

ORIGINAL ARTICLE

Sex ratio and genetic diversity in the dioecious *Pistacia atlantica* (Anacardiaceae)

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Abstract

The levels of genetic variation in dioecious plant species have been reported to differ between male and female populations. This has been attributed to different factors including distribution patterns of individuals, sex ratio and also stochastic events. We measured the levels of genetic diversity in male and female populations of dioecious *Pistacia atlantica* (pistachio, Anacardiaceae) separately for each region and genders in two eco-geographically different regions over 350 km apart and of different population sizes in East Azerbaijan, Iran by randomly sampling 15 individuals of male and female from each of the regions using randomly amplified polymorphic DNAs. The percentage of polymorphic RAPD bands in the male populations of dioecious *Pistacia atlantica* was significantly higher (88.9%) than that of female populations (80.8%), and similarly, male populations had greater genetic diversity (0.387, Shannon; 0.252, Nei's) compared to female populations (0.381, Shannon; 0.249, Nei's). Genetic variation in larger population (Arasbaran) of *P. atlantica* (0.387, Nei's) was greater than that of the smaller (Jazire) population (0.312), indicating the impact of population size on genetic variation. Partitioning the total genetic variation using an analysis of molecular variance indicated that 77% of total genetic diversity was allocated within populations while 23% of this variation was dedicated among populations.

Key words: sex ratio; dioecious; genetic variation; *Pistacia atlantica*; population size

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INTRODUCTION

Less than 4% of all 250,000 flowering plant species are dioecious with separate male and female individuals (Ainsworth 2000). Male and female populations of dioecious plant species display different levels of genetic variations (Hilfiker

et al. 2004, Vandepitte et al. 2010). Several factors cause differences in the levels of genetic diversity between male and female populations of dioecious plants including distribution patterns of individuals across the populations (De Jong and Klinkhamer 2005), sex ratio (Vandepitte et al. 2010), and in small isolated populations, stochastic events also (Engen et al. 2003). The variation of sex ratio in the dioecious plant populations is influenced by environmental conditions since these conditions have a different affect on distribution, survival and growth rates of male and female individuals (Eppley 2001, Stehlik and Barrett 2005, Dudley 2006). According to the ecological causation hypothesis for secondary sexual dimorphism in dioecious plant species, sexes perform differently under environmental stresses and resource availability since males and females have adapted to different ecological niches (Shine 1989, Dudley 2006). For example, in *Salix arctica*, female plants are common in mesic, nutrient-rich habitats while males are distributed in xeric, nutrient-poor sites (Dawson and Bliss 1989b). In *Distichlis spicata*, habitats with female majorities are located at significantly lower elevations in the marsh than are habitats with male majorities (Eppley 2001).

The unbalanced sex ratios can lower the population genetic diversity through both reduced reproductive fitness and by reducing the effective population size, and consequently can result in decreased allelic diversity due to increased genetic drift (Reed and Frankham 2003). In addition, dioecious plant species are expected to have high genetic diversity within populations and low genetic differentiation among populations due to obligate outcrossing (Hamrick and Godt 1996). The amount of genetic variation can have important biological consequences on population fitness and persistence (Bell 1991, Schmid 1994, Reusch et al. 2005). However, the relationship between sex ratios and genetic variation in populations of dioecious plant species has been less well studied (Hilfiker et al. 2004, Vandepitte et al. 2010).

The genus *Pistacia* (pistachio, Anacardiaceae) comprises approximately 12 species, of which three species including *P. atlantica* Defs. are distributed in Iran. *P. atlantica* has Irano-Turanian geographical distribution range extending from Central Asia through the Middle East to North Africa (Zohary 1952). *P. atlantica* is a dioecious tree with unbiased sex ratios (Bogen et al. 1997, Rottenberg 1998) and is a wind-pollinated plant (Inbar and Kark 2007). However,

a monoecious type of sex system has also been reported in this species, where both male and female flowers appear on one individual tree (Kafkas and Peral-Treves 2000).

The current work investigated the patterns of genetic variation in populations of dioecious *Pistacia atlantica* with respect to the sex ratios and population size using randomly amplified polymorphic DNAs (RAPDs).

MATERIALS AND METHODS

Site study

Two different populations of *Pistacia atlantica* were included in this study from eco-geographically different regions over 350 km apart in East Azerbaijan, Iran. Both the populations were located inside the protected areas, one of which, Arasbaran Forestland (altitude: 46° 40 min 0.2 sec; longitude: 39° 40 min 0.12 sec), was located in the southernmost of the Caucasus, and the other, Jazire Islami (altitude: 46° 20 min 0.3 sec; longitude: 38° 10 min 0.12 sec), was situated near the Urmia Lake, a lake well-known for very high salinity. 15 male plants and 15 female plants were randomly sampled from each site making a total of 60 samples. However, 5 samples of females from Jazire site were lost during handling in DNA extraction, and as a result, a total of 55 individuals only were included in the study. Sampling was carried out with approximately 100 meters between each sample in both sites.

