

Conservation genetics of an endemic from the Mediterranean Basin: high genetic differentiation but no genetic diversity loss from the last populations of the Sicilian Grape Hyacinth *Leopoldia gussonei*

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Abstract The Mediterranean Basin is a biodiversity hotspot, housing >11,000 narrowly endemic plant species, many of which are declining due to mass tourism and agricultural intensification. To investigate the genetic resource impacts of ongoing habitat loss and degradation, we characterized the genetic variation in the last known populations of *Leopoldia gussonei*, a self-compatible endangered Sicilian Grape Hyacinth numbering less than 3,000 remaining individuals, using AFLP. Results demonstrated significant genome-wide genetic differentiation among all extant populations ($\Phi_{ST} = 0.05\text{--}0.56$), and genetic clustering according to geographic location. Gene diversity was fairly constant across population (mean $H_E = 0.13$) and was neither affected by current population size nor by spatial isolation. Vegetation analysis showed the presence of known invasive weeds in a quarter of the populations, but we found no relation between genetic diversity and plant community composition. The marked genetic differences among populations and the profusion of rare and private alleles indicate that any further population loss will lead to significant losses of genetic diversity.

Conservation efforts should therefore focus on the preservation of all sites where *L. gussonei* still occurs, yet the deliberate introduction of diverse material into the smallest populations seems unneeded as clonality likely mitigated genetic drift effects thus far. More generally, our findings support the view that endemic plant species with a narrow ecological amplitude, as many specialists in Mediterranean coastal ecosystems, are highly genetically differentiated and that conservation of their genetic diversity requires preservation of most, if not all of their extant populations.

Keywords Mediterranean Basin · AFLP · Asparagaceae · Habitat loss · Habitat degradation · Narrow endemics

Introduction

The Mediterranean Basin is a biodiversity hotspot, harboring ~22,500 vascular plant species, which more than quadruples the number found in all the rest of Europe. More than half of these species are endemic to the region,

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of which most are narrow endemics confined to a small area, such as to any of the ~5,000 islands scattered throughout the Mediterranean Sea (Suc 1984; Greuter 1991; Dallman 1998; Médail and Quézel 1999). Deforestation over thousands of years has considerably altered the inland vegetation of this region and, over the last century, the development of mass tourism and agricultural intensification have also increasingly strained plant diversity in coastal areas, either directly through habitat clearance or indirectly through habitat degradation and the spread of exotic plant species. Today, a sheer 5 % of the Mediterranean Basin is covered by relatively unaffected vegetation. Many narrow endemics, which naturally comprise only a small number of individuals and populations, are currently endangered, if not already extinct (Greuter 1994; Médail and Quézel 1999).

An essential component of biodiversity is the genetic variation held within and among individuals and populations of a species. Its maintenance is crucial to sustain viable populations and to secure the evolutionary potential of the species (Reed and Frankham 2003; Jump et al. 2009). In habitat-specific plant species, habitat destruction and the associated loss of populations will immediately decrease the species' genetic variation (Young et al. 1996). The impact will be especially high when the initial number of populations was already low, as for endemic species, and if the genetic differences among populations are marked (Palop-Esteban et al. 2007). The remaining populations, bound to live in spatially isolated habitat remnants of often degraded quality, may lose genetic variation in a more gradual manner. Populations that remain small for subsequent generations, for instance because suboptimal habitat conditions continuously hamper sexual recruitment or survival (Vergeer et al. 2003; de Vere et al. 2009), are more likely to lose alleles through genetic drift than large populations (Wright 1938; Ellstrand and Elam 1993). When these small populations become spatially isolated due to the loss of nearby populations, gene inflow may no longer replenish the alleles lost and as generations pass, the magnitude of genetic variation will decrease (Ellstrand and Elam 1993; Young et al. 1996). This process, i.e. the accelerated loss of genetic variation due to anthropogenic habitat disturbance, is perceived to occur more rapidly in endemic plant species, which tend to have smaller initial effective population sizes (Gitzendanner and Soltis 2000; Cole 2003; but see Honnay and Jacquemyn 2007) and very specific habitat requirements.

