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Conservation implications of high genetic variation in two closely related and highly threatened species of *Crambe* (Brassicaceae) endemic to the island of Gran Canaria: *C. tamadabensis* and *C. pritzelii*

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We used data from 12 allozyme loci for two endemic Brassicaceae from Gran Canaria (the endangered narrow endemic *Crambe tamadabensis* and its more widespread congener *C. pritzelii*) to assess whether their genetic diversity patterns reflect their phylogenetic closeness and contrasting population sizes and distribution areas, and to derive conservation implications. Genetic diversity values are high for both species and slightly higher in *C. tamadabensis*, despite its narrow distribution in north-western Gran Canaria. At odds with the generally high interpopulation diversity levels reported in Canarian endemics, values of G_{ST} in *C. tamadabensis* and *C. pritzelii* are rather low (0.067 and 0.126, respectively). We construe that the higher genetic structure detected in *C. pritzelii* is mainly a result of unbalanced allele frequencies and low population sizes at the edges of its distribution. The overall high allozyme variation detected in *C. tamadabensis* and *C. pritzelii* is nevertheless compatible with an incipient but consistent genetic differentiation between the two species, modulated by recurrent bottlenecks caused by grazing and drift. Our data suggest that conservation efforts aimed at maintaining the existing genetic connectivity in each species and *ex situ* conservation of seeds are the best strategies to conserve their genetic diversity. © 2016 The Linnean Society of London, *Botanical Journal of the Linnean Society*, 2016, **182**, 152–168

ADDITIONAL KEYWORDS: allozymes – gene flow – genetic differentiation – genetic structure.

INTRODUCTION

Island species are considered to be more prone to extinction and to have lower levels of genetic diversity than their continental congeners, mainly as a result of genetic drift and inbreeding fostered by founder effects and, in many cases, limited gene flow (Ellstrand & Elland, 1993; Frankham, 1997, 1998). The general assumption of low genetic diversity in insular taxa and populations dates back to Stebbins (1942), and the low neutral genetic variation levels often reported for insular endemics are considered to be a collateral effect of rarity (Barrett & Kohn, 1991; Frankham, 1997). Since the seminal allozyme studies on endemics of oceanic archipelagos (e.g. Crawford, Stuessy & Silva, 1987a; DeJoode & Wendel, 1992), it has generally been assumed that rare and insular species hold overall lower levels of genetic variation than common continental species (Karron, 1987; Hamrick & Godt, 1989) and show a high genetic identity with their insular congeners, despite clear morphological and ecological differences (Crawford *et al.*, 2006). Another general tenet derived from allozyme studies of oceanic island species is that most genetic diversity is explained by differences among populations (DeJoode & Wendel, 1992; Francisco-Ortega *et al.*, 2000; Crawford *et al.*, 2001).

Nevertheless, as first noted by Stebbins (1980), allozyme studies of some rare species have revealed levels of variability similar to those of their widespread congeners (e.g. Lewis & Crawford, 1995; Smith & Pham, 1996; Young & Brown, 1996). More

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recently, Gitzendanner & Soltis (2000) have suggested that the view that rare species have less genetic variability than more widespread species may be an over-generalization and that phylogenetic history must be accounted for in population genetic comparisons, rather than using phylogenetically independent or distant taxa.

Ongoing investigations by our group seem to confirm that insular endemics do not necessarily have less genetic variation than their mainland congeners (see García-Verdugo *et al.*, 2014). Furthermore, the general hypothesis that interpopulation genetic variation is much higher in island endemics is at odds with the finding of low interpopulation genetic diversity in species featuring high genetic diversity levels. An investigation of Canarian endemics (Caujapé-Castells, 2010) has strongly suggested that the detection of high levels of genetic fragmentation, as measured by G_{ST} (Nei, 1973) or F_{ST} (Wright, 1951), may be strongly influenced by a spatially non-representative intrapopulation sampling.

Crambe L. section *Dendrocrambe* DC. forms a monophyletic group of 14 species (Francisco-Ortega *et al.*, 2002; Prina & Martínez-Laborde, 2008) endemic to the Canarian and Madeiran archipelagos (nearly all species are single island endemics). *Crambe tamadabensis* A.Prina & Marrero Rodr. and *C. pritzelii* Bolle are two closely related endemics to the island of Gran Canaria which, with *C. santosii* Bramwell, *C. strigosa* L'Hér. and *C. wildpretii* Prina & Bramwell (from La Palma, Tenerife and La Gomera, respectively), formed a monophyletic (albeit poorly resolved) crown group in a phylogenetic analysis of the genus based on internal transcribed spacer (ITS) (Francisco-Ortega *et al.*, 2002). *Crambe tamadabensis* has been described recently (Prina & Marrero, 2001) from vouchers that had been previously ascribed to *C. pritzelii*. It is confined to a few populations in north-western Gran Canaria, whereas *C. pritzelii* consists of fragmented populations widespread throughout the north-eastern half of the island (Fig. 1).

Northern Gran Canaria has been historically much more populated by humans than the southern part, and has suffered many changes in land use that have had a severe negative impact on its vegetation (Kämmer, 1979; Aguilera *et al.*, 1994). At present, most of the original thermosclerophyllous woodlands in which *C. pritzelii* and *C. tamadabensis* occur only exist in the form of secondary vegetation patches (if at all), and some populations of *C. pritzelii* known since the 19th century are nowadays probably extinct (Soto, 2016). Thus, it appears that the main reasons for the historical decline of these *Crambe* spp. in Gran Canaria are changes in land use associated with agricultural expansion and overgrazing; these plants are among the most palatable to goats and some of the first to disappear in the accessible parts of a community grazed by these animals (Marrero & Navarro, 2003; Santana, Naranjo & Soto, 2009). Thus, although some populations have attained a considerable census size in parallel with the decrease in domestic cattle in recent decades, others remain small, most probably because of the persistence of goats and rabbits in their distribution areas (Soto, 2016).

In this investigation, we provide the first exhaustive assessment of the levels and distribution of allozyme genetic diversity in a comprehensive sample of *C. tamadabensis* and *C. pritzelii* with two objectives. First, we explore whether these neutral genetic markers detect population genetic differences between these two phylogenetically close *Crambe* spp., or whether their incipient evolutionary divergence is only manifested by morphological differences and putative phenological barriers. Second, we aim to put forward conservation guidelines consistent with our population genetic findings.

MATERIAL AND METHODS

Plant material

Crambe pritzelii [$2n = 30$ (Ortega & Navarro, 1977), with basic chromosome number $x = 15$ (Warwick & Al-Shehbaz, 2006)] is a woody shrub with *c.* 15 known populations in Gran Canaria and an estimated census size of *c.* 69 000 individuals overall (Santana *et al.*, 2009). It can reach > 1.5 m in height and possesses dry and spiny stems, large and rough lanceolate-elliptical leaves with dentate, pointed edges. It has paniculate and profusely ramified inflorescences with small, white flowers; the fruit is a small silicula with a tetragonous lower half and an ovate, elliptical and apiculate upper half. It inhabits basaltic, phonolitic and traquitic soils in shady places between 200 and 1500 m a.s.l. in north-western, northern and eastern Gran Canaria, always facing the humid north-eastern trade winds. It is found on deep and steep soils where it may become large. It is endangered [EN B1ab(iii,v) + 2ab(iii,v); IUCN, 2015].

