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The conservation significance of natural hybridisation in Mediterranean plants: from a case study on Cyclamen (Primulaceae) to a general perspective

note finali coverpage

(Article begins on next page)

28 April 2024

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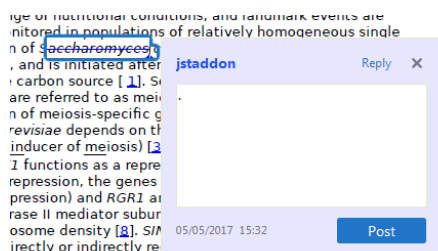
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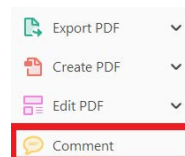
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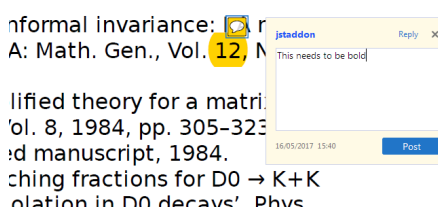
1. Small size (35–250 amino acids).
2. Absence of similarity to known proteins.
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4. Greater than 25% overlap at the N-termin terminus with another coding feature; ov both ends; or ORF containing a tRNA.

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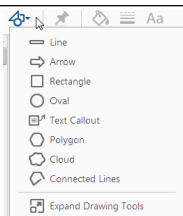Inserts a selected stamp onto an appropriate place in the proof.

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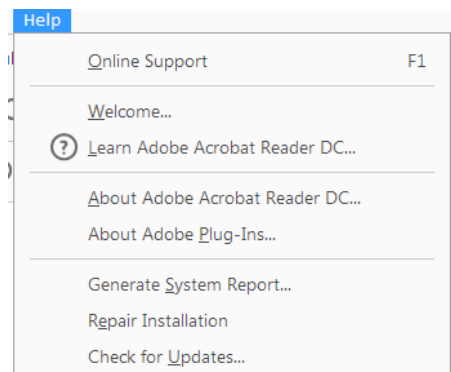
RESEARCH PAPER

The conservation significance of natural hybridisation in Mediterranean plants: from a case study on *Cyclamen* (Primulaceae) to a general perspective

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ABSTRACT

- Hybridisation plays a prominent role in plant evolution due to its influence on genetic diversity, fitness and adaptive potential. We identify a case of on-going hybrid evolution of floral phenotypes in disjunct populations of *Cyclamen balearicum* and *C. repandum* subsp. *repandum* on Corsica and Sardinia.
- Hybrid populations on the two islands contain similar patterns of variation in flower colour and size but are probably at different stages in the evolutionary process of hybridisation, and differences in the frequency of floral types and flower size suggest hybrid vigour that may contribute to the dynamics and maintenance of hybrid forms.
- In a review of cases of hybridisation in Mediterranean plants we found an equivalent number of cases for the contemporary occurrence of mixed hybrid populations, as there are cases of homoploid hybrid species differentiation.
- We argue for the development of a conservation strategy for Mediterranean plants that integrates the need to protect not just pure endemic species (some of hybrid origin) but also mixed populations where adaptive variation and new species are evolving due to contemporary hybridisation.

INTRODUCTION

Rare in animals but common in plants, hybridisation represents a key process in species' divergence (Stebbins 1950; Levin 1983; Abbott 1992; Thompson & Lumaret 1992; Arnold 1997; Rieseberg *et al.* 2003; Marques *et al.* this issue), and can lead to the formation of new species of hybrid origin (Mallet 2007; Soltis & Soltis 2009). Homoploid hybrid establishment may occur rapidly, however speciation may require a long period for a new hybrid species to become stabilised genetically and phenotypically (Abbott *et al.* 2010). The establishment of homoploid hybrids can be facilitated by (and may often require) niche shifts and new ecological tolerances (Rieseberg *et al.* 2003; Marques *et al.* 2007, 2010) and/or karyotype and genetic variation that may set the template for adaptive evolution and speciation (Stebbins 1950; Lewontin & Birch 1966; Rieseberg *et al.* 2003; Abbott *et al.* 2010). Indeed, wild hybrids can produce a mosaic of phenotypes that facilitate rapid divergence (Mallet 2007; Abbott *et al.* 2010). Hybrid speciation in plants also occurs with the process of chromosome doubling to produce allopolyploid plants that are almost immediately recognised as new species due to their immediate reproductive isolation from parental diploids and their enhanced genetic diversity (Thompson 1991; Thompson & Lumaret 1992; Escudero *et al.* 2017). Up to 4% of flowering plants and 7% of ferns are thought to be allopolyploids (Otto & Witton 2000).