DNA extraction, RAPD-PCR amplification and analysis

Nuclear DNA was extracted from seeds and/or seedlings following Miller (2002) with the minor modification of replacing silver sand by liquid nitrogen. This method was previously successfully when used for extraction of nuclear DNA from many plant species. The concentration of DNA samples was measured by both gel electrophoresis and spectrophotometry, and consequently adjusted at 10 ng/ml. 12 arbitrary RAPD primers were tested, of which the 8 primers (Table 1) which had produced the most polymorphic and clearest reproducible bands, were selected for the study. The RAPD profiles were repeated at least three times to insure the reproducibility of the bands. The banding patterns were scored as 1 for presence and 0 for absence 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, and also for genders separately for each sites.

Table 1. Primers sequences

Primer code	Primer sequences	Annealing temperature (°C)
N1	5'GGCGGGTTAG3'	36
N2	5'GACGAGCAGT3'	36
N3	5'GGCTGCCAGT3'	36
N4	5'TGGAGTCCCC3'	36
N5	5'CCCGTCTACC3'	36
E13	5'GTTGCGATCC3'	36
OPA08	5'GTGACGTAGG3'	36
OPO08	5'CCTCCAGTGT3'	36

The levels of genetic variation were separately measured for sites and genders' populations based on Nei's (1973) and Shannon's information index using Popgen (version 1.32). To study the genetic similarity among the male and female populations, the UPGMA (Unweighted Pair-Group Method with Arithmetical Averages) dendrogram was generated based on a matrix of Nei's distances among populations through the SHAN (sequential, hierarchical, agglomerative

and nested clustering of the NTSYS-pc). Total genetic variation was partitioned into, within and among the populations based on analysis of molecular variance (AMOVA) using Arlequin (version 3.11). The significance level for F-statistics analogous was determined using 1023 bootstrap replicates.

RESULTS

The RAPDs profiles were reproducible and clear for scoring (Fig. 1). The levels of polymorphic RAPD bands, genetic diversity separately analysed for the regions and gender-based populations are presented in Table 2. The percentage of polymorphic RAPD bands in male populations of dioecious *Pistacia atlantica* was significantly higher (88.9%) than that of female populations (80.8%), and similarly, male populations had greater genetic diversity (0.387, Shannon; 0.252, Nei's) compared to female populations (0.381, Shannon; 0.249, Nei's).

Comparison of the levels of genetic diversity between male and female populations separately for each region also showed that in both regions the male population had higher genetic variation than the female population. In Arasbaran, male and female populations showed 0.232 and 0.205 Shannon genetic diversity respectively, while these values for Jazire were 0.181 and 0.164, respectively.

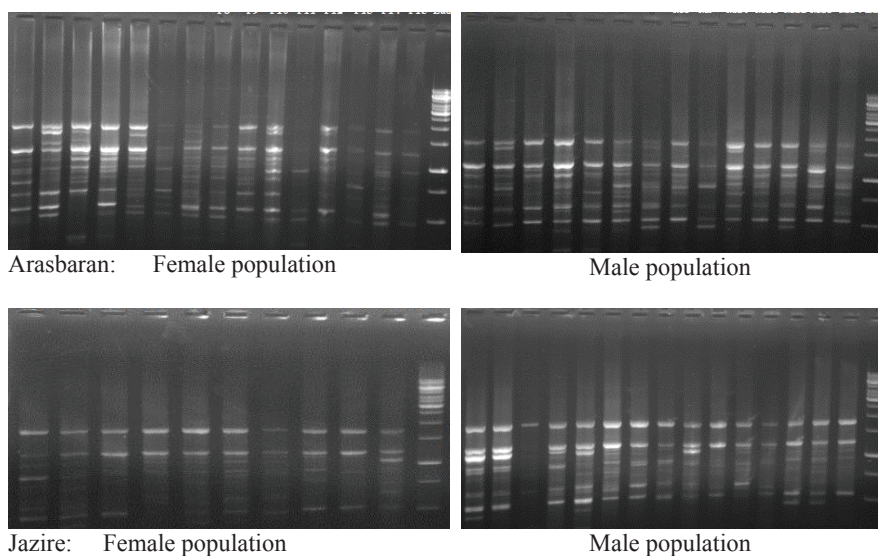


Fig. 1. RAPD patters of male and female populations of *Pistacia atlantica* in two different regions produced by primer N1

Table 2. Polymorphic RAPDs percentage, Shannon and Nei’s genetic diversity in male and female populations of *Pistacia atlantica* in two different regions in East Azerbaijan, Iran

No.	Variable	Population	% Polymorphic RAPDs bands	Shannon’s information index	Nei’s gene index
Gender		Male	88.9	0.387	0.252
		Female	80.8	0.381	0.249
Gender	Arasbaran	Male	68.69	0.232	0.350
		Female	67.68	0.205	0.317
	Jazire	Male	54.55	0.181	0.272
		Female	48.5	0.164	0.248
Region	Arasbaran		88.9	0.248	0.387
	Jazire		66.67	0.205	0.312