Crambe tamadabensis has four known populations in north-western Gran Canaria, with an estimated census size of 13 400 individuals overall (Table 1). It is a microphanerophyte with glabrous stems and obovate-lanceolate, almost glabrous, leaves, wich are smaller than *C. pritzelii*'s. The inflorescences are glabrous and graceful with smaller flowers and fruits (the latter also more apiculate) than *C. pritzelii*. It appears to prefer rupiculous habitats, exclusively related to phonolitic and traquitic rocks of the shield stage in

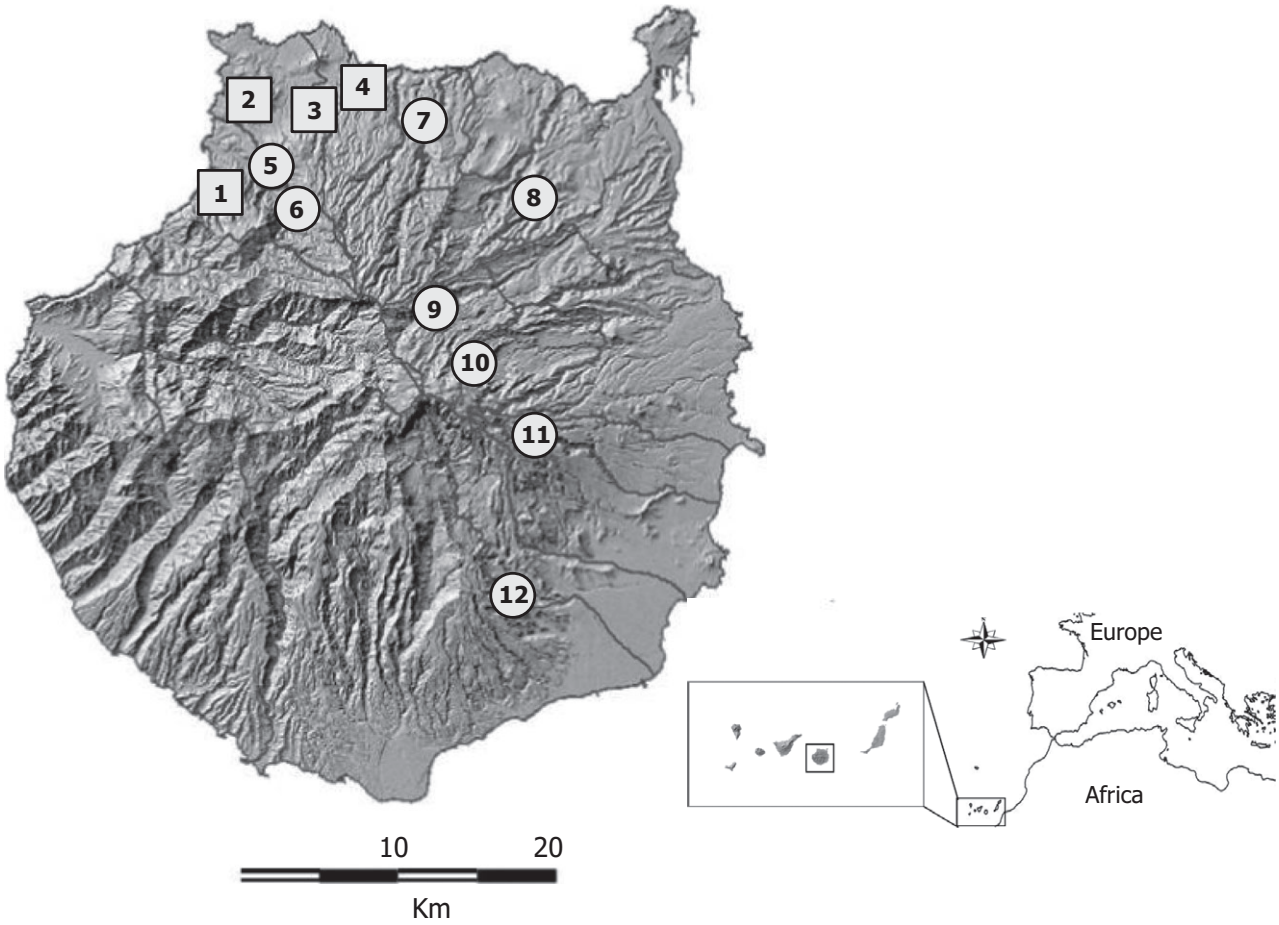

Figure 1. Sample locations of the four known populations of *Crambe tamadabensis* (all sampled for this investigation) (squares) and of the eight sampled populations of *C. pritzelii* (circles) that cover its known distribution area on Gran Canaria. Numbers correspond to those in Table 1.

north-western Gran Canaria, with estimated ages of 13.4–9.7 Myr (Prina & Marrero, 2001). It grows on shady and partially sunny slopes facing north, west, south-west and east, between 250 and 1000 m a.s.l. It is critically endangered [CR B1ab(iii) + 2ab(iii); IUCN, 2015].

Although no reproductive biology studies exist for either species, some experimental data show auto-incompatibility in their congener *C. tatarica* Willd. (East, 1940; Fryxell, 1957), whereas Scott & Randall (1976) reported both outcrossing and selfing in *C. maritima* L. Moreover, high auto-incompatibility has been shown in the Canarian endemic *C. arborea* Webb ex Christ (Calero & Santos, 1988). The fact that a homomorphic sporophytic self-incompatibility system has been reported for many Brassicaceae (Gibbs, 1986, 1988; Barrett, 1988; Byers & Meagher, 1992; Richards, 1997) leads us to consider that both *C. tamadabensis* and *C. pritzelii* are probably predominantly outcrossers.

Sampling

Leaf buds of 721 individuals were collected from the only four known populations of *C. tamadabensis* and from eight natural populations of *C. pritzelii* (Fig. 1, Table 1). Whilst sampling these, we found that one population previously ascribed to *C. pritzelii* fits morphologically with *C. tamadabensis*, and so we considered it as a population of the latter species in subsequent analyses (CTSI in Table 1, in the north-western sector of the island where both species overlap).

Sampling was always preceded by a thorough inspection of plant distribution in each population and was carried out along transects that covered their whole estimated occupancy area, following Caujapé-Castells (2006). In small or homogeneous populations, a single transect was made that covered the whole plant occupancy area; in large and complex populations, different transects were considered.

Table 1. Names, codes and universal transverse Mercator (UTM) coordinates of the sampling locations, with the estimated reproductive individuals (*N*) and the number of individuals sampled per population (*n*) of *Crambe tamadabensis* and *C. pritzelii*

Species/population	Code	UTM	*N*	*n*	Voucher
Crambe tamadabensis					
1. Tamadaba	CTTA	28RDS3003	12 000	84	LPA33086
2. Montaña Amagro	CTAM	28RDS3311	300	32	LPA20074
3. Montaña de Guía	CTGU	28RDS3710	700	41	LPA33088
4. Cuesta de Silva	CTSI	28RDS4012	400	29	LPA33091
Crambe pritzelii					
5. San Pedro – Agaete	CPAG2	28RDS3406	625	39	LPA33094
6. Berrazales – Agaete	CPAG1	28RDS3504	1225	54	LPA19943
7. Azuaje	CPAZ	28RDS4309	3000	95	LPA33471
8. Riscos Jiménez	CPJI	28RDS4906	300	33	LPA19944
9. Antona	CPAN	28RDR4598	600	37	LPA33472
10. Tenteniguada	CPTE	28RDR4794	8425	92	LPA10804
11. Guayadeque	CPGY	28RDR5189	49 525	94	LPA10184
12. El Gallego – Amurga	CPGA	28RDR4880	1800	91	LPA10168

Sample sizes ranged from 29 to 95 individuals, depending on the estimated population sizes (Table 1). Individual samples were placed into zippered plastic bags and stored in a portable cooler until being deposited at −80 °C in the molecular facilities of the Jardín Botánico Canario 'Viera y Clavijo'-Unidad Asociada al Consejo Superior de Investigaciones Científicas (JBCVC-CSIC), where they remained until further use. Representative voucher specimens have been deposited in the Herbarium LPA, at the JBCVC-CSIC (Table 1, Appendix 1).