The evolutionary processes that promote diversification have been increasingly recognised as important elements in biodiversity conservation (Smith *et al.* 1993; Moritz 1994, 2002; Crandall *et al.* 2000; Stockwell *et al.* 2003; Ellstrand *et al.* 2010). As a result of its prominent evolutionary role in plants, hybridisation has become a topic of particular interest in this respect (Allendorf *et al.* 2001; Cozzolino *et al.* 2006; Ellstrand *et al.* 2010; Thompson *et al.* 2010; Stronen & Paquet 2013; Jackiw *et al.* 2015). First, it is critical here to distinguish between hybrids that have a natural or anthropogenic origin because hybridisation among introduced and native species can cause genetic pollution of the latter (Allendorf *et al.* 2001; Jackiw *et al.* 2015). Natural hybridisation can also be a serious threat to rare and endangered species because of the possibility of extensive genetic introgression (Rieseberg & Gerber 1995; Levin *et al.* 1996; Mameli *et al.* 2014). However natural hybridisation is beneficial when it increases genetic diversity, fitness and adaptive potential (Stebbins 1950; Cozzolino *et al.* 2006; Abbott *et al.* 2010; Thompson *et al.* 2010). Hence, the significance of hybridisation for conservation issues may vary and it is critical to distinguish cases of hybridisation that have given rise to the evolution of new species from examples of contemporary and potentially on-going hybridisation that represent a source of new diversity and are potentially incipient species (Harrison 1993; Cozzolino *et al.* 2006; Thompson *et al.* 2010; Mameli *et al.* 2014).

In a given group of species, hybridisation may occur repeatedly at different times and in different locations with diverse evolutionary outcomes (Abbott & Lowe 1996; Fuertes *et al.* 1999; Albach 2007; Sardaro *et al.* 2012; Georgescu *et al.* 2016). In spring-flowering Mediterranean *Cyclamen*, *C. repandum* subsp. *repandum* and *C. balearicum* hybridise in three sites on a small limestone outcrop on Corsica where parental phenotypes can be identified alongside plants with intermediate pale pink or bicoloured flowers (Thompson *et al.* 2010). The presence of chloroplast DNA haplotypes and microsatellite variation characteristic of both parents in these sites (Gielly *et al.* 2001; Thompson *et al.* 2010) confirmed the presence of peripherally isolated *C. balearicum*. Hybridisation has produced a range of introgressed floral types with a higher genetic diversity than in allopatric populations of the parental species (Thompson *et al.* 2010). Recent observations suggest that mixed populations also occur on limestone in northwest Sardinia (E. Farris, unpublished data), indicative of a recurrent, disjunct hybridisation.

The objective of this paper is three-fold. First, we quantify patterns of floral trait variation and the presence of different floral types at sites on Corsica and Sardinia to confirm the occurrence of a hybrid population between *C. balearicum* and *C. repandum* on Sardinia. Second, to explore whether mixed populations on the two islands are at the same stage of evolution we examine whether hybrid phenotypes show enhanced vigour, by assessing flower size and population-scale and patch-scale frequency of the different floral types. Third, we compile a classification of Mediterranean cases of homoploid or polyploid hybridisation in which we distinguish contemporary population-level hybridisation that is in most cases ongoing from reports of taxonomically distinct (mostly endemic) species of hybrid origin. This classification provides a basis to formulate a conservation strategy for either species of hybrid origin or mixed populations subject to contemporary evolution.

MATERIAL AND METHODS

Study species and populations

Cyclamen balearicum and *C. repandum* are two morphologically distinct but very closely related species (Affre & Thompson 1998; Gielly *et al.* 2001; Debussche & Thompson 2002). They have primarily allopatric distributions, other than their recently reported sympatry on Corsica (Thompson *et al.* 2010). *C. balearicum* has a disjunct and narrow endemic distribution in the Balearic Islands and Languedoc-Roussillon (Debussche *et al.* 1995), while *C. repandum* is widespread across the Mediterranean: Tyrrhenian Islands, Italy and the Balkans, southern Greece, Crete, Rhodes and peripherally isolated sites in southern France and Algeria (Debussche & Thompson 2002). In the western Mediterranean, where *C. repandum* occurs as subspecies *repandum*, the two species have little ecological overlap (Debussche & Thompson 2003): *C. repandum* occurs in forest and woodland on a range of acidic bedrock types while *C. balearicum* occurs almost exclusively in evergreen shrublands and open woodlands on rocky limestone substrates. *C. repandum* has large pink flowers with a stigma protruding beyond the mouth of the corolla and a mixed mating system, while *C. balearicum* has small white flowers, a stigma positioned within the corolla and is highly inbred (Affre & Thompson 1997; Affre *et al.* 1997). Leaves of *C. balearicum* are almost unlobed, greyish green, extensively marbled with pale-grey patches, whereas leaves of *C. repandum* have a well-lobed margin, are green and have a greyish hastate patterning.