Knowledge of the distribution of genetic variation and of the factors potentially undermining its maintenance is hence a critical step towards the adequate long-term *in situ* conservation of endangered endemic species (Haig 1998; Schoen and Brown 2001). Yet the conservation genetics of coastal Mediterranean endemics has received little

attention thus far (Palacios et al. 1999; Juan et al. 2004; Palop-Esteban et al. 2007). Here we investigate the conservation genetics of *Leopoldia gussonei* Parl. (Asparagaceae), a self-fertile Grape Hyacinth endemic to Sicily (Italy), whose current conservation status is well-representative for many narrow endemics in coastal areas of the Mediterranean Basin (Médail and Quézel 1999). *Leopoldia gussonei* is now restricted to eight fragmented populations along the Southern coast near Gela (Cundari et al. 2003; Brullo et al. 2011; Fig. 1) but used to be more widespread in the region (Albo 1919; Garbari and Di Martino 1972; Brullo and Marcenò 1974). Greenhouse agriculture and the mechanization of vineyard maintenance imperils the persistence of *L. gussonei*, both directly through habitat destruction, and indirectly through the degradation of adjacent habitats (Brullo et al. 2011). Other immediate threats include tourism infrastructure development, and grazing by feral livestock. A potential threat in the longer term is the invasion of exotic plant species such as the African Hottentot Fig (*Carpobrotus* sp.). As a consequence, the species is red-listed as endangered by the IUCN [B2ab(i,ii,iii,iv,v)] and listed as a priority taxon for conservation in the Habitat Directive 92/43 CEE and the Bern Convention. Seeds have been collected for *ex situ* conservation, yet *L. gussonei* is currently not included in regional or national protection laws or plans (Brullo et al. 2011).

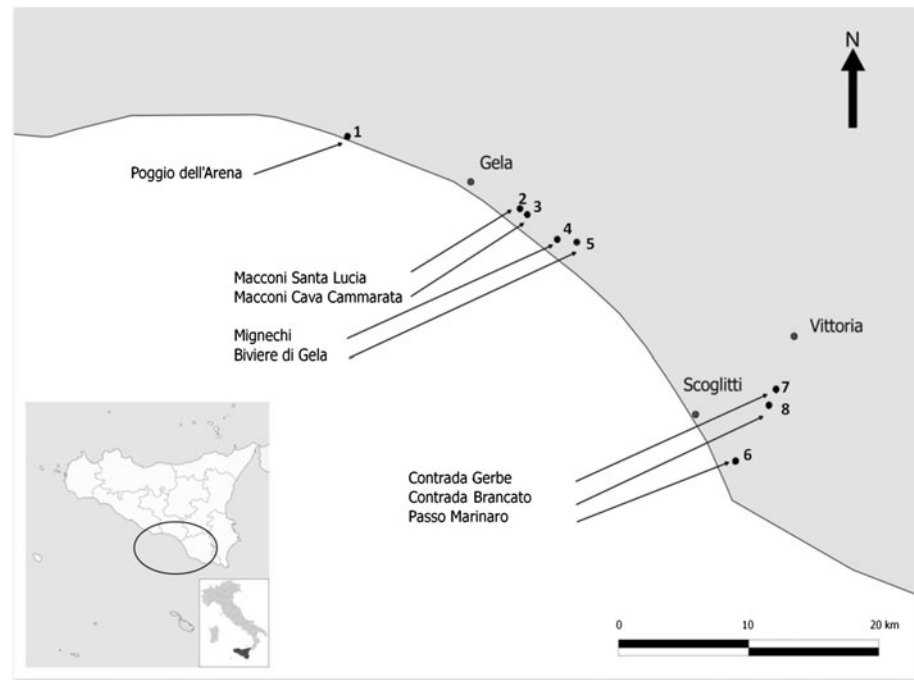
Using Amplified Fragment Length Polymorphism markers (AFLP; Vos et al. 1995), we analyzed all known extant *L. gussonei* populations. We specifically aimed to: (i) quantify the amount and distribution of genome-wide genetic variation in order to delineate conservation units; (ii) infer whether ongoing habitat loss and/or degradation are eroding the adaptive potential of the remaining populations by studying how genetic variation is affected by the size, spatial isolation and habitat quality of the populations; (iii) define conservation priorities.

Materials and methods

Species description

Leopoldia gussonei is a diploid ($2n = 2x = 18$), bulbous geophyte in the Asparagaceae family, subfamily Scilloideae. Leaves sprout from underground bulbs in early spring. The single or double stems are 10–30 cm tall and the upper halves of stems produce urn-shaped flowers. The infertile flowers at the top are blue-colored while the fertile flowers lower down are yellow and less ostentatious, as in the closely related *Muscari* species. Flowers bloom from March to May and set capsular fruits (Garbari and Di Martino 1972). The species is highly self-fertile. The

Fig. 1 Map of the distribution of *Leopoldia gussonei* along the Southern coast of Sicily (Italy)



fruiting success of bagged individuals in the field was ~80 %, comparable to the reproductive success of open-pollinated controls (unpublished data). Seeds have limited dispersal ability (Garbari and Di Martino 1972). Plants can also reproduce clonally through the fragmentation of bulbs (ASG pers. observ.).