Electrophoretic analyses

For each individual sample, a small piece of fresh leaf was ground with a pestle in a glass mortar, using 500 μL of an extraction buffer adequate for the preservation of enzymatic activity (after Shields, Orton & Stuber, 1983). The extracts obtained were absorbed on 4-mm Whatman No. 3 filter paper wicks (Sigma-Aldrich Quimica, Madrid, Spain) that were stored at −80 °C until electrophoretic analysis. Of 12 tested enzymes, eight produced clear, interpretable bands for at least one of the three gel/electrode systems assayed on 12.5% starch gel electrophoresis. Histidine 7.0 (system E in Shields *et al.*, 1983) resolved *phosphoglucomutase* (PGM, EC 5.4.2.2), *phosphoglucoisomerase* (PGI, EC 5.3.1.9) and *esterase* (EST, EC 3.1.1.1). Morpholine-citrate 6.1 (Clayton & Tretiak, 1972) resolved *isocitrate dehydrogenase* (IDH, EC 1.1.1.42), *menadione reductase* (MNR, EC 1.6.99.2) and *malate dehydrogenase* (MDH, EC 1.1.1.37). Lithium borate 8.3 (system C in Shields *et al.*, 1983) resolved *glutamate-oxalacetate-transaminase* (GOT, EC 2.6.1.1.) and *malic enzyme* (ME, EC 1.1.1.40). Staining recipes were based on Arús (1983), Murphy *et al.* (1996) and Wendel & Weeden (1989), with slight modifications in substrate amounts and final pH to enhance band resolution.

Data interpretation

Twelve interpretable loci were scored (*Est-1*, *Got-1*, *Got-2*, *Idh-1*, *Mdh-1*, *Mdh-2*, *Mdh-3*, *Me-1*, *Mnr-1*, *Pgi-1*, *Pgm-1* and *Pgm-2*). For each enzyme, the loci and their associated alleles were labelled following the numeric and alphabetic code (respectively), beginning from the most anodal electromorph. Checking of allele mobilities was carried out by side-to-side comparisons of different electromorphs on the same gel. The number and intensity of bands for almost all cases agreed with the expected quaternary structures of the corresponding enzymes and with the hypothesis of Mendelian co-dominance (Wendel & Weeden, 1989). Therefore, banding patterns were interpreted according to standard practice of Mendelian inheritance for diploid plants. Nevertheless, some enzymes exhibited indirect evidence of duplications, such as: (1) the detection of more loci than expected in PGM [three instead of two (see Soltis, Soltis & Gottlieb, 1987; Wendel & Weeden, 1989; Kephart, 1990)]; (2) the appearance of several unbalanced heterozygous patterns for loci *Est-1*, *Idh-1*, *Mdh-1*, *Mdh-3*, *Mnr-1*, *Pgm-1* and *Pgm-2*; (3) the detection of more electromorphs than expected in a few individuals for *Got-1*, *Got-2*, *Mdh-1* and *Mdh-3*; and (4) the existence of 'ghost bands' or weak electromorphs in *Est-1*, *Idh-1* and *Mdh-3* that might correspond to old duplications in the process of silencing

(Kephart, 1990; Anderson & Warwick, 1999; Williamson & Werth, 1999). On the whole, however, these variations represented < 5% of the samples subjected to analysis and are therefore unlikely to distort the interpretations in accordance with the previously mentioned assumptions.

The resulting genotype matrix was imported to Transformer-4 (T4; Caujapé-Castells *et al.*, 2013) to produce the input files needed to run most of the software programs used for data analyses. The georeferenced genotype matrix used in this article and other relevant information can be found in the genetic diversity digest coded D-ALLOZ-100 (Soto, 2015) in the Demiurge information system (http://www.demiurge-project.org/matrix_digests/100).

Data analysis

Hardy–Weinberg equilibrium and Ewens–Watterson (Watterson, 1978) neutrality tests per locus and population, the number of alleles per locus (A_l), effective number of alleles per locus (A_e), percentage of polymorphic loci (P) [0.95 criterion], observed and expected heterozygosities (H_o and H_e; Levene, 1949), mean fixation index (F_{IS}; Wright, 1978) for all polymorphic loci, Nei's (1978) pair-wise unbiased genetic identities and distances among populations, and Nei's (1987) F-statistics were obtained using Popgene 1.32 (Yeh *et al.*, 1997). Nei's (1973) G_{ST} was calculated using FSTAT version 2.9.3.2 (Goudet, 2002). To estimate gene flow, we used the method of private alleles developed by Slatkin (1985) as implemented in Genepop (Raymond & Rousset, 1995), and Wright's (1951) method as implemented in Popgene 1.32 (Yeh *et al.*, 1997).

We applied the test proposed by Cornuet & Luikart (1996) to detect recent historical bottlenecks using the software Bottleneck-PC (Piry, Luikart & Cornuet, 1998) under the independent allele model (IAM). The rationale of this test is that, as the allele number is reduced more rapidly than gene diversity in a population that has experienced a recent reduction in its effective size, the observed gene diversity (H_e) will be higher than the expected equilibrium gene diversity (H_{eq}) under the test assumptions (Luikart & Cornuet, 1998).

Allele frequencies were subjected to a principal component analysis (PCA) using the software XLSTAT version 7.5.2 (XLSTAT, 2004). We also evaluated the possible influence of 'isolation by distance' (IBD) on interpopulation differentiation through a Mantel (1967) test between genetic and geographical distance matrices, using the program NTSYS-pc version 2.02j (Rohlf, 1998). We carried out an analysis of molecular variance (AMOVA) with Arlequin v. 2.0 (Schneider, Roessli & Excoffier, 2000) to explore the degree and significance of population genetic structure between the two taxa; significance levels were obtained by nonparametric permutations using 16 000 replicates, as suggested by Schneider *et al.* (2000).

To determine the number of genetically distinguishable clusters in the sampled populations, we applied the Bayesian approach implemented in STRUCTURE 2.3.1 (Pritchard, Stephens & Donnelly, 2000), which estimates the likelihood of the individuals being structured in a given number of groups (K) in the absence of previous population information, and provides the proportion of membership (q) of each individual in a given genetic cluster. The program was run ten times from $K = 1$ to $K = 12$; each run consisted of 100 000 iterations of burn-in followed by 1 000 000 Monte Carlo Markov chain (MCMC) iterations. The true value of K was estimated by the maximum value of ΔK, following the method in Evanno, Regnaut & Goudet (2005). The admixture ancestry model with correlated allele frequencies (Falush, Stephens & Pritchard, 2003) was selected as the most appropriate option for the analysis, as it is considerably flexible and often improves clustering for closely related populations (Pritchard, Wen & Falush, 2010), such as those of *C. tamadabensis* and *C. pritzelii*. We also activated the LOCPRIOR model (Hubisz *et al.*, 2009), which uses sampling locations as prior information to improve clustering in datasets where the signal of structure may be relatively weak.

To explore the boundaries of genetic differentiation among populations and to compare these with the STRUCTURE results, we used the software BARRIER version 2.2 (Manni, Guérard & Heyer, 2004), which maps genetic barriers using Delaunai's triangulation (Brassel & Reif, 1979) and Monmonier's (1973) algorithm. To assign a barrier, this algorithm applies the 'maximum difference' criterion of the given distance measure among the edges of neighbouring populations and iterates the process across adjacent edges until a boundary is formed (Manni & Guérard, 2004). We used Nei's (1978) unbiased genetic distance matrix between all population pairs, and we chose the 'virtual points' option to avoid the detection of false barriers caused by genetic distances among remote populations (see Manni & Guérard, 2004). The robustness of the computed barriers was assessed with 100 bootstrap replicates prior to barrier validation, following Manni & Guérard (2004). Only barriers supported by bootstrap values higher than 50% were considered.