Mixed populations with plants with a morphological resemblance to *C. balearicum* intermingled with plants of *C. repandum* are known on Corsica, in three populations occurring near the town of St. Florent on a single limestone massif of ~5 km² (Thompson *et al.* 2010) and have recently been observed on limestone in northwest Sardinia (E. Farris, unpublished observations; Fig. 1). Both sites represent peripherally isolated populations for *C. balearicum* – but within the distribution range of *C. repandum* and ecological conditions that are marginal for *C. repandum* subsp. *repandum* – but typical of *C. balearicum* habitats (Debussche & Thompson 2003). A previous study has shown that Corsican populations growing on this limestone massif are all genetically introgressed (Thompson *et al.* 2010).

### Relative frequency of floral types on Sardinia and Corsica

We quantified the frequency of different floral types in populations on the two islands by walking through populations and randomly sampling flowers at least 1 m apart. Flowers were classed into six floral types (Thompson *et al.* 2010; Fig. 2): dark pink flowers (RSE) that resemble parental *C. repandum*, three hybrid floral types – bicoloured flowers with either a narrow basal corolla ring (BIN) or a wide basal corolla ring (BIW) and pale pink flowers (PAL), and two types of white flowers with either an exerted stigma (BSE) or, like parental *C. balearicum*, an inserted stigma (BSI). Sampling was done in the three St Florent populations on Corsica studied by Thompson *et al.* (2010) and in ten populations in N.W. Sardinia between the 9 April and 14 April 2015 (Table 1). White flowers were classed as having an inserted or exerted stigma because this difference represents an important trait for species delimitation in spring-flowering *Cyclamen* (Affre & Thompson 1998; Debussche & Thompson 2002).

To explore among-population variation in the frequency of different floral types, we ran principal components analysis and because of the low number of populations on Corsica we tested for a statistical difference between populations on the two islands with a non-parametric multiple ANOVA with 999 permutations (np-Manova; McArdle & Anderso 2001). We also compared the ratio of white flowers with either inserted or exerted stigmas among and within islands using Chi-square tests.

Hybrid vigour

To examine whether hybrid floral types have enhanced vigour relative to other floral types we quantified whether hybrid floral types occur at a higher frequency than parental floral types in small plots (as a surrogate for flower number per plant). It is impossible to count the number of flowers per plant on *Cyclamen* species in the wild, due to the intermingling of flowers from different tubers. We also quantified flower size in the different floral types to test whether they may have greater pollinator attractivity.
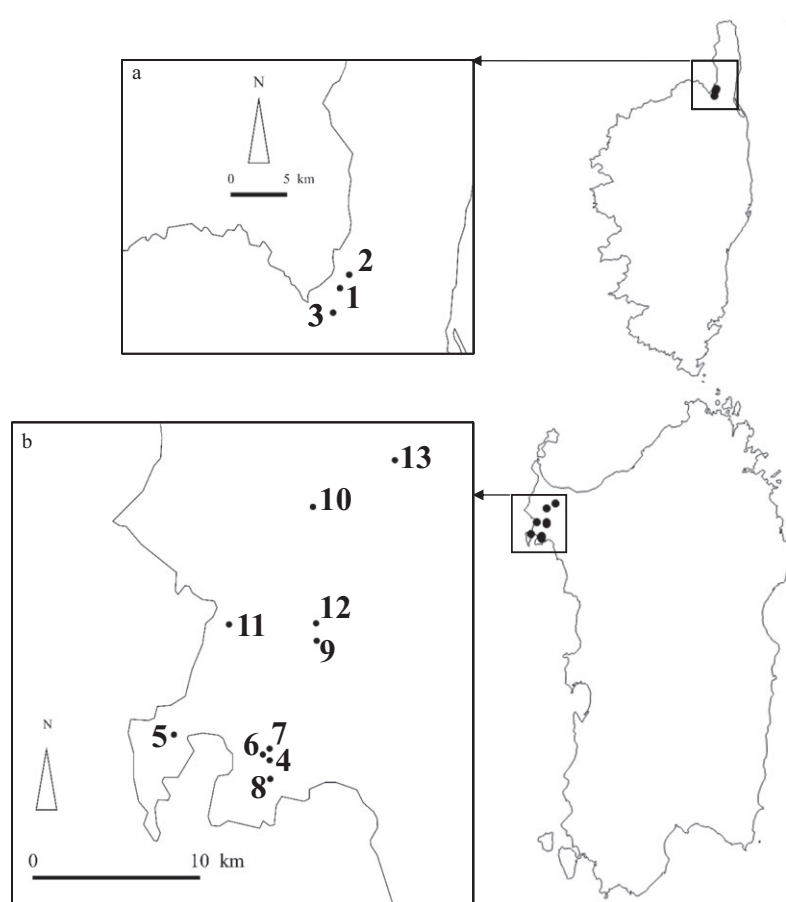
Fig. 1. The location of study sites of mixed populations of *Cyclamen balearicum* and *C. repandum* subsp. *repandum* on Corsica a: and Sardinia b.

