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ORIGINAL ARTICLE

Low genetic diversity and high levels of inbreeding in the Sinai primrose (*Primula boveana*), a species on the brink of extinction

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Abstract The Sinai primrose (*Primula boveana*) is one of the most endangered plant species worldwide, with less than 200 wild individuals surviving in the Sinai mountains of Egypt. There has been a decline in both the number and size of its populations in recent times, possibly caused by threats that include habitat aridification and the impact of human activities. Studying the standing genetic variation and extent of inbreeding of *P. boveana* is necessary for the design of appropriate conservation strategies for this species. In the present work, we used a set of seven, recently developed, polymorphic microsatellite markers to characterize the genetic variation and levels of inbreeding of the extant populations of *P. boveana*. We found low levels of genetic variation ($H_T = 0.470$), high differentiation between populations ($F_{ST} = 0.737$, $R_{ST} = 0.935$), and very elevated levels of inbreeding ($F = 0.862$) due to recurrent selfing. These results may be the reflection of low levels of genetic variation and high levels of inbreeding over a long evolutionary period, suggesting that the current genetic pool of the species may enable *P. boveana* to persist in a habitat where water availability and pollinator services are restricted. Nevertheless, in sight of its rapidly dwindling abundance, it seems prudent to adopt swift measures, including habitat restoration and ex-situ conservation, to prevent the impending extinction of this emblematic species.

Keywords Aridification · Genetic variation · Inbreeding · Mount St. Catherine · Sinai primrose · Human-driven environmental change

Introduction

With ca. 430 species distributed mainly in the Northern Hemisphere, primroses (*Primula* L.) have long been a model for the study of evolution of plant breeding systems (e.g., Darwin 1862, 1877; Charlesworth and Charlesworth 1979; Barrett 1992; de Vos et al. 2012) and represent an important group of gardening plants (Richards 2003). *Primula* section *Sphondylia* (Duby) Rupr. comprises eight species with patchy distributions endemic to mountainous regions from the West Himalayas to Ethiopia, including Afghanistan, Egypt, Iran, Saudi Arabia, Turkey and Yemen (Wendelbo 1961; Richards 2003). This section is remarkable for its intra- and inter-specific variation of floral morphologies, which is not as marked in other sections of the genus (Al Wadi and Richards 1993; Richards 2003). Within section *Sphondylia*, floral syndromes range from distyly (a floral polymorphism characterized by the reciprocal placement of male and female organs in two different floral morphs) to incomplete distyly (see below) and homostyly (only one floral morph, with both male and female organs at the same level within the corolla tube; Al Wadi and Richards 1993).

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Within section *Sphondylia*, *Primula boveana* Decne ex Duby ($2n = 18$), also known as the Sinai primrose, is particularly interesting for its reproductive biology and extreme rarity. First, *P. boveana* deviates from the classic definition of distyly, typical of most other species of *Primula*, by displaying incomplete distyly, for its plants are characterized by flowers with anthers in a fixed, intermediate position, but styles that are either long or short. Such combination of character states for the style and anthers has sometimes been interpreted as a transitional stage between homostyly and distyly (Al Wadi and Richards 1993; but see Mast et al. 2006). As observed in other non-distylous primroses (Richards 2003), *P. boveana* is self-fertile (Al Wadi and Richards 1993). In addition, *P. boveana* is currently found in only five localities on Gebel Katarina (Mount St. Catherine), which is included in the Saint Catherine Protectorate national park situated in the mountainous area of the southern Sinai Desert of Egypt. Due to its geographic isolation, at least 1,400 km away from other species from section *Sphondylia*, *P. boveana* is a key element for understanding the biogeographic connections within the genus *Primula*. Population sizes are very small, below 100 plants, although this species is known to have been more abundant in the recent past (Al Wadi 1993; Richards 2003). Such a narrow distribution and scarcity prompted Richards (2003) to refer to *P. boveana* as "one of the rarest plant species". The IUCN lists it under the category deficient data (DD; García et al. 2010). Due to its extreme rarity, *P. boveana* is considered as a priority target for conservation at a national level in Egypt (Radford et al. 2011).

