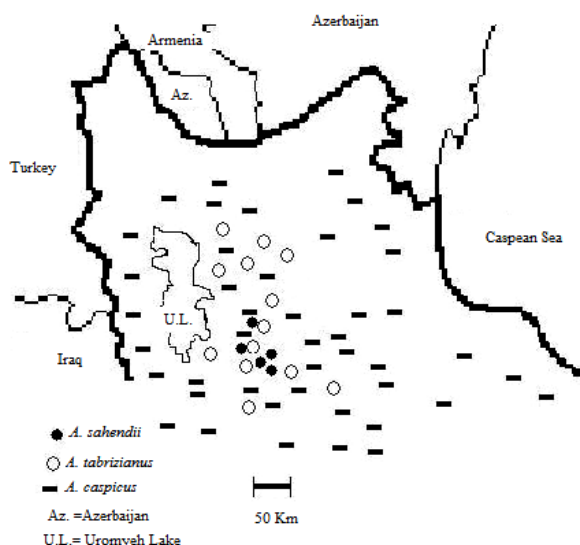
of genetic diversity. However, some other comprehensive reviews showed no association between geographical distribution range and population genetic diversity [31]. Studying the factors shaping the geographical extent of species is one of the main interests in plant science [11], while this is less well studied in biogeography [22].

This work aimed to study the relationship between geographical distribution range and levels of genetic variation in three *Astragalus* species of narrow-restricted endemic, wider-endemic and widespread geographical distribution range using Randomly Amplified Polymorphic DNAs (RAPDs). The genus *Astragalus* L. (Fabaceae) includes over 2500 species [24, 25], and is distributed mainly in cool arid regions of the Northern Hemisphere and South America [34, 37]. Many *Astragalus* species are narrow endemics, often preferentially distributed in marginal habitats. This provides an opportunity for studying evolutionary processes on an early unique scale [34].

### MATERIALS AND METHODS

#### Species and site study

Three *Astragalus* species of *A. sahendii*, *A. tabrizianus* and *A. caspicus* investigated in the current study are sympatrically distributed around Sahand Mountains-Chain situated in some 30km Southeast of Tabriz City (East Azerbaijan, Iran) with altitude ranging from 1500 to 2700m. These species have very different geographical distribution range. *A. sahendii* is a local endemic species with very narrow-restricted distribution, occurring exclusively in the uplands of the study site with altitude over 2000m, while *A. tabrizianus* has relatively a wider distribution range covering larger areas with altitude between 1600 and 2000m. *A. caspicus* has very widespread and covers much larger areas covering some part of study site with altitude below 1700m and extends beyond the site to the North West of the Country (Fig. 1). The sampling



**Figure 1.** Geographical distribution range of three *Astragalus* species in Sahand Mountain-Chain in Northwest of Iran. *A. sahendii* has narrow-restricted endemic, *A. tabrizianus* wider-endemic and *A. caspicus* has widespread distribution.

was carried out at the same area in order to minimize the impact of environmental changes. A number of 10 individual plants of each species were randomly sampled in 2012 from the study site.

#### DNA extraction and RAPD-PCR profile

Genomic DNA was extracted from seeds/seedlings or leaf material following [28]. The concentration of DNA samples was estimated by both gel electrophoresis and spectrophotometry, and adjusted to 10ng/ml wherever necessary. Twelve decamer arbitrary RAPD primers (CinnaGen, Iran) were examined, of which five primers producing the most polymorphic bands were selected for further analysis (N1=5'-GGCGCGTTAG-3', N25'-GACGAGCGG-3', N35'-GGCTGCCAGT-3', N4=5'-TGGAGTCCCC-3', N5=5'-CCCGTCTACC-3'). PCR reaction consisted of 5 µl of 10ng/ml template DNA plus 1 µl of 100pm/µl primer and 19 µl Master Mix (CinnaGen PCR MasterKit, Cat. No. PR8251C). Amplifications were performed in a Biometra thermal cycler for an initial 4 min denaturation at 94°C followed by 40 cycles of 1 min at 93°C (denaturation), 1 min at 40°C (annealing) and 1.5 min at 72°C (synthesis). All PCR products were separated by electrophoresis on 1.5% w/v agarose gels in 1X TBE buffer, stained with ethidium bromide, viewed under ultraviolet light and photographed using UV Transilluminator (UVP, USA). PCR reactions and electrophoresis were repeated at least twice in each case to ascertain the reproducibility of the bands.