Fig. 2. The six floral types: a: large pink flowers typical of *Cyclamen repandum* subsp. *repandum* (RSE), bicoloured flowers with b: a wide (BIW) or c: a narrow (BIN) basal ring, d: pale-pink flowers (PAL), and white flowers with e: exerted (BSE) or f: inserted (BSI) stigma.

Corolla length of each floral type was quantified in the three populations on Corsica and in five populations on Sardinia (Table 1). We randomly sampled flowers of each floral type at least 1 m apart and measured corolla lobe length with digital callipers to 0.1 mm. Corolla length was measured on 141 to 164 flowers per population; the total number of measurements per floral type ranged from 91 for white flowers with an exerted stigma (BSE) to 341 for pink flowers that resemble parental *C. repandum* (RSE).

For each floral type we fitted a linear mixed effect model, with corolla length as a response variable, island (Corsica or Sardinia) as fixed explanatory variables and population as a random factor. To test the statistical significance of the fixed

factors, we ran an analysis of deviance based on type II Wald chi-square tests (Nelder & Baker 1972). To test for differences among floral types on each island, we fitted a linear mixed effect model, with corolla length as a response variable, floral morph as fixed explanatory variables and *population* as a random factor. We repeated the statistical significance of the fixed factors and ran *post-hoc* tests to quantify pair-wise differences between all levels of the fixed factor (function *glht* of R package *multcomp*; see Bretz *et al.* 2010) and create groups of significantly different floral types. In each case, *P*-values for multiple tests were made with a Bonferroni correction (Shaffer 1995).

Second, we quantified the frequency of each floral type at a local scale by counting all flowers in 30 randomly placed 1-m²

Table 1. Details concerning the location of study sites, the numbers of flowers used for floral measures, the total number of flowers counted in the random walk (population count) and the total number of flowers counted in the 30 quadrats in each population (quadrat count).

population code	island	latitude	longitude	flower size	quadrat count	population count
1	Corsica	42.69292	9.33043	141	345	222
2	Corsica	42.70372	9.34050	145	635	248
3	Corsica	42.67324	9.32290	144	324	219
4	Sardinia	40.61062	8.24240	164	576	252
5	Sardinia	40.62436	8.17458	146	764	213
6	Sardinia	40.61369	8.23756	154	670	226
7	Sardinia	40.61683	8.24233	–	553	255
8	Sardinia	40.60057	8.24282	–	317	242
9	Sardinia	40.67476	8.27575	143	590	209
10	Sardinia	40.74659	8.27306	–	539	211
11	Sardinia	40.68354	8.21360	142	345	255
12	Sardinia	40.68418	8.27529	–	818	272
13	Sardinia	40.77163	8.33115	–	898	238

quadrats in each of the 13 populations where it was possible to access plants with minimal physical disturbance (low and dense shrub vegetation characterise many sites!) except for a small number that were chosen to include rare floral types in a site. We used these data in two ways. First, we tested for a statistical difference between Corsican and Sardinian populations with a non-parametric multiple ANOVA with 999 permutations. Then, for all floral types we tested whether the frequency of flowers in quadrats (surrogate for flower number per plant) was different from the frequency of flowers quantified in the random walk (surrogate for the frequency of each floral type in a population), using a two-sided non-parametric Wilcoxon paired test (Hollander & Wolfe 1999). Then, in regard to previous field observations of larger flowers for the two bicoloured floral types, and smaller flowers in the pale-pink floral type, we tested whether the frequency of flowers at the local scale was higher than the frequency of individuals at the population scale for bicoloured flowers, and whether frequency of flowers at local scale was lower than the frequency of individuals at the population scale for pale-pink flowers. We used one-sided non-parametric Wilcoxon paired test with alternative *greater* and *lower*, respectively.

Statistical analyses were made using R software version 3.3.3 (R Core Team 2017), with packages *ade4* (Dray & Dufour 2007), *lme4* (Bates *et al.* 2015), *multcomp* (Hothorn *et al.* 2008) and *vegan* (Oksanen *et al.* 2017).

Literature review

A search of the literature for examples of hybridisation among species in Mediterranean plants since 2000 was performed using the Web of Science with the 'plant science' option, and either 'hybridisation' with 'Mediterranean' or 'allopolyploidy' with 'Mediterranean' as key words. Our objective here was not to have an exhaustive and complete selection of papers on hybridisation in Mediterranean plants (see Marques *et al.* 2017) but to have a representative sample of papers on the occurrence of hybridisation (including allopolyploidy) in Mediterranean flora. Autopolyploidy was not included since it does not involve hybridisation between distinct species. We separated cases of homoploid hybridisation from allopolyploidy and for each of these two processes we distinguished

cases in which a new species has been clearly identified from those in which hybridisation is contemporary, *i.e.* occurs in mixed populations and in most cases is on-going. Some additional historical references in Thompson (2005) and two very recent references proposed by a reviewer were also included.