RESULTS

Fifty-five alleles were scored for the 12 interpreted loci (see Appendix 2). Only two loci (*Me-1* and

Mdh-2) were monomorphic throughout the 12 populations. Eight of the alleles in polymorphic loci were exclusive to *C. tamadabensis* (*Idh-1c*, *Mnr-1c*, *Mnr-1d*, *Mdh-1b*, *Mdh-3d*, *Mdh-3f*, *Got-1a*, *Got-1d*) and eight were exclusive to *C. pritzelii* (*Pgm-1c*, *Pgi-1a*, *Pgi-1f*, *Est-1a*, *Est-1b*, *Mdh-3b*, *Got-1f*, *Got-2b*). No private alleles were either monomorphic or present in all populations of the corresponding taxa; therefore, they are of no diagnostic value.

Overall, high levels of genetic diversity were detected across all populations of the two species (Table 2), with the populations of Tamadaba (CTTA) (A_l = 3.4; P = 83.3; H_o = 0.320; H_e = 0.411) and Tenteniguada (CPTE) (A_l = 3.4; P = 75.0; H_o = 0.329; H_e = 0.397) showing the highest allozyme diversity values. The lowest genetic variation was detected in population 'Riscos Jiménez' of *C. pritzelii* (A_l = 1.9; P = 41.7; H_o = 0.178; H_e = 0.168). On average, *C. tamadabensis* displayed higher genetic variation values (A_l = 2.9; P = 81.2; H_o = 0.298; H_e = 0.401) than *C. pritzelii* (A_l = 2.7; P = 66.7; H_o = 0.259; H_e = 0.322), despite its much more restricted distribution. All populations of *C. tamadabensis* and three populations of *C. pritzeli* showed evidence of a recent bottleneck according to the test of Cornuet & Luikart (1996) (Table 2). The fixation index (F_{IS}) was slightly higher than zero in both taxa (0.237 and 0.192 for *C. tamadabensis* and *C. pritzelii*, respectively), indicating a heterozygote deficiency. Consistently, deviations of Hardy–Weinberg proportions were detected in some populations (data not shown). All loci could be considered neutral according to Ewens–Watterson tests (data not shown).

The five most variable populations of *C. pritzelii* formed a distinctive group differentiated from the least variable ones (CPJI and CPGA) on the scatter diagram defined by the first two PCA axes. These two components explained 54.63% of the variance among populations (Fig. 2). All populations of *C. tamadabensis* formed another distinct group that did not overlap with the populations of *C. pritzelii*. The interpopulation apportionment of genetic variation, as inferred from Nei's (1973) G_{ST}, was much lower for *C. tamadabensis* (G_{ST} = 0.067) than for *C. pritzelii* (G_{ST} = 0.126).

Genetic identities between population pairs of the same species (I_{NEI} = 0.929 ± 0.027 for *C. tamadabensis* and I_{NEI} = 0.923 ± 0.043 for *C. pritzelii*) were significantly higher than the average genetic identity between population pairs of both taxa (I_{NEI} = 0.877 ± 0.034) (P < 0.0001, Kruskal–Wallis test).

The AMOVA (Table 3) detected a substantial and significant within-population genetic variation (80.25%, P < 0.001). The variability between taxa was lower, but still significant (8.84% of the total variation, P < 0.001). The variability among populations of the same taxon was 10.91% (P < 0.001). Overall, the Mantel test supported a weak IBD (r = 0.391, P < 0.01), which was slightly higher within *C. pritzelii* (r = 0.503, P < 0.05; data not shown).

The average gene flow between population pairs with Wright's (1951) method was four-fold higher than that estimated by the private alleles method (N_m = 3.526 vs. N_m = 0.874, Table 4). The values obtained through Wright's method were significantly higher between population pairs of the same species (N_m = 5.126 for *C. tamadabensis* and N_m = 4.373 for *C. pritzelii*) than between population pairs of different species (N_m = 2.484; P < 0.05, Kruskal–Wallis test). There was no difference between mean intra- and interspecific gene flow by private alleles (N_m = 1.209, 0.846 and 0.835, respectively; P = 0.186, Kruskal–Wallis test).

ΔK following Evanno *et al.* (2005) showed a maximum for K = 3, indicating that there are three main genetic clusters (I, II and III in Fig. 3) in the model pre-defined by STRUCTURE. Whereas all populations of *C. tamadabensis* could be assigned unambiguously to cluster I (q = 0.808–0.939), the assignment of the five most variable populations of *C. pritzelii* to clusters II or III was difficult because they had similar membership coefficients (Fig. 3). These populations also showed small to moderate coefficients of admixture to cluster I (q = 0.042–0.223). BARRIER analysis based on Nei's (1978) genetic distances strongly supported (high bootstrap values) only one major genetic barrier separating the populations of both species into two main groups (D_{NEI} = 0.136–0.099, Fig. 3). Other barriers were not supported by bootstrap values (Fig. 3).

DISCUSSION

Levels of genetic variability

The overall levels of allozyme variation detected for *C. tamadabensis* and *C. pritzelii* are comparatively much higher than the average values reported for endemic plants in general (A_l = 1.39; P = 26.0; H_e = 0.063; Hamrick & Godt, 1989). These unexpectedly high levels of intrapopulation genetic diversity are even more noteworthy when compared with other studies involving narrow endemic island species (e.g. López-Pujol *et al.*, 2013). The detected values are slightly higher than those obtained in other Canarian Brassicaceae sampled exhaustively (Table 2). Thus, as in these other cases, the estimates of intrapopulation genetic variation in *Crambe* spp. are substantially higher than the averages for the Canarian endemics included in the review of

Table 2. Basic indicators of allozyme variation in *Crambe tamadabensis*, *C. pritzelii* and other endemic Canarian taxa of Brassicaceae

Species/population	A_l	N_e	P	H_o	H_e	F_{IS}	Bottleneck test		
							L	H_d/H_e	P
Crambe tamadabensis									
1. CTTA	3.4 (1.6)	2.0 (0.8)	83.3	0.320 (0.215)	0.411 (0.249)	0.183	10	3/7	**0.016**
2. CTAM	2.6 (1.2)	1.9 (0.8)	75.0	0.242 (0.256)	0.396 (0.277)	0.371	9	1/8	**0.005**
3. CTGU	3.1 (1.4)	1,9 (0.7)	83.3	0.278 (0.210)	0.410 (0.247)	0.310	10	3/7	**0.042**
4. CTSI	2.6 (1.2)	1.8 (0.7)	83,3	0.350 (0.253)	0.386 (0.234)	0.082	10	3/7	**0.009**
Crambe pritzelii									
5. CPAG2	2.7 (1.2)	1.7 (0.7)	66.7	0.227 (0.217)	0.331 (0.270)	0.302	9	2/7	0.102
6. CPAG1	2.8 (1.3)	1.8 (0.7)	75.0	0.246 (0.209)	0.369 (0.257)	0.319	10	1/9	**0.003**
7. CPAZ	3.2 (1.6)	1.6 (0.7)	66.7	0.284 (0.291)	0.316 (0.256)	0.077	10	4/6	0.312
8. CPJI	1.9 (0.8)	1.3 (0.5)	41.7	0.178 (0.237)	0.168 (0.216)	−0.034	8	5/3	0.473
9. CPAN	2.5 (1.4)	1.8 (0.7)	75.0	0.330 (0.292)	0.384 (0.253)	0.126	9	0/9	**0.001**
10. CPTE	3.4 (1.6)	2.0 (0.8)	75.0	0.329 (0.266)	0.397 (0.275)	0.219	10	2/8	**0.007**
11. CPGY	3.1 (1.4)	1.9 (0.8)	66.7	0.271 (0.273)	0.356 (0.283)	0.283	10	3/7	0.097
12. CPGA	2.3 (1.1)	1.5 (0.6)	66.7	0.206 (0.237)	0.255 (0.242)	0.241	9	3/6	0.082
Averages									
Crambe tamadabensis	2.9	1.9	81.2	0.298	0.401	0.237			
Crambe pritzelii	2.7	1.7	66.7	0.259	0.322	0.192			
Other Canarian endemic Brassicaceae									
Matthiola bolleana (Sánchez *et al.*, 2006)	2.2	–	67.0	0.237	0.262	–			
Parolinia ornata (Fernández-Palacios *et al.*, 2006)	2.9	1.6	64.1	0.184	0.288	0.371			
Parolinia glabriuscula (Fernández-Palacios *et al.*, 2004)	1.8	–	46.2	0.250	0.204	−0.206			
Parolinia platypetala (Fernández-Palacios *et al.*, 2004)	2.4	–	64.5	0.230	0.292	0.157			
Erysimum albescens (Vilches *et al.*, 2004)	1.6	–	40.0	0.162	0.165	0.018			
Lobularia canariensis (Borgen, 1997)	2.3	–	74.0	0.213	0.278	0.222			

Values in parentheses are standard deviations.