This Hyacinth is currently restricted to an area of approximately 40 km² along the southern coast of Sicily (Fig. 1), which is a hot, semi-arid Mediterranean region. It occurs on coastal and inner sand dunes on stabilized substrate as a characteristic species of the unique *Vulpio-Leopoldietum gussonei* vegetation (order *Malcolmettalia*) (Brullo and Marcenò 1974).

Data collection

All eight extant *L. gussonei* populations, identified based on available records (Brullo and Marcenò 1974) and through field surveys in the region, were visited in spring 2011. Distances between populations ranged from 787 m to 50.26 km (mean: 18.60 km \pm 15.50). Population sizes were determined by estimating the number of flowering individuals. The area occupied by each *L. gussonei* population was measured using GPS. In order to characterize local habitat quality, all plant species were identified and their abundance (% cover) was visually estimated in two to three 10 \times 10 m plots per population.

For AFLP genotyping (Vos et al. 1995), we sampled leaves of 25–30 individuals per population, separated by >50 cm as to avoid the sampling the same genet (total sample size was 221 individuals). Plant material was dried

on silica and DNA was extracted using the Nucleospin DNA-extraction kit (Macherey–Nagel, Germany). *EcoRI* and *MseI* were used for DNA digestion. Each individual plant was fingerprinted with four primer combinations: *EcoRI*-ACGT (Hex) + *MseI* + CCAT, *EcoRI*-ACCG (Hex) + *MseI*-CCTG, *EcoRI*-ACGT (Hex) + *MseI* + CCGA and *EcoRI*-ACAG (Fam) + *MseI* + CCCC ('Fam' and 'Hex' correspond to the fluorochromes used for primer labelling). Fluorescently labeled AFLP-fragments were separated on an ABI3130xl sequencer on 50 cm capillaries (Applied Biosystems) and scored using GeneMapper 3.7 software (Applied Biosystems). For each primer combination, unambiguous markers were scored in the size range 50–450 bp [see Vandepitte et al. (2012) for details on the scoring procedure]. Five samples were duplicated from independent DNA extractions. After confirmation of reproducibility, the percentage of non-matching bands within pairs was 3.9 %, repeats were removed from the final binary dataset. Out of the 221 attempted individuals, 193 (87 %) produced scorable patterns for all four primer combinations and were retained in further data analysis.

Data analysis

The distribution of genetic variation was examined using the Analysis of molecular variance method (AMOVA; Excoffier et al. 1992; Huff et al. 1993) as implemented in GENALEX 6.41 (Peakall and Smouse 2006), which partitions total genetic variation based on binary distances within versus among populations. Significance of overall and of pairwise genetic differentiation (Φ_{ST}) was tested

using 9,999 permutations. To visualize the distribution of among-population genetic variation, we performed a Principal component analysis (PCA) on the pairwise matrix of Φ_{ST} .

We also computed population genetic parameters based on the estimation of allele frequencies from the observed AFLP-fragment frequencies using the Bayesian approach of Zhivotovsky (1999) in AFLPSURV (Vekemans 2002). The deduced allele frequencies, were used to quantify the gene diversity per population (H_E) and the magnitude of genetic differentiation between populations (F_{ST}) assuming different deviations from Hardy–Weinberg equilibrium ($F_{IS} = 0, 0.5$ and 1). Because H_E estimates for different values of F_{IS} were very similar ($R = 0.97$; $P < 0.01$) and because the species is self-fertile, we retained the values for $F_{IS} = 0.5$ for further analysis.

To further characterize the overall genetic structure, a model-based clustering method was applied using the software STRUCTURE 2.2.3. This Bayesian method identifies, loosely speaking, subgroups that have distinctive allele frequencies without making a priori groupings according to sampling locations (Pritchard et al. 2000). Portions of the genome of individuals are then probabilistically assigned to each of the inferred genetic clusters for a given value of K , the optimal number of clusters. We applied the admixture model for dominant data with 20^4 burn-ins, 20^4 iterations and 15 repetitions for each value of K , from $K = 1$ – 8 (the number of locations sampled). The use of the admixture model allows estimating the number of natural genetic clusters and has the ability to detect historical population admixture (Falush et al. 2007). The optimal number of clusters in the dataset was identified based on the evolution of the likelihood of the data $\text{Pr}(X|K)$ versus K using the method outlined in Evanno et al. (2005).