RESULTS

Relative frequency of floral types

The frequency distribution of the six floral types showed significant differences between the two islands (np Manova with 999 permutations, $F_1 = 5.5$, $P = 0.003$; Figs 3 and 4). On Corsica 50% of floral types are typical of parental *C. repandum* (RSE) whereas on Sardinia the dominant floral types are bicoloured flowers (BIW and BIN). It is noteworthy that the only three Sardinian populations where the floral type akin to parental *C. repandum* has a relative higher percentage are those where the BSI floral type is at very low frequency.

Axis 1 of the principal components analysis (PCA) accounts for 43.3% of the total variation in frequency of the six floral types in the complete dataset (Fig. 4a). From left to right, it represents a gradual decrease of the frequency of bicoloured and pale-pink flowers, and an increase in the frequency of the floral type typical of parental *C. repandum* (Fig. 4b). Axis 2 of the PCA accounts for 31.5% of the variation in the dataset, and is mainly driven by the frequency of white flower types, decreasing from top to bottom. We used the function *s.class* from R package ADE4 (Dray & Dufour 2007) to group populations by island and observed a marked discrimination between Corsica and Sardinia on axis 1, with Corsican populations tending to exhibit relatively fewer bicoloured and pale-pink flowers but more flowers typical of *C. repandum* (Figs 3 and 4). Bicoloured flowers with a wide basal ring represent <11% of Corsican populations but >18% of Sardinian populations (Fig. 3). The proportion of pale-pink flowers is always <10% except for one population on Sardinia. There is no discrimination between the two islands for axis 2; both the highest and lowest frequencies of white flowers are found on Sardinia.

Chi-squared tests of homogeneity of the relative frequency of the two white floral types (BSE & BSI), showed no differences among populations on Corsica ($\chi^2 = 2.7$, df = 2,

Fig. 3. Frequency distribution of the six floral types in a: population counts and b: quadrat counts on Corsica (populations 1–3) and Sardinia (populations 4–13). Each bar (left to right) represents a floral type: large pink flowers typical of *Cyclamen repandum* subsp. *repandum* (black bar): RSE, bicoloured flowers with a wide or narrow basal ring (grey bars): BIW and BIN, pale-pink flowers (doted bar): PAL, and white flowers with exerted or inserted stigma (white bars): BSE and BSI.
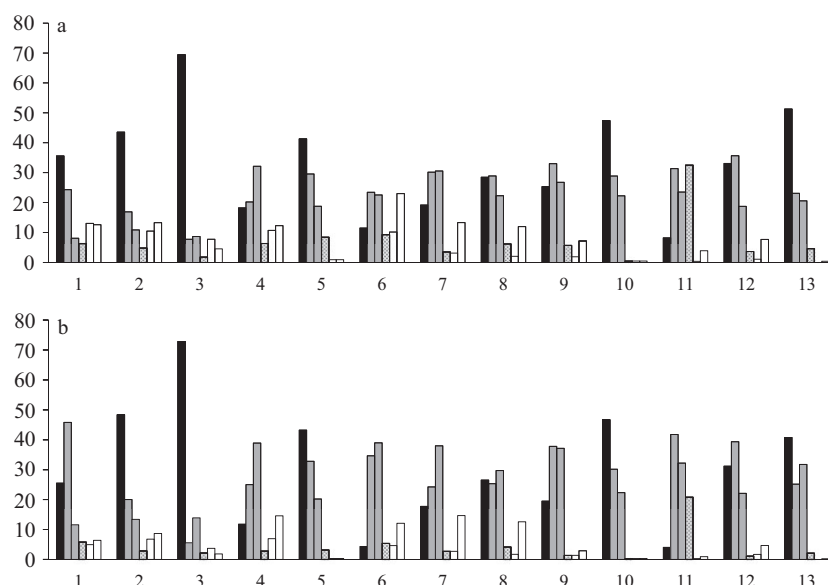
Fig. 4. Principal components analysis of the frequency of floral types in mixed populations of *Cyclamen balearicum* and *C. repandum* subsp. *repandum* on Corsica and Sardinia. Axis one summarizes 43.3% and axis two 31.5% of the total variation in the dataset. a: A projection of individuals grouped by their respective island (Corsica or Sardinia) and b: the correlation circle of the variables.
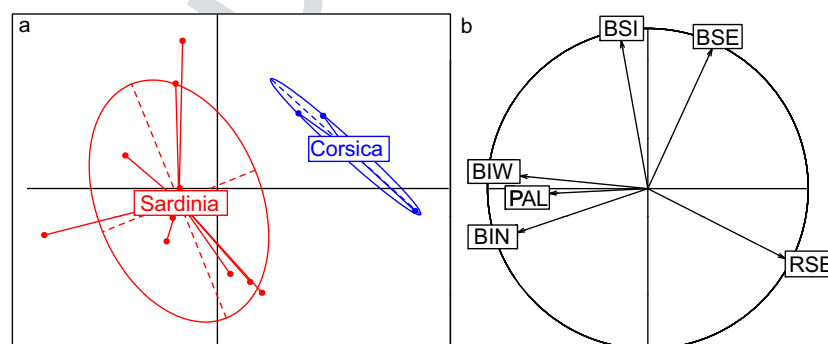
$P = 0.265$), but a significant difference among Sardinian populations ($\chi^2 = 22.2$, df = 9, $P = 0.0084$) and a significant difference ($\chi^2 = 20.5$, df = 1, $P = 5.844e^{-06}$) in the proportion of the two white floral types on the two islands (Fig. 5). On Sardinia, the frequency of both types of white flowers varies markedly among populations, from an almost absence in several populations to frequencies similar to those on Corsica. As the frequency of white flowers decreases on Sardinia, we observed that the relative proportion of flowers with an inserted stigma (BSI) increases, hence the differences among populations in their relative proportion on Sardinia.