A_l, mean number of alleles per locus; N_e, effective number of alleles per locus; P, percentage of polymorphic loci (0.95 criterion); H_o and H_e, observed and expected heterozygosities; F_{IS}, fixation index; L, number of polymorphic loci used in the bottleneck tests; H_d/H_e, number of loci with heterozygote deficiency and excess (respectively) according to the independent allele model (IAM); P, probability of the test (bold values indicate significance).

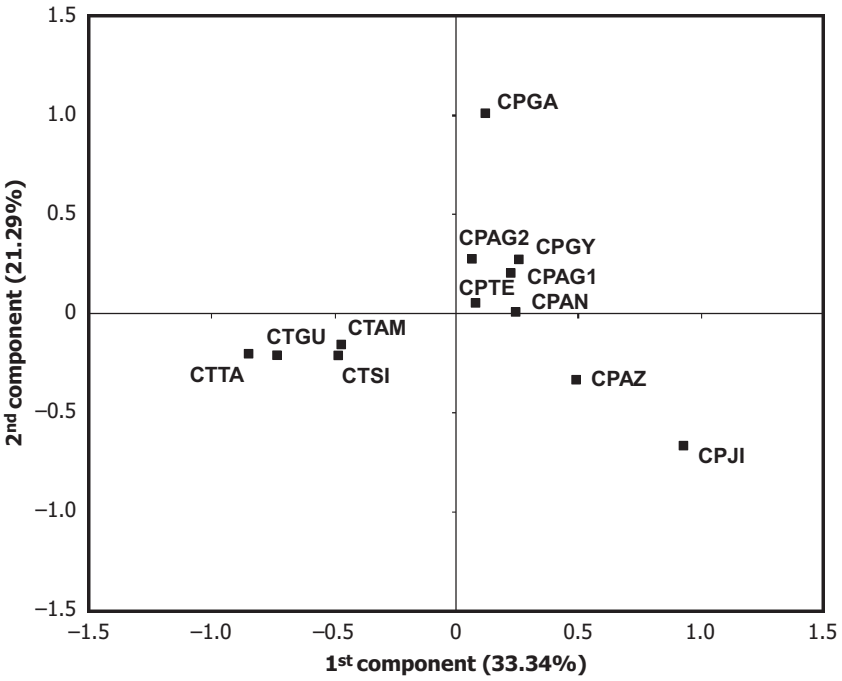

Figure 2. Principal component analysis of *Crambe tamadabensis* and *C. pritzelii* populations. The proportion of total variation explained by each component is indicated in parentheses.

Table 3. Analysis of molecular variance (AMOVA) and values of the genetic variation at the three hierarchical levels considered for this study

Source of variation	Sum of squares	Variance components	Percentage variation	P
Among species	121.08	0.229	8.84	< 0.001
Among populations within species	327.15	0.283	10.91	< 0.001
Within populations	2742.51	2.082	80.25	< 0.001
Total	3190.74	2.594		

Pérez de Paz & Caujapé-Castells (2013), who emphasized the high genetic variability of the Canarian flora. Considering that many species included in that review were not sampled intensively, it is probable that the average genetic diversity in the Canarian flora is still much higher than reported [see Caujapé-Castells (2010) and Pérez de Paz & Caujapé-Castells (2013)].

Possibly, the phylogenetic assignment of these taxa also contributes to their high genetic variation (Webb, 1984; Karron, 1987, 1988; Gitzendanner & Soltis, 2000), as previous studies of population genetic variability in tribe Brassiceae have found overall high levels of genetic variation and moderate among-population variability, e.g. in *C. maritima* (with inter-simple sequence repeat (ISSRs); Bond, Daniels & Bioret, 2005), *Raphanus raphanistrum* L. (with isozymes; Kercher & Conner, 1996), *Brassica oleracea* L. (with isozymes and randomly amplified polymorphic DNA (RAPDs); Lannér-Herrera *et al.*, 1996) or the *B. oleracea* complex (with isozymes; Lázaro & Aguinagalde, 1998). In the cases of *C. maritima* and *R. raphanistrum*, self-incompatibility is argued to be one of the possible causes to explain both high variability and low differentiation among populations. Although there are no reproductive biology studies available for these *Crambe* spp., we believe that they can be tentatively considered as self-incompatible (see Methods), as reported for species of other Canarian endemic outcrossing Brassicaceae, including *Parolinia* Webb (Fernández-Palacios, 2009) and *Descurainia* Webb & Berthel (Goodson, Santos-Guerra & Jansen, 2006).

The narrowly distributed *C. tamadabensis* maintains substantially higher average levels of population genetic variation than the more widespread

Table 4. Estimates of N_m between all pair-wise combinations of populations, as inferred by the private allele method (Slatkin, 1985; above the diagonal) and Wright's *F*-statistics (Wright, 1951; below the diagonal)

	CTTA	CTAM	CTGU	CTSI	CPAG2	CPAG1	CPAZ	CPJI	CPAN	CPTE	CPGY	CPGA
CTTA		1.894	1.461	0.835	1.481	2.591	0.665	0.336	1.058	1.009	0.816	0.430
CTAM	4.310		1.534	0.738	0.584	1.049	0.925	0.157	0.298	1.654	0.448	0.202
CTGU	10.388	3.091		0.794	1.889	1.528	0.885	0.216	1.073	1.047	1.160	0.817
CTSI	5.958	3.064	3.946		0.704	0.729	0.695	0.260	0.629	0.647	0.399	0.348
CPAG2	2.297	3.408	2.219	2.790		1.644	1.009	0.534	0.768	1.277	1.129	0.634
CPAG1	2.581	3.625	2.767	3.149	9.295		0.828	0.453	1.085	0.872	1.038	0.469
CPAZ	2.080	2.591	2.192	2.726	2.505	4.806		0.547	0.982	1.285	1.102	0.339
CPJI	0.955	0.978	1.002	1.135	1.092	1.604	2.128		0.337	0.593	0.414	0.245
CPAN	2.423	2.671	3.183	2.625	5.010	8.915	4.362	1.679		0.874	1.866	0.528
CPTE	3.186	4.377	3.910	3.779	9.179	10.229	4.715	1.531	9.057		0.781	0.896
CPGY	2.592	2.663	3.130	2.392	4.076	7.657	4.904	1.472	7.040	8.169		1.159
CPGA	1.515	1.357	1.446	1.732	1.864	2.606	1.282	0.754	1.798	2.006	2.717	

C. pritzelii and the same number of private alleles. Despite all populations of *C. tamadabensis* and some of *C. pritzelii* showing evidence of genetic bottlenecks (Table 2), both taxa have similar population sizes (generally > 500 individuals per population, Table 1) on secluded cliffs that may have acted as refugia. Only two populations of *C. pritzelii* (CPJI and CPGA) showed a loss of variability associated with small population sizes. A similar example in the same tribe is found in *Brassica* L. (Lázaro & Aguinagalde, 1998), in which low genetic diversity is found in several narrow endemics and in some extremely small populations of common species. As in *C. tamadabensis*, the Canarian endemic *B. bourgeaui* Kuntze possesses high levels of isozyme variation (Lázaro & Aguinagalde, 1998), although it only consists of a single small population on La Palma.