The potential negative impact of spatial isolation and habitat degradation on the magnitude of genetic variation was examined using linear regression models (e.g. Van-depitte et al. 2007). Dependent variables were gene

diversity (H_E) and the percentage of polymorphic loci (P). Explanatory variables were population size (log-transformed), spatial isolation quantified as the distance to the nearest population (DIST), and plant community composition. We used Detrended Correspondence Analysis (DCA) on the vegetation relevés to quantify the plant community composition of each population, as a proxy for local habitat quality (Hill and Gauch 1980). Cover values of the plant species were averaged across all plots for each population. The scores of each population on the first and second DCA axis (DCA1 and DCA2) were used as measures for plant community composition in the regression analysis. Forward selection ($P > 0.1$) was applied for model inclusion.

Results

Distribution of genetic variation

The four primer combinations used rendered a total of 105 polymorphic AFLP-bands. High-quality profiles were obtained for all four AFLP primer combinations in 193 *Leopoldia gussonei* individuals. The mean number of bands per individual was 40.51 ± 3.20 (range: 34–51). All populations exhibited rare alleles (i.e. bands present in maximal 2 populations), and six out of eight populations displayed private alleles (Table 1). The percentage of polymorphic loci (P) ranged between 38.10 and 62.86 (mean: 45.95 ± 2.83 SD), and H_E averaged 0.13 ± 0.02 SD (range: 0.10–0.16; Table 1).

AMOVA demonstrated that genetic differentiation among populations was highly significant ($\Phi_{ST} = 0.30$, $P < 0.0001$) and comparable to the F_{ST} -values based on allele frequencies instead of upon binary distances [$F_{ST(FIS=0)} = 0.21$, $F_{ST(FIS=0.5)} = 0.25$ and $F_{ST(FIS=1)} = 0.27$]. Pairwise Φ_{ST} -values were between 0.05 and 0.56 (Table 2). All

Table 1 Population genetic variation of *Leopoldia gussonei*

Location	ID	Area (m ²)	Size	<i>N</i>	Private alleles	Rare alleles	<i>P</i>	<i>H_E</i>
Poggio dell'Arena	1	4,810	1,000	28	1	3	39.05	0.10
Macconi Santa Lucia	2	858	100	24	0	2	38.10	0.11
Macconi Cava Cammarata	3	670	300	28	0	8	51.43	0.13
Mignechi	4	1,286	300	27	3	8	62.86	0.16
Biviere di Gela	5	231	50	15	3	5	43.81	0.14
Passo Marinaro	6	4,703	300	26	2	2	41.90	0.14
Contrada Brancato	7	1,546	500	24	2	5	44.76	0.12
Contrada Gerbe	8	571	50	21	6	7	45.71	0.12

Size: estimated as number of flowering individuals, *N* number of samples successfully genotyped with four AFLP primer combinations. Private alleles: unique bands, Rare alleles: bands present in <3 populations. *P*: percentage of polymorphic loci, H_E gene diversity for $F_{IS} = 0.5$

Table 2 Pairwise genetic differentiation (Φ_{ST}) between *Leopoldia gussonei* populations

ID	1	2	3	4	5	6	7
2	0.387**						
3	0.372**	0.114**					
4	0.354**	0.150**	0.110**				
5	0.392**	0.212**	0.168**	0.048*			
6	0.506**	0.316**	0.246**	0.222**	0.255**		
7	0.558**	0.331**	0.280**	0.264**	0.328**	0.195**	
8	0.547**	0.335**	0.279**	0.199**	0.240**	0.176**	0.205**