Hybrid vigour

We detected significantly larger flower size on Sardinia for both types of bicoloured flowers (Fig. 5), *i.e.* with a narrow pink ring (analysis of deviance, $\chi^2 = 7.2$, df = 1, $P = 0.043$) and with a wide pink ring (analysis of deviance, $\chi^2 = 11.2$, df = 1, $P = 0.005$). There was no significant difference in flower size between the two islands for pale-pink flowers (analysis of deviance, $\chi^2 = 0.1$, df = 1, $P = 1$), flowers resembling *C. repandum* (analysis of deviance, $\chi^2 = 0.5$, df = 1, $P = 1$), white flowers with an exerted stigma (analysis of deviance, $\chi^2 = 2.3$, df = 1, $P = 0.757$) and white flowers with an inserted stigma (analysis of deviance, $\chi^2 = 6.4$, df = 1, $P = 0.067$).

The comparison of mean flower size revealed significant differences between floral types on both Corsica (analysis of deviance, $\chi^2 = 119.7$, df = 5, $P = < 0.001$) and Sardinia (analysis of deviance, $\chi^2 = 271.9$, df = 5, $P = < 0.001$). The patterns of variation were different on the two islands. On Corsica, *post-hoc* means tests showed that flowers typical of *C. repandum* are significantly larger than flowers of all the other floral types (Fig. 5, Table 2). Pale-pink flowers were significantly smaller than the *C. repandum* type flowers, bicoloured flowers with a wide corolla ring and white flowers with an exerted stigma. White flowers with an inserted stigma were significantly smaller than white flowers with an exerted stigma. In addition, pale-pink flowers and white flowers with an inserted stigma were not significantly different from each other. On Sardinia, flowers typical of *C. repandum* were significantly larger than flowers of the other floral types, with the exception of bicoloured flowers with a wide ring that were also significantly larger than all other floral types (Fig. 6, Table 2). Pale-pink flowers were significantly smaller than all the other floral types. Unlike on Corsica the two types of white flowers were not significantly different in size.

The mean number of flowers per 1-m² quadrat varied from ten to 29 across the 13 populations. The bicoloured flower types showed the highest frequency of flowers in quadrats in nine of the 13 populations and the floral type typical of
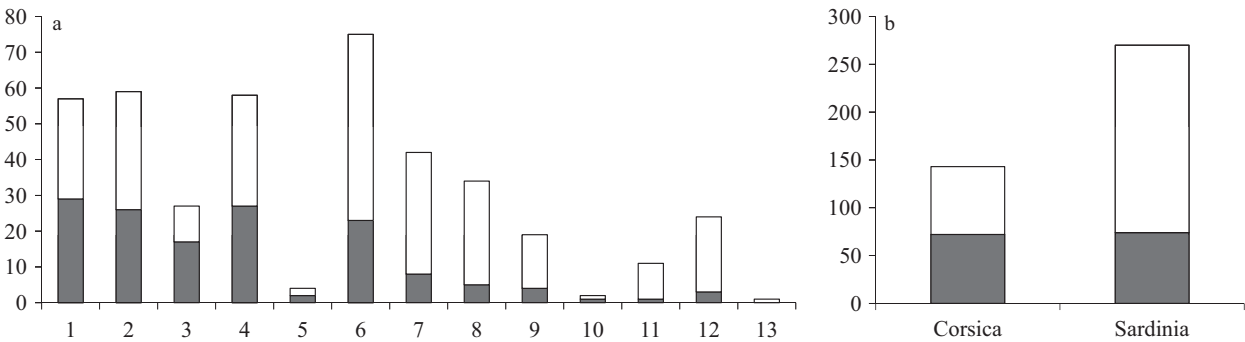
Fig. 5. Number of white flowers with inserted stigma (white bar) and exerted stigma (black bar) in the 13 mixed populations of *Cyclamen balearicum* and *C. repandum* subsp. *repandum* on Corsica (populations 1–3) and Sardinia (4–13): a: per population and b: the total number on each island (2373 flowers on Sardinia and 689 flowers on Corsica).