The alarming, recent decrease in the population sizes of *P. boveana* seemingly corresponds to the increasing aridification of its habitat, namely granitic mountains dissected by steep-sloped wadis (i.e., valleys) at high elevation (i.e., >1,700 m a.s.l.) and irrigated by springs mostly fed by meltwater (Moustafa et al. 2001). The climate in the Sinai is Saharan-Mediterranean, with the mountainous regions characterized by hot summers, relatively cold winters and precipitation regimes of 60 mm/year. The highest peaks receive more water, part of it in form of snow, which increases precipitation to up to 300 mm/year (Grainger 2003). Human activities in the past few decades, especially water consumption for the local bedouin populations and a fast-developing tourist industry (until recent political events), have severely depleted local water reserves (Grainger 2003). This human-driven aridification, together with the prediction of warmer and drier conditions due to climate change both in mountains generally (Nogués-Bravo et al. 2007) and in the Eastern Mediterranean specifically (Alpert et al. 2008; Giorgi and Lionello 2008; Issar 2008), forecasts a further fall in the number and extent of the populations of *P. boveana*.

Rare species such as *P. boveana* are typically characterized by their low genetic variation (Hamrick and Godt 1989; Nybom 2004; but see Gitzendanner and Soltis 2000). In many cases, low population sizes and scarce gene flow among fragmented populations result in high levels of inbreeding in a species, which frequently cause a drop in fitness due to inbreeding depression (e.g., Oostermeijer et al. 1994; Crnokrak and Roff 1999; Luijten et al. 2000; Frankham 2005) and losses of rare, potentially valuable alleles due to genetic drift (Ellstrand and Elam 1993; Honnay and Jacquemyn 2007). Within *Primula*, several studies have reported that habitat fragmentation can negatively affect gene flow among populations (e.g., Van Rossum and Triest 2006; Van Geert et al. 2008) and reproductive success (e.g., Matsumura and Washitani 2000; Valdés and García 2011) by reducing the number and size of their populations, especially in distylous species that depend on sufficient frequencies of both floral morphs for efficient seed production. Previous empirical and theoretical work illustrates that inbreeding and low genetic variation may limit a species' ability to adapt to environmental changes, eventually resulting in its extinction (e.g., Booy et al. 2000; Frankham 2005; Heller and Zavaleta 2009), although they may also produce genomes that are adapted to specific, constant ecological conditions (Lande and Schemske 1985). Consequently, there exists a need for an accurate characterization of the genetic variation and levels of inbreeding in *P. boveana*, since this information is crucial for the development of effective management programs for the survival of rare species (Schemske et al. 1994; Sydes and Peakall 1998; Frankham et al. 2002), especially in light of predicted, human-driven climatic changes that may be too fast for species to adapt to rapid habitat changes (Stockwell et al. 2003).

To provide useful information for conservation programs, we sampled all extant populations of *P. boveana* and assessed their genetic diversity and levels of inbreeding using a set of seven putatively neutral microsatellite markers. Microsatellites display elevated rates of mutation caused by insertion/deletion events that occur during DNA replication (Li et al. 2002; Ellegren 2004), and thus are suitable for detecting genetic variation even in extremely rare or genetically bottlenecked species. Furthermore, microsatellites are co-dominantly inherited, which is an indispensable trait for the estimation of the levels of inbreeding based on population genetic parameters (Selkoe and Toonen 2006). To summarize, our goals were to: (1) quantify the standing genetic variation of *P. boveana* and describe how it is partitioned within and among populations; (2) determine the extent of inbreeding in natural populations of *P. boveana*; and (3) propose specific conservation guidelines for *P. boveana* in light of our results.