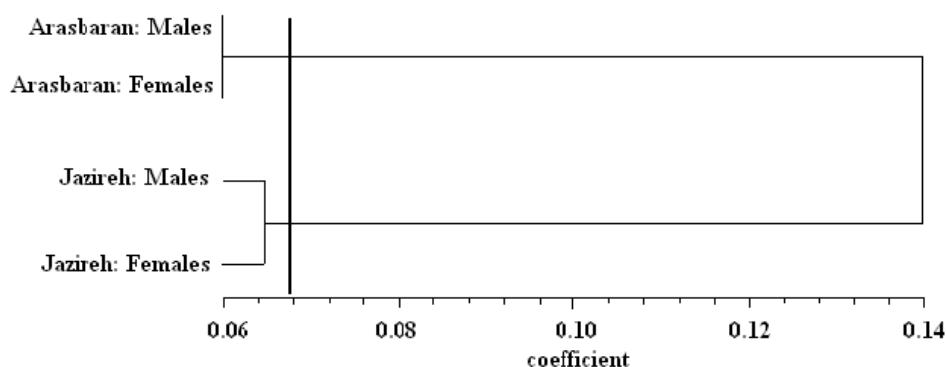


Fig. 2. UPGMA Nei’s based-dendrogram showing similarity among males and females populations of *Pistacia atlantica* in two different regions in East-Azerbaijan, Iran

The comparison of genetic variation between populations of *P. atlantica* (regardless of gender) between the two regions indicated that the Arasbaran population had higher genetic variation (0.387, Nei’s) than the Jazire population (0.312).

Studying the genetic similarity between all male and female populations of *P. atlantica* showed that the male population of Arasbaran was more similar to its female population than to the male population of the Jazire region. Similarly, Jazire male and female populations indicated more similarity to each other than to those populations in Arasbaran (Fig. 2).

Partitioning the total genetic variation using analysis of molecular variance (AMOVA) indicated that 77% of total genetic diversity was allocated within populations while 23% of this variation was dedicated among populations.

DISCUSSION

This study shows that in dioecious *Pistacia atlantica* the level of genetic variation in male populations was always greater than that of females. This could have resulted from two reasons: first, in both regions the numbers of male plants were slightly greater than those of females. Second, the male individuals were more evenly distributed across the areas compared to the female individuals. There are some reasons for the male biased population of *P. atlantica* in this study. First, male plants in dioecious plant population always encounter less local resource competition (Taylor 1994). When a dioecious plant produces a female seed, then the seeds produced by this daughter plant are dispersed in her seed shadow. While a dioecious plant produces a male seed, the pollen produced by

this son is first transferred to a female plant, after which the seeds are dispersed. In higher plants, the male gametophytes (pollen grains) disperse, whereas the female gametophyte (the egg cell) does not. If a dioecious plant produces male seeds, its F_1 progeny are more evenly spread out over the whole population than if it produces female seeds (De Jong and Klinkhamer 2005). Producing more male seeds always reduces local resources competition because pollen grains are dispersed to further distances than the seed (Hu and Ennos 1997). Moreover, another reason for male-biased sex ratios especially in long-lived dioecious species has been attributed to differences in the costs of reproduction in different genders (Lloyd and Webb 1977) since a greater proportion of resources in females are usually allocated to reproduction than males (Stehlik and Barrett 2005). This can make female individuals more susceptible to environmental stress, and consequently could result in higher mortality in females (Meagher 1981). This is the reason why female-biased sex ratios are less common in dioecious species (Lloyd 1974). In addition, the female plants of *P. atlantica* in the study regions are, because of their edible fruits, more attractive to herbivores, especially bears. This causes damage to the female plants, leading to a male-biased population. Therefore, sex ratios in populations of dioecious flowering species often deviate from 1:1 (Stehlik and Barrett 2005), and in most cases male populations have greater individual numbers (Vandepitte et al. 2010). A review survey of the sex ratios in dioecious flowering plants has confirmed this idea by showing that 29% of species had a ratio 1:1 while 57% of species were male-biased populations (Rottenberg 1998, Delph 1999).

In the current study the high level of genetic variation found within populations (77%) compared to the lower level of this variation among populations has resulted from the obligate outcrossing in dioecious *P. atlantica*. In dioecious plant species, obligate outcrossing should naturally maintain a high genetic diversity within populations and low genetic differentiation among populations compared to self-compatible plants (Hamrick and Godt 1996).

In this study the higher level of genetic variation detected in the Arasbaran population of *P. atlantica* can be attributed to population size as the population size in this region was larger than that of the Jazire site. The lower levels of genetic diversity, in turn, impact negatively on the fitness, through high inbreeding depression

and accumulation of deleterious mutations caused by genetic drift and genetic incompatibility (Frankham et al. 2002). Small populations and consequently lower genetic diversity lead to genetic drift, higher risks of inbreeding, lower evolutionary potential, and subsequently result in a higher risk of extinction (Reed and Frankham 2003). Studying the pattern of genetic variation and its relationship with environmental factors in dioecious plants can help in both better understanding of the evolution of this breeding system in plants, and better management of conservation.

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