* $P < 0.01$, ** $P < 0.0001$

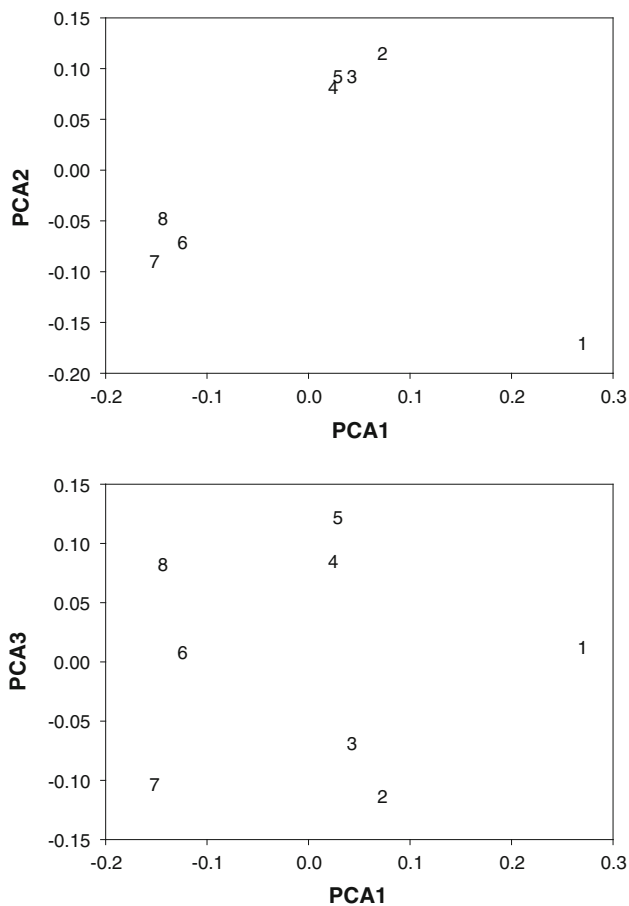


Fig. 2 Principal component analysis (PCA) plots based on pairwise genetic differentiation (Φ_{PT}) among all known *Leopoldia gussonei* populations. 1 Poggio dell'Arena, 2 Macconi Santa Lucia, 3 Macconi Cava Cammarata, 4 Mignechi, 5 Biviere di Gela, 6 Passo Marinaro, 7 Contrada Bracanto and 8 Contrada Gerbe, as in Fig. 1. The first two PCA axes (top) account for 38.92 and 22.60 % of the variance respectively. The third axis (bottom) explained 15.11 % of the variance

pairwise comparisons were significant ($P < 0.0001$). Nearby populations tended to cluster together in the PCA plot based on pairwise genetic differentiation (Fig. 3). The first two PCA axes, which explained 38.92 and 22.60 % of the variance respectively, subdivided the populations into

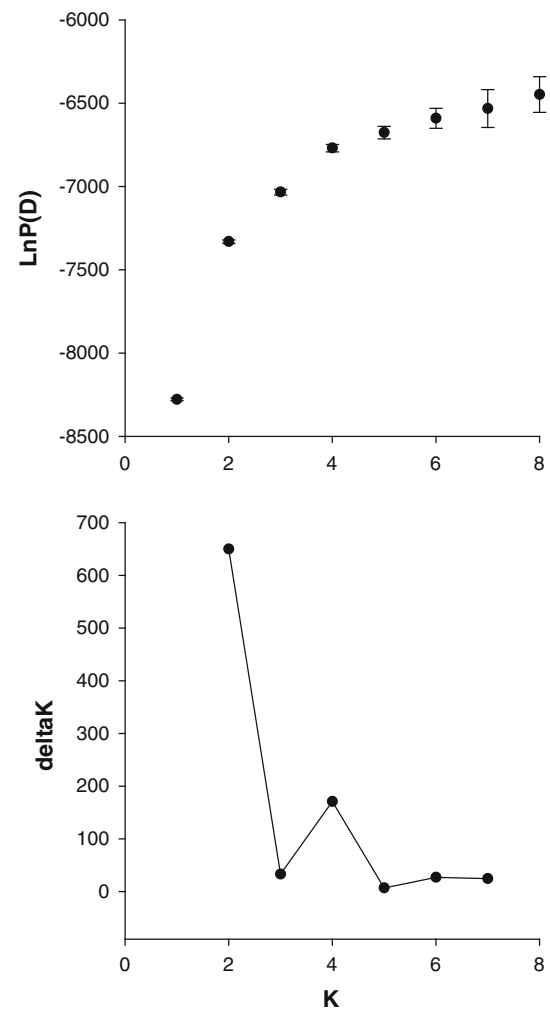
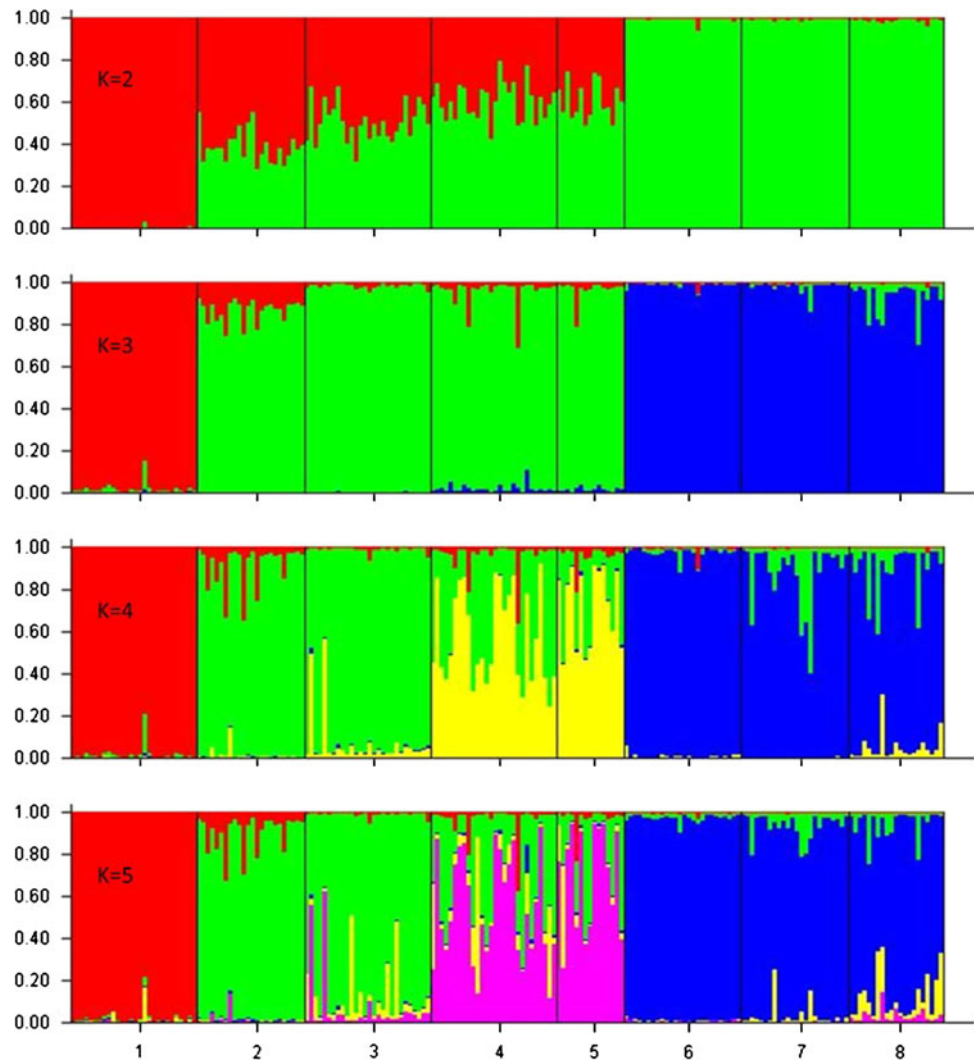