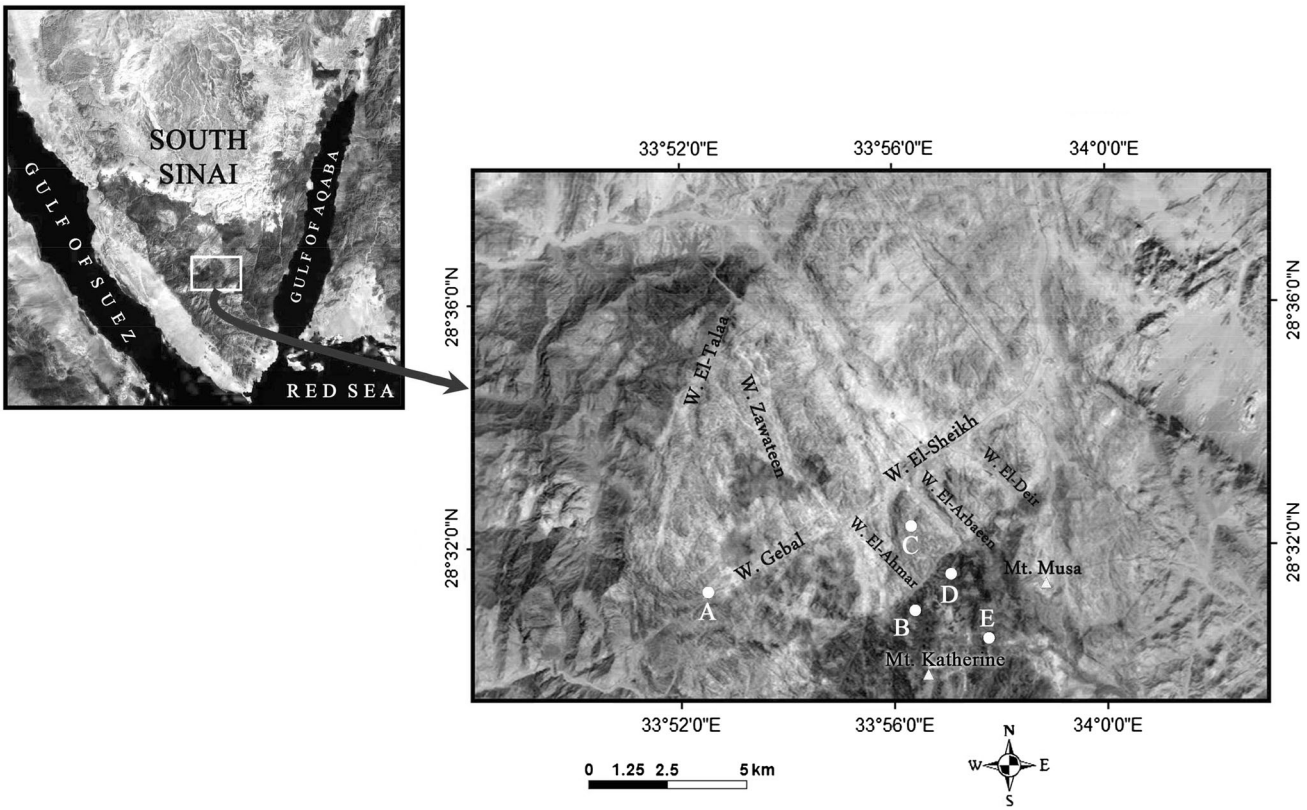

Fig. 1 Locations of the five known populations of *Primula boveana* in Mount St. Catherine, Egypt. **a** W. Geb (Wadi Gebal); **b** AinSh (Ain Shennarah); **c** KaGhs (Kahf El-Ghoula); **d** ShaMo (Shaq Mousa); **e** W. Grag (Wadi Garagniah)

Materials and methods

Populations studied and sampling procedure

We sampled adult plants (both blooming and non-blooming) from four out of the five populations of *P. boveana* in Mount St. Catherine, Egypt (Fig. 1), in late summer 2011. The largest population sampled, Ain Shennarah (abbreviated AinSh), with 195 observed adult individuals, is found in a small gully fed mostly by meltwater in Ain Shennarah at the headwaters of Wadi Shaq Mousa, above 2,000 m a.s.l. (Table 1), and corresponds to the population reported by Al Wadi (1993). The other four known populations (Wadi Shaq Mousa, abbreviated ShaMo; Wadi Gebal, abbreviated W. Geb; Kahf El-Ghoula, abbreviated KaGhs; and Wadi Garagniah, abbreviated W. Grag) are found at lower altitudes and include fewer individuals than AinSh (Table 1). In AinSh, we randomly sampled 33 individuals out of the 195 adult plants observed during our field season in 2011, whereas in ShaMo, W. Geb and KaGhs we sampled all adult plants available (39, 29 and five individuals, respectively). In total, we sampled 106 individuals. Unfortunately, all plants of the W. Grag population were found dead, apparently as a consequence of extreme water scarcity, thus no samples from this population were available for genetic analysis. A recent census carried out in April 2013 revealed no regeneration of the latter population and a decrease in population sizes in the other four localities, including the withering of all plants in KaGhs, also apparently as a consequence of severe drought (Table 1). One to two green leaves per individual were collected and directly dried in silica gel until DNA extraction. One voucher specimen (S. A. Gamal El-din 340) from population AinSh has been deposited in the herbarium at the Suez Canal University (SCU).