Table 2. Synthesis of *post-hoc* tests for differences in floral size of different floral types in mixed populations of *Cyclamen balearicum* and *C. repandum* subsp. *repandum* on (a) Corsica and (b) Sardinia. The lower panel presents the difference between morphs (and its SE The upper panel presents the *P*-value associated with each *post-hoc* test and its significance level after a Bonferroni correction (ns, non-significant; *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$). Floral types are large pink flowers typical of *Cyclamen repandum* subsp. *repandum* (RSE), bicoloured flowers with a wide basal ring (BIW), bicoloured flowers with a narrow basal ring (BIN), pale-pink flowers (PAL), white flowers with exerted stigma (BSE), and white flowers with inserted stigma similar to *C. balearicum* (BSI).

	RSE	BIW	BIN	PAL	BSE	BSI
(a) Corsica						
RSE		0.00226**	<0.001***	<0.001***	<0.001***	<0.001***
BIW	−1.7394 (0.4636)		0.55419	0.00160**	0.99645	<0.001***
BIN	−2.6100 (0.4047)	−0.8705 (0.5272)		0.06089	0.83171	0.05835
PAL	−4.3247 (0.5818)	−2.5853 (0.6731)	−1.7148 (0.6207)		0.00488**	0.99617
BSE	−2.0124 (0.4433)	−0.2730 (0.5605)	0.5976 (0.4985)	2.3123 (0.6507)		0.00304**
BSI	−4.0015 (0.4483)	−2.2621 (0.5606)	−1.3916 (0.5007)	0.3232 (0.6529)	−1.9891 (0.5386)	
(b) Sardinia						
RSE		0.99826	<0.001***	<0.001***	0.02624*	<0.001***
BIW	0.1151 (0.2753)		<0.001***	<0.001***	0.00830**	<0.001***
BIN	−1.5822 (0.2717)	−1.6974 (0.2573)		<0.001***	0.99884	0.00693**
PAL	−4.3703 (0.3319)	−4.4854 (0.3140)	−2.7881 (0.3162)		<0.001***	<0.001***
BSE	−1.4091 (0.4626)	−1.5242 (0.4478)	0.1732 (0.4506)	2.9612 (0.4880)		0.06146
BSI	−2.7736 (0.3621)	−2.8888 (0.3424)	−1.1914 (0.3450)	1.5967 (0.3918)	−1.3645 (0.4956)	

C. repandum had the highest frequency in four populations (Fig. 3). We detected a significant difference between Corsica and Sardinia in the relative frequency of the different floral types in the quadrat survey (np Manova with 999 permutations, $F_1 = 3.4837$, $P = 0.045$).

We found a significant difference between relative flower frequency in quadrats compared to the random population count for two floral types: bicoloured flowers with a wide corolla ring (two-sided Wilcoxon paired test, $V = 91$, $P = 0.0015$) and pale-pink flowers (two sided Wilcoxon paired test, $V = 2$, $P = 0.0066$) but not for the other floral types (Fig. 7). For the three hybrid floral types we tested whether bicoloured flowers presented a higher frequency in quadrats than in the overall random population count and whether pale-pink flowers show the opposite trend. We found a significantly higher frequency of bicoloured flowers with a wide corolla ring in quadrats than in the overall population count (one sided Wilcoxon paired test, $V = 0$, $p = 0.0011$) but no significant trend for bicoloured flowers with a narrow corolla ring (one-sided Wilcoxon paired test, $V = 19$, $P = 0.3065$). Finally, we detected a significantly lower frequency of pale flowers in quadrats than in the overall

population count (one-sided Wilcoxon paired test, $V = 89$, $P = 0.0033$).

Literature review

Our literature search revealed 60 clear-cut cases of hybridisation in native Mediterranean plants (Table 3), with 44 cases of homoploid hybridisation, 22 of which involve fully established new species and 22 in which hybrids occur in mixed populations where hybridisation bears a contemporary signature and may be on-going. This indicates that examples of contemporary evolution are prevalent in the literature, and as far as the recent literature is concerned, at an equivalent prevalence as examples of known species of hybrid origin. We also detected an almost equivalent number (16 examples) of well-documented cases of allopolyploidy, all of which concern the documentation of a clearly defined species. Allopolyploidy is generally thought to be more frequent than homoploid speciation (Abbott *et al.* 2010) and a more extensive review of the older literature on chromosome variation would probably prove this to be true in Mediterranean plants.