Fig. 3 Evolution of the STRUCTURE model likelihood vs. K (top) and K vs. ΔK (bottom), the second order rate of change, as outlined in Evanno et al. (2005)

three main clusters: the northernmost population (ID_1), the four central populations (ID_2 to ID_5) and the three southernmost populations (ID_6 to ID_8). The third axis, which still accounted for 15.11 % of the variance, subdivided the central populations.

Fig. 4 STRUCTURE barplots of individual assignment results for $K = 2$ to $K = 5$, based on AFLP data of *Leopoldia gussonei*. 1 Poggio dell'Arena, 2 Macconi Santa Lucia, 3 Macconi Cava Cammarata, 4 Mignechi, 5 Biviere di Gela, 6 Passo Marinaro, 7 Contrada Bracanto and 8 Contrada Gerbe, as in Fig. 1. Different colours represent distinct genetic clusters. K is the number of genetic clusters



In agreement with the PCA results (Fig. 2), STRUCTURE clustered individuals and populations mainly according to their geographic location and revealed the presence of four main genetic clusters. The model likelihood steeply increased until $K = 4$ and then evened out while the variance among runs increased (Fig. 3a), as is also indicated by the second order rate of change (Fig. 3b). Assignment results for $K = 4$ showed that individuals from the isolated population *Poggio dell'Arena* (ID_1 in Fig. 1) in the north of the species' distribution range, were assigned to a distinct genetic cluster (Fig. 4). A second main cluster essentially comprised the central populations *Macconi Santa Lucia* (ID_2), *Macconi Cava Cammarata* (ID_3) and part of the genomes of individuals collected at *Mignechi* (ID_4) and *Biviere di Gela* (ID_5), located a little more to the south. The *Mignechi* (ID_4) and *Biviere di Gela* (ID_5) individuals were further assigned to a separate third genetic cluster, while individuals collected at the three southernmost sites, *Passo Marinaro* (ID_6), *Contrada Gerbe*

(ID_7) and *Contrada Bracanto* (ID_8) were mostly allocated to a fourth cluster. The assignment of individuals to three instead of four genetic clusters ($K = 3$) assigned the four central populations to a single cluster while the allocation of individuals to more than four clusters rendered patterns comparable to $K = 4$ (Fig. 4).