DNA extraction, microsatellite amplification and genotyping

Prior to DNA extraction, about 20 mg of dry leaf tissue per individual was ground with stainless steel beads using a MM 3000 shaker (Retsch GmbH, Germany). Total genomic DNA was extracted using the DNeasy Plant Mini Kit (Qiagen, Switzerland) following the manufacturer's guidelines. We amplified seven polymorphic microsatellites developed for *P. boveana* (Mansour et al. 2013) in all the individuals sampled following the single-reaction, nested PCR method of Schuelke (2000), a cost-efficient method best suited for projects with small to moderate number of samples (Blacket et al. 2012). PCRs were

Table 1 Geographic coordinates, altitude and population size evolution between 2007 and 2013 of the five known populations of *Primula boveana* in Mount St. Catherine, Egypt

Acronym	Locality	Latitude (*N*)	Longitude (*E*)	Altitude (m a.s.l)	Number of individuals		
					2007	2011	2013
KaGhs	Kahf El-Ghoula	28°32′08.57″	33°56′10.80″	1,803.0	17	5	0
W. Geb	W. Gebal	28°33′19.08″	33°52′36.06″	1,831.5	41	29	12
ShaMo	W. Shaq Mousa	28°31′58.20″	33°57′56.98″	1,807.0	53	39	17
AinSh	Ain Shennarah	28°31′24.98″	33°57′37.60″	2,082.1	225	195	86
W. Grag	W. Garagniah	28°30′36.80″	33°58′46.50″	1,810.5	11	0	0
				Total	336	268	115

performed in 25 µL containing 2.5 µl of 10× reaction buffer, 1 µl of $MgCl_2$ (50 mM), 0.5 µl of a mix of all 4 dNTPs (10 mM), 0.2 µl of the forward primer including the extended M13-tail (10 µM; Schuelke 2000), 0.5 µl of the reverse primer (10 µM), 0.5 µl of the universal M13 primer (10 µM; Schuelke 2000) labeled with a fluorophore (FAM, NED, VIC or PET), 0.1 µl of Taq DNA polymerase (Bioline; 50 U/µl), 1.0 µl of BSA (bovine serum albumin; 20 mg mL^{-1}), 1.0 µl of 10 ng/µL genomic DNA, and sterilized water up to the final volume of 25 µl. All PCRs were carried out in singleplexes using a TPersonal Thermocycler (Biometra, Germany) under the following conditions: initial denaturation at 94 °C for 3 min, 30 cycles of 94 °C for 30 s, 55 °C for 45 s and 72 °C for 1 min, 8 cycles of 94 °C for 30 s, 53 °C for 45 s, and 72 °C for 1 min, and a final extension step of 72 °C for 5 min. The resulting fluorescently labeled PCR products were run in multiplexes on a 3130xl Genetic Analyzer (Applied Biosystems, USA) using LIZ500 (Applied Biosystems, USA) as a size standard and scored using GeneMapper 4.1 (Applied Biosystems, USA).

Analysis of genetic variation and estimation of levels of inbreeding

Genetic variation in *P. boveana* was assessed by estimating the number of alleles per locus (*A*) and the total heterozygosity (H_T; used as measurement for total genetic diversity). Genetic differentiation among populations was assessed with F_{ST} (Weir and Cockerham 1984) and R_{ST}, an analogue of F_{ST} developed for microsatellite loci evolving under the stepwise mutation model (Slatkin 1995). The accuracy of F_{ST} and R_{ST} is contingent on the microsatellite mutation mode and on the variance of the estimation of these parameters, which in turn depends on the sample size and the number of loci involved in the analyses (Balloux and Lugon-Moulin 2002). An analysis of molecular variance (AMOVA; 9,999 permutations) was used to assess the population genetic structure of *P. boveana* (Excoffier et al. 1992; Michalakis and Excoffier 1996). The number of reproductively successful migrants per generation (N_m) was estimated using the private allele method (Barton and Slatkin 1986). The observed heterozygosity (H_o), expected heterozygosity (H_e) under Hardy–Weinberg equilibrium, and Wright's (1943) fixation index $F = 1-H_o/H_e$ were calculated for each locus in each population to determine deviations from Hardy–Weinberg equilibrium as a measure of inbreeding. Concordance of genotype frequencies with Hardy–Weinberg equilibrium was tested using Chi-squared tests. All these analyses were carried out using GenAlEx 6.1 (Peakall and Smouse 2006) except for H_T, which was calculated as per Nei (1973) and weighted for the number of individuals per population.