#### Analysis of genetic variation

The banding patterns were recorded as 1 for present and 0 for absent of a band. Then the data were entered in a binary matrix for cluster analysis using the NTSYS-pc (Numerical Taxonomy and Multivariate Analysis System, ver. 2.02). The number and percentage of polymorphic RAPD bands were obtained for each population. Genetic diversity within each

population was estimated using Nei's [30] and Shannon's [23] information index (Popgen ver. 1.32).

#### RESULTS

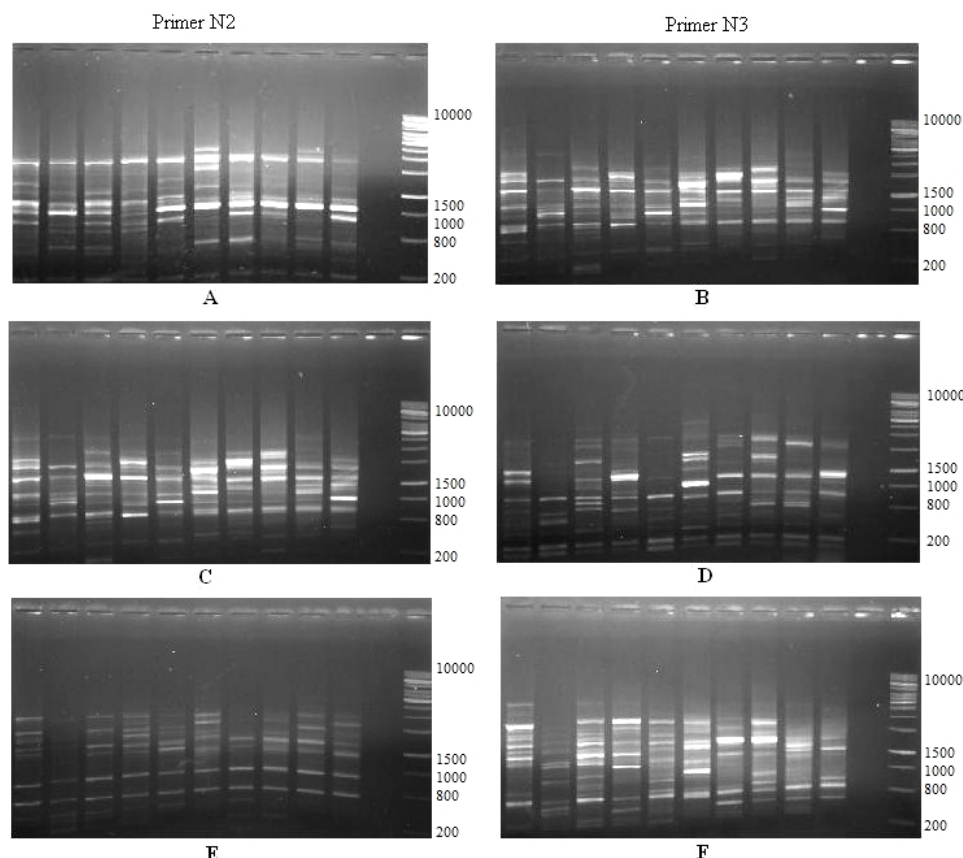
Using five primers in 30 individuals belonging to the three *Astragalus* species produced 54 clear, polymorphic and reproducible RAPD bands (Fig. 2). The number and percentage of polymorphic RAPD loci within the populations of species were 56 and 46.08% in *A. sahendii*, 48 and 44.12% in *A. tabrizianus*, and 70 and 63.73% in *A. caspicus*. The smallest within-population Nei' genetic diversity (0.122) was detected in *A. tabrizianus* population, while the greatest diversity (0.200) was revealed in *A. caspicus* population. Similarly, the lowest (0.217) and highest (0.310) within-population Shannon's genetic variations were found in *A. tabrizianus* and *A. caspicus*, respectively (Table 1).

#### DISCUSSION

This study showed that the higher level of genetic diversity detected in *Astragalus caspicus* with larger geographical distribution is consistent with the general conclusion made by [14] based on extensive review of the literatures. They have shown a strong correlation between extent of geographic distribution and levels of genetic diversity at both the species and population levels. In contrast, *A. sahendii* with very restricted-endemic distribution presented a higher genetic diversity compared to that of *A. tabrizianus* with wider-endemic distribution. Similar patterns have also been reported from other endemic plant species with very restricted geographical distribution. Similarly, the high genetic variability has been reported from endemic species of *Sequoiadendron giganteum* and *Eriogonum apricum* [36], *Adenophorus periens* [32], *Polygonella* [22], *Allium aaseae* [35], *Argyroxiphium sandwicense* [10], *Daviesia suaveolens* [38], while their widespread con-generic species showed lower variation.