Impact of habitat quality and fragmentation on population genetic variation

Detrended correspondence analysis (DCA) showed strong differences in plant community composition between the most spatially isolated populations *Poggio dell'Arena* (ID_1) and *Passo Marinaro* (ID_6) and the other six populations (Fig. 5). Common species were annuals and grasses characteristic of stable coastal dunes communities (order *Malcolmietalia*) such as *Erodium laciniatum* (Cav.) Willd., (Geraniaceae); Brassicaceae like *Maresia nana* (DC.) Batt. and *Brassica tournefortii* Gouan; Poaceae

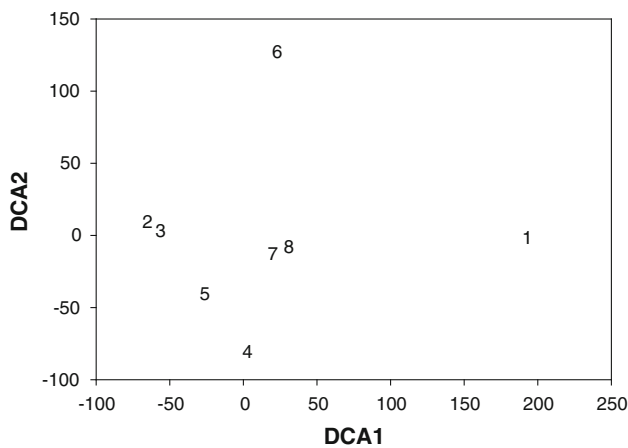


Fig. 5 Community composition of all known *Leopoldia gussonei* populations based on a Detrended correspondence analysis (DCA) of the species abundance data. 1 Poggio dell'Arena, 2 Macconi Santa Lucia, 3 Macconi Cava Cammarata, 4 Mignechi, 5 Biviere di Gela, 6 Passo Marinaro, 7 Contrada Bracanto and 8 Contrada Gerbe, as in Fig. 1

(*Lagurus ovatus* L., *Cutandia divaricata* (Desf.) Benth., *Anisantha rigida* (Roth) Hyl., *Vulpia fasciculata* (Forssk.) Fritsch and Fabaceae (*Ononis diffusa* Ten., *Lotus halophilus* Boiss. & Spruner, *Medicago littoralis* Rohde ex Loisel., *Ononis variegata* L., and *Coronilla repanda* Boiss.). The African Hottentot Fig (*Carpobrotus* sp. pl.) covered 4 % of the centrally located MCC population. Wild sugarcane (*Saccharum spontaneum* subsp. *aegyptiacum* (Willd.) Hack.) originating from Asia but spreading into many natural habitats around the world (USDA, NRCS 2010), was recorded at low densities (<2 %) in *Macconi Cava Cammarata* (ID_3) and *Biviere di Gela* (ID_5).

Populations were generally small (mean: 325 ± 314 SD). The largest population *Poggio dell'Arena* (ID_1) was estimated 1,000 individuals and the two smallest populations *Biviere di Gela* (ID_5) and *Contrada Gerbe* (ID_7) were below 100 individuals. There were no significant relationships between the magnitude of within-population genetic variation (P or H_E) and population size (log-transformed), plant density, the distance to the nearest population as a proxy of spatial isolation (ISO), or the first two DCA axes (all $P > 0.1$). DCA1 was strongly correlated to ISO (Pearson-R: 0.96, $P < 0.01$).

Discussion

Leopoldia gussonei is an endangered Grape Hyacinth found in small fragmented populations along the south-western coast of Sicily. Historical records support the loss of populations over the last century, mostly from sites along the southeastern coast where the species went extinct (Albo 1919; Brullo and Marcenò 1974). In all, less than

3,000 individuals remain (Table 1), distributed over a small number of populations in three main areas (Fig. 1). The decaying habitat quality of sites where *L. gussonei* still occurs is well-representative of the current poor state of many coastal ecosystems in the Mediterranean Basin (Médail and Quézel 1999). Extant populations are located in the direct vicinity of tourism infrastructure (*Poggio dell'Arena*), greenhouse agriculture (*Mignechi*, *Contrada Bracanto*, *Contrada Gerbe*) and an industrial site (*Macconi Santa Lucia* and *Macconi Cava Cammarata*) and, one quarter of the extant populations faces the immediate threat of encroachment by the African Hottentot Fig (*Carpobrotus* sp) and/or Wild Sugarcane (*Saccharum spontaneum*), two known invasive weeds (Weber and D'Antonio 1999; USDA, NRCS 2010). Our results therefore provide valuable insights into genetic conservation requirements in light of the critical status of *L. gussonei* in particular, and other endangered endemic plant species in the coastal landscapes of the Mediterranean Basin more in general.