Results

The largest population, AinSh (33 out of 195 individuals sampled in 2011, Table 1), showed polymorphism at three of the seven microsatellite loci examined. The populations with an intermediate size, ShaMo (39 individuals, all of them sampled) and W. Geb (29 individuals, all of them sampled) were polymorphic for four loci, whereas the smallest population, KaGhs (five individuals, all of them sampled), was monomorphic for all of the seven loci (Table 2). The mean *A* was 1.57 for each of the AinSh, ShaMo and W. Geb populations, and 1.00 in KaGhs. The AinSh, ShaMo and W. Geb populations had two private alleles each, whereas all the alleles found in KaGhs were present at least in one other population (Online Resource 1). Total heterozygosity, averaged for all loci and populations, was $H_T = 0.470$.

Genetic differentiation among populations was high according to the global value of F_{ST} (=0.737) and even higher according to R_{ST} (=0.935). The results of AMOVA also indicated that the greatest genetic differentiation occurred among populations (94 %, $P = 0.010$), whereas it was much lower both among individuals (5 %, $P = 0.010$) and within individuals (1 %, $P = 0.010$). In agreement with these high values of differentiation among populations, the estimated number of migrants per generation was very low ($N_m = 0.017$).

Table 2 Measures of genetic variation and Hardy–Weinberg equilibrium in the four extant populations of *Primula boveana*

Population	*N*	Locus	*A*	H_o	H_e	*F*
AinSh	33	Prim45b	1	0	0	–
		Prim64	2	0	0.213	1***
		Prim59	1	0	0	–
		Prim54	1	0	0	–
		Prim49	1	0	0	–
		Prim 61	3	0.091	0.473	0.808***
		Prim 66	2	0.152	0.483	0.687***
		Mean ± SE	1.57 ± 0.28	0.034 ± 0.022	0.167 ± 0.079	
ShaMo	39	Prim45b	2	0	0.184	1***
		Prim64	2	0	0.295	1***
		Prim59	2	0	0.142	1***
		Prim54	2	0	0.295	1***
		Prim49	1	0	0	–
		Prim61	1	0	0	–
		Prim66	1	0	0	–
		Mean ± SE	1.57 ± 0.19	0.000 ± 0.000	0.131 ± 0.036	
W. Geb	29	Prim45b	1	0	0	–
		Prim64	2	0	0.067	1***
		Prim59	1	0	0	–
		Prim54	1	0	0	–
		Prim49	2	0	0.128	1***
		Prim61	2	0.034	0.034	−0.018 n.s.
		Prim66	2	0	0.067	1***
		Mean ± SE	1.57 ± 0.19	0.005 ± 0,005	0.042 ± 0.017	
KaGhs	5	Prim45b	1	0	0	–
		Prim64	1	0	0	–
		Prim59	1	0	0	–
		Prim54	1	0	0	–
		Prim49	1	0	0	–
		Prim61	1	0	0	–
		Prim66	1	0	0	–
		Mean ± SE	1.00 ± 0.00	0.000 ± 0.000	0.000 ± 0.000	

N sample size, *A* number of alleles, H_o observed heterozygosity, H_e expected heterozygosity, *F* Wright's (1943) fixation index (*n.s.* non significant)
*** $P < 0.05$

All loci, except Prim61b in W. Geb, significantly departed from Hardy–Weinberg equilibrium in all populations due to a deficit of heterozygotes, as shown by the positive values of the fixation index *F*, which ranged from 0.687 to 1.000 (Table 2) and averaged a value of 0.862 across all loci and populations.

Discussion

Genetic variation and differentiation in *P. boveana* populations

The three biggest populations, AinSh, ShaMo and W. Geb, displayed low levels of allele richness (*A*) and gene diversity (H_e), and the smallest population, KaGhs, was genetically monomorphic (Table 2). Considering that the maximal theoretical value for total genetic diversity is one when estimated with highly variable markers, including microsatellites (Hedrick 1999), the total genetic diversity of *P. boveana* ($H_T = 0.470$) can be considered very low. These results are in line with the observations for other rare and geographically restricted species (e.g., Hamrick and Godt 1989; López-Pujol et al. 2013), including primroses (e.g., Glover and Abbott 1995; Shao et al. 2009).