Field inspection on the levels of fruit set in the three *Astragalus* species showed that seed set in *A. sahendii* is very low since majority of legume fruit in plants was found to be empty, while this was not the case in *A. tabrizianus*. The very narrow restricted geographical range distribution in *A. sahendii* could most likely be attributed to failure of seed/fruit formation. On the other hand, The two species *A. sahendii* and *A. tabrizianus* investigated in this study with restricted geographical range distribution may indicate the founder effect. This means that these species have recently originated in this area, and have not had time to increase their genetic diversity and distribution range.

The impact of low seed/fruit set on restricting geographical distribution has been reported in many plant species. Moreover, differences in the extent of geographical distribution between congeneric species have been mostly attributed to seeds production levels [3, 8], and also to geographical barriers to dispersal,



**Figure. 2.** Patterns of RAPDs variations in populations of three *Astragalus* species with different geographical distribution range. A&B= *A. caspicus*, C&D= *A. tabrizianus*, E&F=*A. sahendii* produced by primers N2 and N3 (the first and second lanes from right are size markers and negative control, respectively).

**Table 1.** Number and percentage of polymorphic RAPDs loci, and levels of genetic variation in three *Astragalus* populations

Species	No. polymorphic bands	No. polymorphic specific bands	%Polymorphic loci	Shannon's Information Index	Nei's diversity
<i>A. sahendii</i>	56	13	46.08%	0.217	0.139
<i>A. tabrizianus</i>	48	11	44.12%	0.195	0.122
<i>A. caspicus</i>	70	26	63.73%	0.310	0.200

and in part to the biological traits and ecological requirements of particular species [5, 6, 19, 21, 26]. For example, interspecific comparison between restricted endemic and widespread species in the western Mediterranean indicated that the endemic species generally have lower seed/fruit set than widespread ones [21]. The levels of reproductive success and fitness in plants significantly vary within and among populations due to diverse ecological and genetic causes [16]. Because ecological factors in plants pollinated by animal can have a strong impact on reproductive success [20], as levels of pollinator activity can also strongly limit the number and quality of seeds produced through affecting on levels of pollen transfer [4].

Studies on the relationship between distributional range and genetic variations in plants have revealed very diverse patterns. Comparison between 11 pairs of endemic and widespread congeneric species has shown that genetic variability is dramatically lower in rare species compared to their widespread congeners [17]. However, in a review of the literatures based on

RAPD, Nybom and Bartish [31] revealed no association between geographical distribution range and population genetic diversity, and similarly, in a review based on 34 pairs of rare and widespread congeneric plant species, Gitzendanner and Soltis [12] have shown that levels of genetic diversity in rare and widespread congeners are closely related at both the population and species levels.

This could be attributed to differences between these two species in different life history traits especially pollination mode. However, Karron' review [17] on several rare and widespread congeners has shown that outcrossing rates in rare species were not significantly lower than their widespread congeners

The low genetic diversity within species of narrow endemic distribution has been attributed to strong genetic drift and higher inbreeding depression due to small size of the isolated populations [2, 7]. In addition, low genetic variation within narrow endemic species could be caused by the founder events associated with recent speciation [38], or by bottleneck effect related to fragmentation of populations [30]. In

contrast to taxa with narrow endemic distribution range, widespread species are expected to exhibit higher levels of genetic diversity, since the large continuous populations of widespread taxa are theoretically less susceptible to genetic drift [14]. Moreover, widespread species tend to have more continuous population systems in a broader range of environments. These species might be expected to show less genetic structure due to increased influences of gene flow even though they cover a much greater geographic range. The widespread species do exchange fewer genes across their geographic range and have overall higher levels of population divergence than geographically restricted species [13].

The current study along side the others indicate that a rare endemic plant species could have either lower or higher genetic diversity compared to their widespread congeners, since many other factors e.g. pollination mode, reproductive fitness and eco-geography can affect on population genetic structure. Therefore, to interpret the relationship between genetic variation and geographical range distribution in plants it would be useful to include other factors affecting on the patterns of genetic variations such as population reproductive fitness and pollination mode.

Our results alongside all other reports on relationship between genetic variation and distributional range indicate that the patten is too complex and there must be other strong factors effecting on this relationship.

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