As we sampled exhaustively the whole distribution area of all populations, it seems that the moderately high fixation indices (F_{IS}, Table 2) detected in our study indicate a strong within-population genetic structure, rather than other processes which may lead to homozygote excess (e.g. the Wahlund effect, which implies the grouping of individuals that belong to independent reproductive cohorts as a sole population).

Genetic variation and structure

The interpopulation apportionment of genetic variability, as inferred from Nei's G_{ST} values (0.067 for *C. tamadabensis* and 0.126 for *C. pritzelii*) and AMOVA results (Table 3), indicate a high genetic cohesion within each species, in sharp contrast with the high fragmentation levels estimated for the Canarian flora (G_{ST} = 0.280; Francisco-Ortega *et al.*, 2000). Caujapé-Castells (2010) and Pérez de Paz & Caujapé-Castells (2013) indicated that many of the average values of G_{ST} reported by Francisco-Ortega *et al.* (2000) might be overestimates resulting from a restricted sampling in terms of the number and distribution of individuals. In contrast, genetic differentiation estimates obtained by us with these populations of *Crambe* are based on an exhaustive sampling of their occurrence areas and are much closer to those of outbreeding endemic species in general (G_{ST} = 0.179; Hamrick & Godt, 1997).

In *C. pritzelii*, the two least variable populations (CPJI, CPGA) account for most of the species interpopulation divergence as estimated by G_{ST} (see also PCA, Fig. 2). Thus, the genetic divergence within this species seems to reflect both: (1) the effects of population fragmentation, more probably in the surroundings of CPJI (at the edge of the distribution area of the species), where several populations that once occurred nearby were not sampled in the present study because they no longer exist, and (2) relatively recent founder events. The latter possibility is more probable in populations such as CPGA, situated in the isolated Amurga massif, in south-eastern Gran Canaria. Indeed, CPJI and CPGA seem to account for the low correlation detected between geographical and genetic distances in the Mantel test (data not shown).

Some of the lowest values of N_m obtained with the private alleles method occur among genetically close populations (Table 4). Feasibly, this result is a consequence of recurrent bottlenecks that may have affected most populations examined (Table 2), as suggested by the detection in each taxon of exclusive alleles that are not present in all populations (Appendix 2). This scenario is in conflict with the generalized high levels of gene flow inferred by

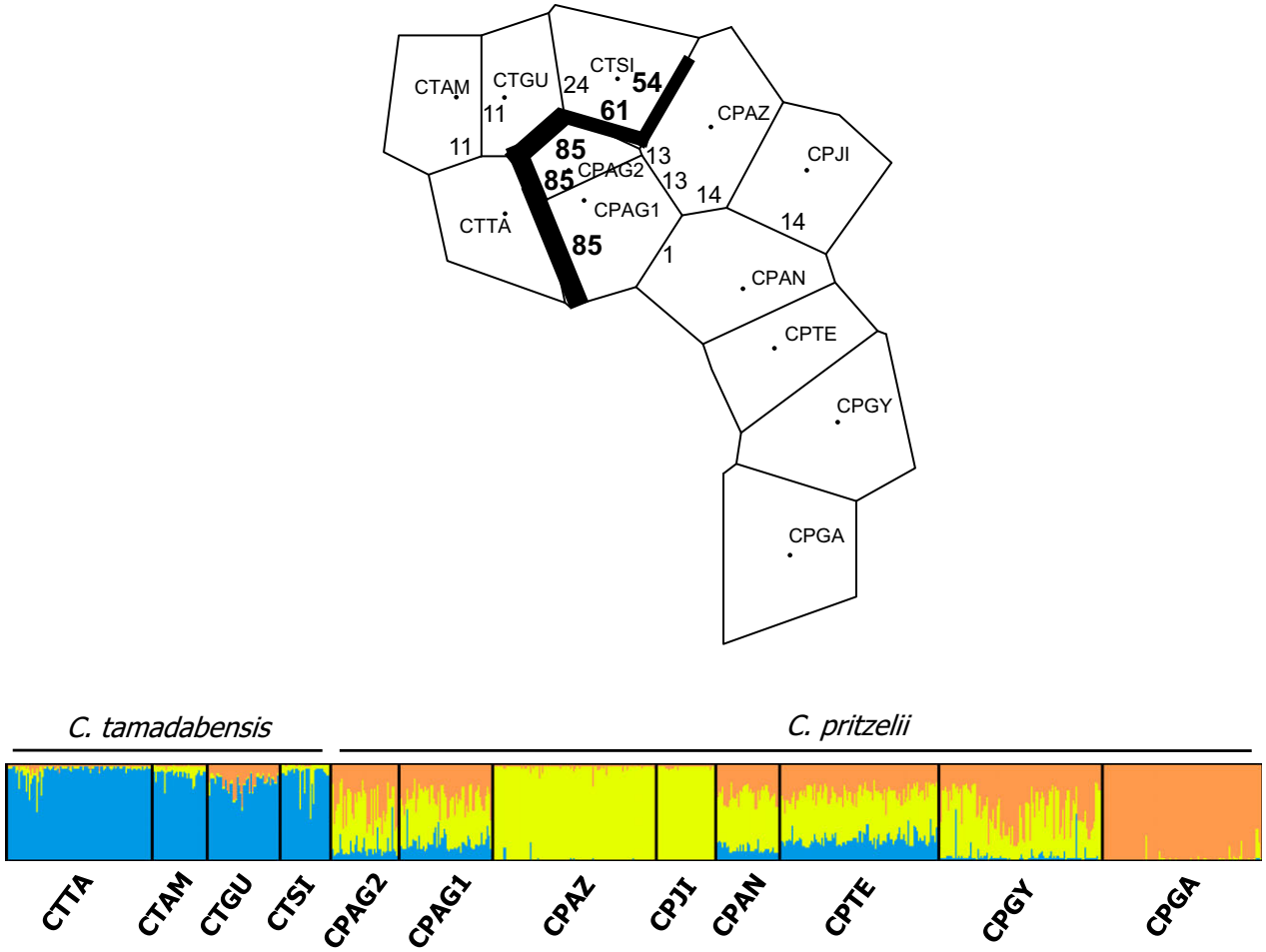

Figure 3. Top: map of boundaries detected with BARRIER (Voronoi tesselation); only barriers with 50% support or more from 100 bootstrapped matrices are represented by thick lines over the edges between neighbouring populations. Numbers indicate the proportion of barriers (out of 100 assessed) that passed over a border. Bottom: diagram representing the results of STRUCTURE analyses; each individual is represented by a thin vertical line, with coloured segments that indicate the estimated membership of an individual (q) in the assumed three clusters (blue, yellow and orange for clusters I, II and III, respectively).

Wright's (1951) F-statistics (Table 4). Despite criticisms by Bossart & Prowell (1998), Whitlock & McCauley (1999) and Jost (2008), indirect measures of gene flow are meaningful when they are used as a comparative measure of population divergence, and they have provided relevant comparisons in many cases (Waples, 1987; Slatkin & Barton, 1989; Cockerham & Weir, 1993; Bohonak *et al.*, 1998; Bohonak, 1999; Neigel, 2002). The practical problems in estimating rare allele frequencies in electrophoretic studies (as observed previously by Waples, 1987) suggest that F_{ST} is likely to be a more relevant comparative method than the private allele approach (Slatkin & Barton, 1989). Therefore, we contend that levels of interpopulation gene flow within either *Crambe* sp. are high overall.