Reproductive strategies are known to affect levels of genetic diversity in general, and, specifically, in rare primroses. For instance, the two heterostylous and, hence, most likely outcrossing primroses *P. apennina*, endemic to Italy (Crema et al. 2009), and *P. interjacens*, endemic to China (Xue et al. 2004), display high levels of genetic variation, whereas the homostylous and putatively self-

compatible *P. scotica*, endemic to Scotland, displays low levels of genetic variation (Glover and Abbott 1995). Since *P. boveana* is self-fertile (Al Wadi and Richards 1993) and exhibits low levels of genetic diversity, it fits the pattern found in other rare *Primula* species that can produce seeds autonomously.

Another factor contributing to the low genetic variation of *P. boveana* may be the small size of its populations (Table 1). Species with small and declining population sizes are expected to display reduced levels of genetic variation as a consequence of genetic drift and inbreeding (Ellstrand and Elam 1993; Keller and Waller 2002; Smyser et al. 2012). Although collections and reports on *P. boveana* are scarce, the abundance of this species seems to have decreased dramatically ever since its discovery. According to Richards (2003), when first discovered by Nicolas Bové in 1832, *P. boveana* was abundant on the north face of Mount St. Catherine. Over 150 years later, in 1991, the population in Ain Shennarah near the summit of the mountain, which was assumed to be the only extant population of this species, consisted of nearly 2,000 individuals (Al Wadi 1993). A further decrease in the abundance of *P. boveana* was detected in the past few years, with counts of five populations, for a total of 336 adult individuals, in 2007; four populations, for a total of 268 adult individuals, in 2011; and three populations, for a total of 115 adult individuals, in 2013 (Table 1). This drastic and fast decline of *P. boveana* may have thus contributed to the low genetic variation that we observed in this study through the random elimination of alleles. Similar losses of genetic variation have been reported for other *Primula* species affected by habitat fragmentation, in which genetic variation decreases with population size (Van Rossum et al. 2004; Van Geert et al. 2008), although newly fragmented populations can still preserve high levels of genetic variation due to recent gene flow or historical variation (Van Geert et al. 2008).

All the metrics used to assess genetic differentiation in *P. boveana* suggest that gene flow between populations is extremely low. Both F_{ST} (=0.737) and R_{ST} (=0.935) values were very high, thus reflecting a high degree of genetic differentiation between populations. In accordance with these results, the AMOVA also indicates that 95 % of the genetic variation occurs among populations. These findings are corroborated by low migration rates ($N_m = 0.017$) between populations, since genetic differentiation increases with lower levels of gene flow among populations. Furthermore, we found two private alleles in AinSh, ShaMo and W. Geb (Online Resource 1), which is a relatively high proportion of private alleles, considering the small distribution area of *P. boveana* (Fig. 1) and that only two to four alleles were scored per locus (Online Resource 1). These results are consistent with the trend of high inter-population differentiation in rare, endemic species (Hamrick and Godt 1989), and similar patterns have been previously reported for other plants in the Sinai mountains as well (e.g., Wolff et al. 1997).

In seed plants, gene flow among populations consists of two components: seed dispersal and pollen dispersal. A low dispersibility of both seeds and pollen in *P. boveana* could explain our results of high differentiation among populations. Seed dispersal has been reported to be limited in several *Primula* species, with most seeds falling close to the mother plant (e.g., Weeda et al. 1985; Crema et al. 2009). *P. boveana* produces small seeds with an average weight of 3.1 mg (Moustafa et al. 2001). However, despite their size, seeds of *P. boveana* lack any obvious adaptations for wind-driven, long-distance dispersal and are thus likely dispersed by gravity over short distances. Pollen flow represents the main source of gene flow in other primroses (e.g., Crema et al. 2009) and depends on the abundance, timing and foraging behavior of pollinators. Habitat fragmentation and distance between populations impose a limit for pollinator flight among populations, thus negatively affecting gene flow (Van Rossum and Triest 2006; Van Geert et al. 2008; Van Rossum et al. 2011). The natural fragmentation of the habitat of *P. boveana* and the extremely steep topography of Mount St. Catherine very likely represent the main factors limiting pollinator flight and, therefore, pollen flow among the populations of this species. Considering that the migration rate that we found in *P. boveana* ($N_m = 0.017$) is well below the value of at least one individual per generation theoretically necessary to prevent drift-mediated genetic differentiation (Spieth 1974), genetic drift due to small population sizes and limited gene flow could lead to a further impoverishment of genetic variation in the populations of *P. boveana*.