Results demonstrated overall low within-population genetic variation, and significant genetic differentiation among populations and among the three main geographic locations. Within-population genome-wide genetic variation, in terms of polymorphism and gene diversity (mean $P = 45.95$ %, mean $H_E = 0.13$; Table 1), was fairly constant across populations and not affected by current population size, local plant community composition or degree of spatial isolation, suggesting no losses of genetic diversity from small, isolated or degraded populations (Ellstrand and Elam 1993). Overall, levels of genetic diversity were somewhat on the lower bound of values reported for endemic plant species using dominant markers [mean $H_E = 0.20$ in Nybom (2004)]. In agreement with the many rare or even private bands the populations contain, among-population genetic differentiation was significant ($\Phi_{ST} = 0.30$; $F_{ST} = 0.21$ – 0.27 , Table 2) and somewhat higher than the value reported by Nybom (2004) for endemic species analyzed using dominant markers ($\Phi_{ST} = 0.26$ and mean $F_{ST} = 0.18$), and most other recent AFLP based studies of endemic plant species [e.g. *Medicago citrina* (Font Quer) Greuter, $\Phi_{ST} = 0.20$ (Juan et al. 2004); *Scalesia affinis* Hook.f., $\Phi_{ST} = 0.49$ (Nielsen 2004); *Myricaria laxiflora* (Franch.) P.Y.Zhang & Y.J.Zhang, $\Phi_{ST} = 0.45$ (Liu et al. 2006); *Astragalus albens* Greene, $\Phi_{ST} = 0.01$ (Neel 2008); *Astragalus ampullarioides* (S.L.Welsh) S.L.Welsh, $\Phi_{ST} = 0.18$ (Breinholt et al. 2009); *Hieracium eriophorum* Lapeyr., $\Phi_{ST} = 0.09$ (Frey et al. 2012)]. At the higher spatial level, individuals cluster according to geographic location (Fig. 2, 4).

The low within-population genetic variation and the high among-population genetic differentiation most likely primarily result from self-pollination in combination with natural rarity; characteristics that decrease effective

population sizes and reduce among-population gene flow (Hamrick and Godt 1996). As the habitat patches that fulfill the environmental requirements of the species are naturally fragmented (Brullo and Marcenò 1974), we can assume that the current fragmented distribution of *L. gussonei* is at least partially natural (Fig. 1). This is supported by the genetic clustering of individuals according to their geographic location (Figs. 3, 4). These results corroborate the view that most endemic plant species are characterized by a high degree of among-population differentiation and low levels of within-population genetic variation, relative to widespread species (reviewed in Hamrick and Godt 1996; Gitzendanner and Soltis 2000; Cole 2003; Nybom 2004, but see e.g. Crema et al. 2009). This difference is generally perceived to arise from the narrow ecological amplitude and associated fragmented distribution of many endemic plant species, and restricted gene flow among populations (Cole 2003).

Nevertheless, the marked genetic differences among the few *L. gussonei* populations that still remain and the profusion of rare and private alleles highlight that any further population loss, even of the small-sized populations, will lead to significant losses of genetic diversity. The loss of genetic diversity will be particularly evident if any of the main four genetic clusters (Fig. 4), which likely represent different evolutionary legacies of the species, goes extinct (Waples 1995; Segarra-Moragues and Catalán 2010). Management actions should therefore focus on the conservation of all main sites where *L. gussonei* still occurs. Active conservation includes monitoring and elimination of spreading exotic plant species, the containment of greenhouse agriculture and the use of pesticides, and the prevention of habitat disturbance and nutrient pollution. Additional management actions such as the interchange of genetic material among extant populations seems currently unneeded as there were no indications of accelerating losses of genetic variation or decreasing population viability. Seed viability in the populations was found to be >80 % and varied very little among populations (unpublished data). Due to the persistence of old established genotypes through clonal fragmentation of bulbs, long overlapping generation times may have tempered the impact of genetic drift in the smallest populations of this perennial clonal herb and, to some extent, have precluded severe population differentiation (Luijten et al. 2000; Honnay et al. 2006).

Coastal ecosystems harbour many endemic plants (van der Maarel and van der Maarel-Versluys 1996), of which many are rare specialists of any specific successive stages in the course of dune formation (vanden Berghen 1964; Bakker 1976; Géhu and Franck 1985). A high degree of among-population differentiation and low levels of within-population genetic variation may be at least partially

natural in these species due to their high habitat specificity. Our findings of pronounced genetic structuring, and the concomitant considerable conservation genetic consequences of further population loss, may therefore apply to a wide range of endangered rare endemic plant species in coastal ecosystems of the Mediterranean Basin, as seems also supported by the few adequate population genetic studies available (e.g. Palacios et al. 1999; Juan et al. 2004). Whether or not these populations of endemics are also susceptible to gradual loss of genetic variation due to genetic drift in declining populations can be expected to be mediated by the species' plant traits (Leimu et al. 2006; Aguilar et al. 2008). As demonstrated before, we can assume that clonality and longevity likely mitigate loss of genetic variation from these small populations.

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