The populations of *C. tamadabensis* are genetically homogeneous according to STRUCTURE analysis (Fig. 3). In contrast, those of *C. pritzelii* show higher levels of admixture and their members are mostly assigned to two clusters. As shown by Rosenberg *et al.* (2002), the STRUCTURE software detected first isolated populations with lower variability (CPJI and CPGA), whereas individuals of highly variable populations have partial memberships in both clusters, probably reflecting genetic admixture of neighbouring populations, consistent with the scenario of high levels of gene flow. Our interpretation of these results is that the two least variable populations of *C. pritzelii* diverge so much in allele frequencies that they interfere in the estimation of the number of clusters. Notably, populations CPJI and CPGA are

two of three (the third being Azuaje, CPAZ) assigned unambiguously by the program to clusters II or III. In contrast, the results obtained with the software BARRIER do not support a strong gene disruption among populations of *C. pritzelii*, because of the lower genetic distances among CPJI, CPGA and neighbouring populations with respect to those among interspecific populations.

In these two *Crambe* spp., the average genetic identities between and within taxa are significantly different (I_{NEI} = 0.877 and I_{NEI} = 0.929–0.923, respectively) and the average genetic identity between them is closely similar to the interspecific values found in other Canarian endemics, such as *Parolinia* (Brassicaceae) (I_{NEI} = 0.754–0.914; Fernández-Palacios *et al.*, 2006), in *Lotus* L. (Fabaceae) from the Gran Canarian pine forest (I_{NEI} = 0.822–0.939; Oliva-Tejera *et al.*, 2005) or in coastal *Lotus* spp. (I_{NEI} = 0.823–0.894; Oliva-Tejera *et al.*, 2006).

As argued by Crawford *et al.* (2006), the incipient intra- and interspecific genetic divergence detected in *C. pritzelii* and *C. tamadabensis* probably followed rapid morphological differentiation. We believe that this process may have been aided by the appearance of phenological barriers (we did not detect hybrids, even though several populations of these species are spatially close). The explanation often invoked for the high similarity at allozyme loci among congeneric oceanic island species is that recent and rapid radiation following establishment has been too fast to allow for substantial differences at neutral or near-neutral allozyme loci (Crawford, Whitkus & Stuessy, 1987b; Crawford & Stuessy, 1997).

Thus, partially at odds with the phylogenetic closeness found with ITS by Francisco-Ortega *et al.* (2002), the high allozyme variation detected in *C. tamadabensis* and *C. pritzelii* points towards an incipient, but consistent, genetic differentiation probably influenced by recurrent bottlenecks. Despite the fact that populations of *C. tamadabensis* occur in the oldest parts of Gran Canaria, we argue that these species represent a recent speciation event, tentatively from an ancestor closely related to *C. scoparia* Svent. (Francisco-Ortega *et al.*, 2002). In this context, it appears that the genetic similarity between some populations of *C. tamadabensis* and *C. pritzelii* is a reflection of ancestral gene flow and recent speciation (see Table 4).

Conservation implications

Despite both species having succeeded in maintaining high neutral genetic variation and within-species interpopulation cohesion (Tables 2 and 3), the general impact of intensive human activities (related to grazing by introduced herbivores, deforestation and agriculture) may have affected them overall. Even the largest populations in this study have been subjected to historical fluctuations in size, as also reflected by the bottleneck tests (Table 2). Consequently, the overall high values of the genetic diversity indicators are best interpreted as the effect of overall high levels of interpopulation gene flow.

In this context, the fact that some small populations (such as CPAN or CPAG2 in *C. pritzelii*) contain higher levels of genetic variability than other populations of similar size, such as CPJI and CPGA (Tables 1 and 2), is feasibly a result of their spatial proximity to large, highly variable conspecific populations, which fosters the incorporation of genetic variation through high levels of gene flow (Table 4). Consequently, environmental stochasticity jeopardizes population survival only when it generates small isolates that cannot maintain sufficient levels of gene flow with other populations, as detected with *Atractylis preauxiana* Sch.Bip. (Asteraceae) by Caujapé-Castells *et al.* (2008).

Our results therefore suggest that these *Crambe* spp. have a remarkable capacity for a fast demographic and genetic recovery if the current high levels of within-species gene flow are preserved, and suggest that *in situ* protection and control of grazing will be the most effective ways to protect these species. As underscored earlier, these taxa still contain high levels of neutral genetic variation, which should be preserved *ex situ* in the germplasm bank at JBCVC-CSIC for use in future reinforcements or reintroductions, following the indications in Bacchetta *et al.* (2008). According to the formula $P = 1 - (G_{ST})^n$ (Hamrick *et al.*, 1991), the minimum numbers of populations (n) needed to conserve 99% of the detected genetic variability in *C. tamadabensis* and *C. pritzelii* are two and three, respectively. We thus suggest the intensive collection of seeds in populations CTTA and CTGU for *C. tamadabensis* and CPAG1, CPTE and CPAN for *C. pritzelii*. These populations display the highest values of expected heterozygosity in their respective species, and are thus those that warrant the representation of most natural genetic diversity in *ex situ* facilities.

As we aim to maintain the existing genetic connectivity in these species as the most appropriate *in situ* conservation strategy, the above-mentioned populations plus CPGY are also those on which conservation efforts should be mainly focused, as they contribute most to the current genetic cohesion in their respective species [G_{ST} (Nei, 1973), θ (Weir & Cockerham, 1984), see Appendix 3]. However, we believe that the isolated population CPGA also deserves special *in situ* protection, because it is genetically and ecologically distinct.

Apart from these guidelines based on the neutral markers used, it is important to attempt to identify

the genetic basis and to conserve adaptive traits (i.e. selectively important variation), as suggested in several recent investigations (Ouborg *et al.*, 2010; Hunter, Wright & Bomblies, 2013; Schlötterer *et al.*, 2015).

Finally, as extensively argued in other conservation works for Canarian endemics (e.g. Rumeu *et al.*, 2014), the success of these suggested *in situ* guidelines is contingent upon the implementation of actions that effectively enforce habitat protection through the eradication of the impact of introduced vertebrate herbivores (especially feral goats and rabbits). These actions should be accompanied by new research on the effect of grazing on these species (such as the fenced enclosure of control populations).

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Appendix 1 Herbarium Vouchers

LPA33086: *Crambe tamadabensis.* Andenes de La Breña, Guayedra, Tamadaba. Moisés Soto. 13.v.2006. LPA20074: *Crambe tamadabensis.* Islas Canarias, Gran Canaria, Gáldar, Amagro. 28RDS3311. B. Navarro, J. Naranjo, J. Navarro, F. Oliva, M. Soto, O. Saturno. 27.xi.2003. LPA33088: *Crambe tamadabensis.* Montaña de Guía, cara oeste. Moisés Soto. 11.v.2006. LPA33091: *Crambe tamadabensis.* Risco Alto del Cabro, Barranco del Calabozo (Cuesta de Silva). Moisés Soto. 12.v.2006. LPA33094: *Crambe pritzelii.* Las Escaleras, al norte de San Pedro, Agaete, 300 m. Moisés Soto. 12.v.2006. LPA19943: *Crambe pritzelii.* Agaete, Los Berrazales. A Roca, J. Naranjo, J. Navarro. 31.iii.2000. LPA33471: *Crambe pritzelii.* Barranco de Azuaje, tramo por debajo de la carretera, 175 m. Moisés Soto. 28.iv.2015. LPA19944: *Crambe pritzelii.* Teror, Riscos de Jiménez. J. Naranjo, F. Oliva, B. Vilches, J. Navarro. 29.vi.2001.