Extent of inbreeding of *P. boveana*

Our genetic results indicate that *P. boveana* is markedly inbred, with an average value of Wright's (1943) fixation index $F = 0.862$, reflecting a pronounced deficit of heterozygotes. Inbreeding in self-compatible plants, such as *P. boveana*, may be caused by an excess of selfing, either autonomous (i.e., without pollinator assistance) or induced by foraging pollinators (i.e., with pollinators depositing pollen from the anthers to the stigmas of the same plant), and/or by crossing between related individuals (i.e., biparental inbreeding). Although there are no published reports on the diversity and abundance of insects that pollinate *P. boveana*, we have observed very few visits to *P. boveana* by the solitary bee *Anthophora pauperata* in its range (H. Mansour, personal observation). In two other species of *Primula* with limited distribution (the distylous, narrow endemic *P. allioni,* restricted to the southwestern Alps,

Minuto et al. 2013; and the homostylous *P. halleri*, restricted to the Alps, Carpathian mountains and Balkan region, deVos et al. 2012), longevity of anthesis and low competition for pollinators (due to scarcity of other co-flowering plants) allow for at least some allogamy, despite sporadic pollinator services. Indeed, in *P. boveana*, flowers remain open for longer than a month in greenhouse conditions (B. Keller, pers. observation) and few other species co-flower in its natural habitat in the Sinai (H. Mansour, pers. observation). Nevertheless, considering the likely low frequency of pollinator visits to *P. boveana* and its very small population sizes, it is reasonable to suggest that recurrent autonomous selfing might be the main cause for the high level of inbreeding observed in this species. Furthermore, preliminary observations show that herkogamy (i.e., the spatial separation between anthers and stigmas within a flower) varies widely during flower development in *P. boveana* (B. Keller, personal observation). Decreasing herkogamy during anthesis has been observed in the homostylous, alpine *P. halleri*, where it has been proposed as a mechanism to favor reproductive assurance in an environment with unreliable pollinator services (de Vos et al. 2012). Therefore, despite the incomplete distyly that has been attributed to *P. boveana* flowers (Al Wadi and Richards 1993), which might suggest a mainly outcrossing breeding system, some selfing might be facilitated by the progressively shorter distance between anthers and stigma as the flowers age. We are currently performing greenhouse experiments aimed at determining whether varying herkogamy during flower development favors autonomous selfing also in *P. boveana,* possibly as an evolutionary response to pollinator scarcity.

High levels of inbreeding frequently have a negative impact on population viability due to inbreeding depression (e.g., Oostermeijer et al. 1994; Crnokrak and Roff 1999; Frankham 2005). Accordingly, inbreeding as a consequence of infrequent pollinator services and small population sizes has been suggested as one of the causes of limited seed set in several heterostylous primroses (Matsumura and Washitani 2000; Van Rossum et al. 2002; Valdés and García 2011). In contrast, *P. boveana* has been reported to produce large seed sets after self-fertilization (79 % of selfed ovules developing into seeds; Al Wadi and Richards 1993), thus suggesting that selfing is not coupled with strong negative effects on reproductive fitness in this species. Furthermore, the high F values found in all populations (Table 2) suggest that high levels of inbreeding are not likely to represent a new phenomenon caused by a recent decrease of pollinator services in *P. boveana*, but rather a feature of the species' reproductive biology acquired during its evolutionary history to overcome pollinator scarcity. Under this scenario, deleterious alleles (i.e., genetic load; Crnokrak and Barrett 2002) in homozygosis after selfing would have been purged, and homozygous genotypes carrying non-deleterious alleles would have become essentially fixed within each population (Lande and Schemske 1985). Thus, inbreeding might have actually helped *P. boveana* to persist in its habitat. The proposed explanation is in agreement with the low levels of allele richness A and low levels of observed heterozygosis H_o detected in the populations of *P. boveana* (Table 2).

Implications for conservation

The low levels of genetic variation that we found in *P. boveana* could potentially have a negative impact on its survival because, in the long term, genetic variation is necessary for species to adapt to changing environmental conditions (Booy et al. 2000; Frankham 2005; Jump et al. 2009). In addition, the high levels of inbreeding detected in *P. boveana* may also result detrimental for the survival of this species, if the species is suffering from inbreeding depression, which we cannot exclude based on our currently available data. Inbreeding depression can lead to a decline of population viability (Keller and Waller 2002; Vilas et al. 2005; Frankham 2005) and, in turn, result in higher vulnerability to stochastic events (Frankham 2005), such as particularly intense arid spells, thus increasing the risk of extinction. The impact of inbreeding depression is often higher on self-incompatible than on self-compatible plants (e.g., Busch 2005). Unfortunately, studies on the effects of inbreeding depression in self-compatible *Primula* species are not available. Although the large seed sets produced by *P. boveana* after self-fertilization (Al Wadi and Richards 1993) suggest that this species is not strongly affected by inbreeding depression, it has been demonstrated that the presence of non-lethal, weakly deleterious mutations accumulated after long periods of inbreeding can produce detrimental effects even in predominantly self-fertilizing populations (Husband and Schemske 1996). Consequently, it is necessary to carry out experiments specifically aimed at determining the possible impact of inbreeding depression on the survival of *P. boveana*.

Since the negative effects of genetic erosion and inbreeding tend to operate in the long run, the recent and steep demographic decline observed in *P. boveana* is likely caused by environmental changes in the past few decades. Habitat deterioration as a consequence of global warming trends is a general threat for the survival not only of *P. boveana*, but also of other species endemic to the Sinai mountains (Hoyle and James 2005). Both temperatures and aridification are expected to increase in the Mediterranean region in the next decades (Alpert et al. 2008; Giorgi and Lionello 2008; Issar 2008), and predictive models forecast a high extirpation risk for species in mountains, especially

in arid areas (McCain and Colwell 2011). Less precipitation throughout the year would unavoidably reduce the volume of the water flows to which *P. boveana* is intimately linked, therefore reducing the number and size of habitat patches suitable for this species. Furthermore, rising human demands on the environment would aggravate the problem of water availability. Besides the direct effect of low water availability on plant survival, an increase in temperatures could definitely affect the flowering phenology of the species and further disrupt the already irregular pollination services (Root et al. 2003). Finally, the reduction of genetic variation and gene flow between populations might ultimately render selfing insufficient to offset the negative consequences of aridification.

One of the natural strategies that may buffer *P. boveana* against the risk of extinction is the build-up of seed banks (Moustafa et al. 2001; Zaghloul 2008), a strategy previously reported for other primroses (e.g., Milberg 1994, Shimono et al. 2006). Seed banks in arid habitats allow seeds to stay dormant in dry years and germinate when conditions are more favorable to growth and reproduction. In addition, seed banks can also act as reservoirs of genetic variation, thus delaying the loss of genetic variation and maintaining the evolutionary potential of populations (Zaghloul 2008). Therefore, as long as dry periods are interspersed by moister intervals, seed banks could buffer the genetic and demographic erosion of *P. boveana*. However, as explained above, both temperatures and aridification are expected to increase in the Sinai mountains in the very near future. Given our lack of detailed knowledge on the reproductive biology of *P. boveana*, its demographic dynamics and its ability to recover from seed banks, we recommend the adoption of swift, active measures to guarantee the conservation of this species in the short and long term. These measures, mainly focused on habitat preservation, should include a careful management of water resources in the region, restoration of the habitats potentially suitable for *P. boveana* and, if necessary, occasional artificial irrigation of the populations. In addition, fencing the populations would protect the plants from threats of lesser concern currently affecting *P. boveana*, such as sporadic collections for medicinal uses (González-Tejero et al. 2008) and grazing (H. Mansour, personal observation). Lastly, given the extreme rarity of this plant and the high genetic differentiation among its populations, these measures should be complemented by a thorough sampling of seeds from as many populations and individuals as possible (Holsinger and Gottlieb 1991). Part of these seeds should be incorporated in a germplasm bank for long-term, ex-situ conservation and eventual population reinforcements and reintroductions, whereas other seeds should be used to implement a breeding program aimed at determining the consequences of inbreeding depression in *P. boveana*. Because of the importance of the seed bank in preserving genetic diversity, soil samples should also be taken from the natural populations. Specifically, sampling the seed bank could be extremely useful in recovering the seemingly extinct populations in Wadi Garagniah and Kahf El-Ghoula and any potential genetic variation lost from living plants in the three other populations in the last years. As a whole, these measures would greatly aid in preventing the extinction of the emblematic Sinai primrose.

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