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Appendix 2 Allele frequencies at the 12 loci interpreted in the 12 populations of *Crambe tamadabensis* and *C. pritzelii* sampled on the island of Gran Canaria

	C. tamadabensis				*C. pritzelii*							
Alleles	CTTA	CTAM	CTGU	CTSI	CPAG2	CPAG1	CPAZ	CPJI	CPAN	CPTE	CPGY	CPGA
Est-1a	0.000	0.000	0.000	0.000	0.030	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Est-1b	0.000	0.000	0.000	0.000	0.000	0.000	0.011	0.030	0.000	0.000	0.018	0.000
Est-1c	0.289	0.611	0.160	0.600	0.909	0.872	0.978	0.970	0.792	0.885	0.881	0.919
Est-1d	0.689	0.389	0.800	0.367	0.061	0.128	0.011	0.000	0.208	0.103	0.095	0.081
Est-1e	0.022	0.000	0.040	0.033	0.000	0.000	0.000	0.000	0.000	0.011	0.006	0.000
Got-1a	0.034	0.000	0.125	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Got-1b	0.514	0.304	0.550	0.404	0.237	0.449	0.729	0.982	0.500	0.428	0.709	0.848
Got-1c	0.075	0.018	0.000	0.000	0.026	0.061	0.000	0.000	0.000	0.072	0.025	0.000
Got-1d	0.034	0.000	0.000	0.288	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Got-1e	0.301	0.375	0.313	0.115	0.605	0.388	0.217	0.018	0.500	0.394	0.266	0.152
Got-1f	0.000	0.000	0.000	0.000	0.105	0.020	0.000	0.000	0.000	0.056	0.000	0.000
Got-1g	0.041	0.304	0.013	0.192	0.026	0.082	0.054	0.000	0.000	0.050	0.000	0.000
Got-2a	0.000	0.017	0.000	0.000	0.053	0.000	0.012	0.000	0.000	0.060	0.012	0.000
Got-2b	0.000	0.000	0.000	0.000	0.000	0.000	0.262	0.000	0.114	0.055	0.042	0.000
Got-2c	0.544	0.414	0.622	0.431	0.316	0.224	0.065	0.000	0.271	0.412	0.458	0.231
Got-2d	0.056	0.000	0.000	0.000	0.000	0.000	0.006	0.081	0.000	0.000	0.012	0.000
Got-2e	0.344	0.569	0.378	0.569	0.632	0.776	0.655	0.919	0.614	0.396	0.476	0.769
Got-2f	0.056	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.077	0.000	0.000
Idh-1a	0.013	0.323	0.027	0.000	0.000	0.000	0.000	0.000	0.000	0.079	0.000	0.000
Idh-1b	0.279	0.274	0.500	0.220	0.444	0.598	0.813	0.900	0.778	0.483	0.714	0.160
Idh-1c	0.000	0.032	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Idh-1d	0.675	0.371	0.365	0.780	0.542	0.272	0.148	0.083	0.208	0.202	0.115	0.583
Idh-1e	0.019	0.000	0.000	0.000	0.014	0.065	0.011	0.00	0.000	0.000	0.000	0.000
Idh-1f	0.000	0.000	0.068	0.000	0.000	0.000	0.023	0.017	0.014	0.124	0.170	0.215
Idh-1g	0.013	0.000	0.041	0.000	0.000	0.065	0.006	0.000	0.000	0.112	0.000	0.042
Mdh-1a	0.127	0.047	0.037	0.000	0.000	0.029	0.000	0.000	0.000	0.000	0.000	0.000
Mdh-1b	0.000	0.000	0.085	0.179	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Mdh-1c	0.012	0.047	0.000	0.036	0.013	0.000	0.091	0.000	0.000	0.124	0.006	0.005
Mdh-1d	0.861	0.906	0.878	0.786	0.987	0.971	0.909	1.000	1.000	0.876	0.994	0.995
Mdh-2a	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Mdh-3a	0.036	0.177	0.207	0.017	0.077	0.173	0.102	0.000	0.143	0.080	0.128	0.075
Mdh-3b	0.000	0.000	0.000	0.000	0.000	0.000	0.005	0.000	0.000	0.006	0.000	0.000
Mdh-3c	0.602	0.452	0.524	0.603	0.615	0.531	0.457	0.955	0.429	0.529	0.445	0.586
Mdh-3d	0.000	0.016	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Mdh-3e	0.331	0.355	0.183	0.328	0.308	0.265	0.419	0.045	0.429	0.368	0.396	0.339
Mdh-3f	0.018	0.000	0.061	0.017	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Mdh-3g	0.012	0.000	0.024	0.034	0.000	0.031	0.016	0.000	0.000	0.017	0.030	0.000
Me-1a	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Mnr-1a	0.072	0.000	0.098	0.052	0.000	0.130	0.011	0.000	0.311	0.038	0.022	0.000
Mnr-1b	0.910	1.000	0.878	0.948	1.000	0.870	0.989	1.000	0.689	0.962	0.978	1.000
Mnr-1c	0.000	0.000	0.012	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Mnr-1d	0.018	0.000	0.012	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Pgi-1a	0.000	0.000	0.000	0.000	0.000	0.000	0.161	0.000	0.015	0.017	0.034	0.000
Pgi-1b	0.169	0.500	0.293	0.214	0.770	0.606	0.283	0.212	0.456	0.590	0.443	0.250
Pgi-1c	0.519	0.194	0.524	0.589	0.149	0.144	0.489	0.303	0.265	0.275	0.295	0.085
Pgi-1d	0.281	0.306	0.183	0.196	0.081	0.250	0.056	0.000	0.059	0.096	0.085	0.523

Appendix 2. *Continued*

	C. tamadabensis				*C. pritzelii*							
Alleles	CTTA	CTAM	CTGU	CTSI	CPAG2	CPAG1	CPAZ	CPJI	CPAN	CPTE	CPGY	CPGA
Pgi-1e	0.031	0.000	0.000	0.000	0.000	0.000	0.011	0.485	0.191	0.022	0.142	0.142
Pgi-1f	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.015	0.000	0.000	0.000
Pgm-1a	0.480	0.703	0.539	0.500	0.486	0.337	0.461	0.766	0.543	0.550	0.208	0.060
Pgm-1b	0.520	0.297	0.461	0.500	0.446	0.616	0.539	0.234	0.357	0.438	0.612	0.773
Pgm-1c	0.000	0.000	0.000	0.000	0.068	0.047	0.000	0.000	0.100	0.013	0.180	0.167
Pgm-2a	0.361	0.688	0.098	0.083	0.090	0.149	0.324	0.000	0.000	0.089	0.211	0.000
Pgm-2b	0.291	0.234	0.354	0.708	0.321	0.436	0.582	0.667	0.432	0.487	0.222	0.038
Pgm-2c	0.152	0.078	0.427	0.208	0.410	0.362	0.060	0.076	0.527	0.405	0.467	0.925
Pgm-2d	0.196	0.000	0.122	0.000	0.179	0.053	0.033	0.258	0.041	0.019	0.100	0.038

Shaded frequencies are the exclusive alleles detected in each taxon.

Appendix 3 Effects of the removal of each population on the value of the interpopulation component of genetic variation

Species	G_{ST} (Nei, 1973)	θ (Weir & Cockerham, 1984)
Crambe tamadabensis	0.067	0.074
Excluding CTTA	0.077	0.114
Excluding CTAM	0.044	0.053
Excluding CTGU	0.061	0.084
Excluding CTSI	0.056	0.068
Crambe pritzelii	0.126	0.134
Excluding CPAG2	0.128	0.136
Excluding CPAG1	0.140	0.147
Excluding CPAZ	0.125	0.131
Excluding CPJI	0.093	0.116
Excluding CPAN	0.136	0.140
Excluding CPTE	0.138	0.154
Excluding CPGY	0.136	0.153
Excluding CPGA	0.098	0.096