Fig. 6. Comparison of corolla length of the six floral types in mixed populations of *Cyclamen balearicum* and *C. repandum* subsp. *repandum* on Corsica (white bars) and Sardinia (black bars). Stars indicate significant differences between islands for a given floral type. A common letter in different bars means no significant difference (see Table 2).
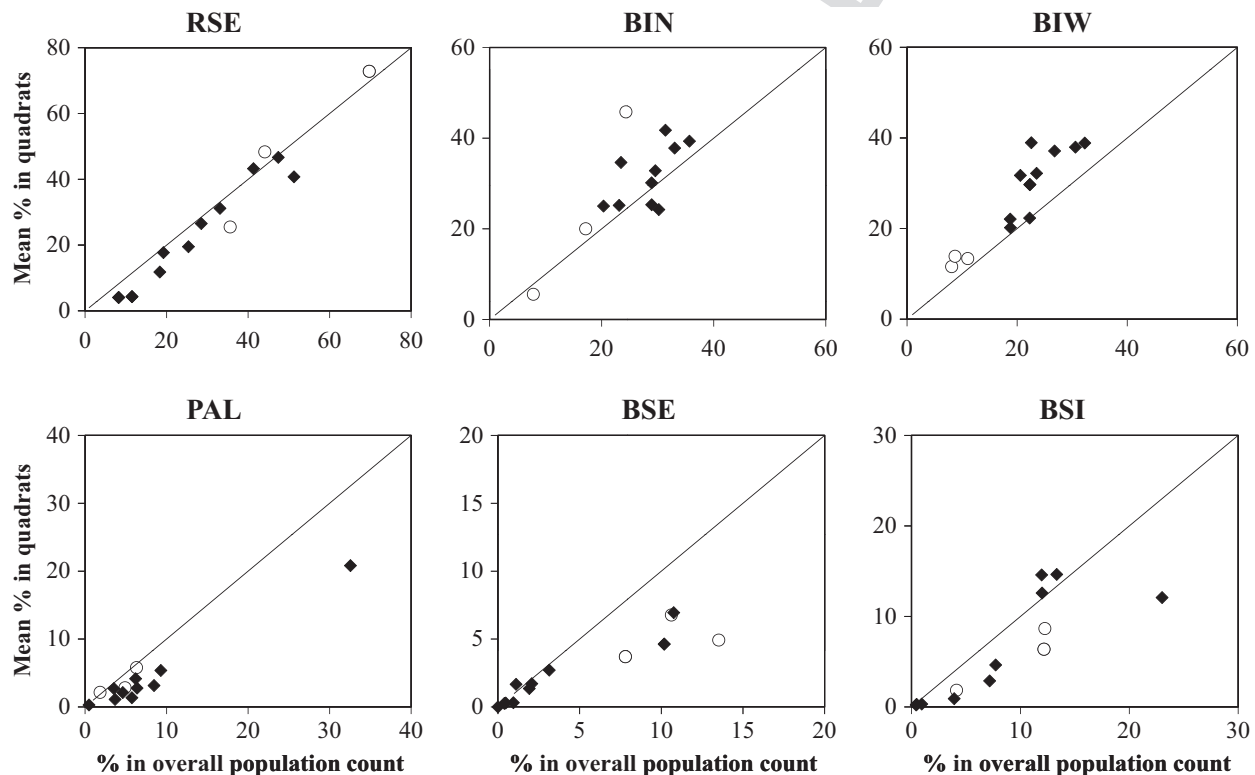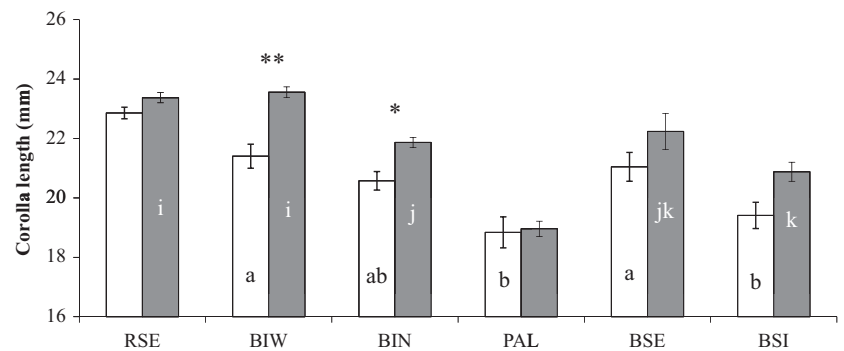
Fig. 7. Mean frequency of each floral type in mixed populations of *Cyclamen balearicum* and *C. repandum* subsp. *repandum* on Corsica (white symbols) and Sardinia (black symbols). The *y*-axis represents the mean frequency of a given floral type in the 1-m² plots and the *x*-axis depicts the frequency of the floral type in the random population count. The black line represents the bisector (equal frequency in the two types of count). RSE – large pink flowers typical of *Cyclamen repandum* subsp. *repandum*, bicoloured flowers with a wide (BIW) or narrow (BIN) basal ring (grey bars), PAL – pale-pink flowers and white flowers with an exerted (BSE) or inserted (BSI) stigma (white bars).

Several large genera which have shown important radiation and divergence in the Mediterranean Basin show recurrent hybridisation between different pairs of species, *e.g.* in genera such as *Senecio* (Abbott *et al.* 2010), *Anthemis* (Lo Presti & Oberprieler 2011), *Antirrhinum* (Vargas *et al.* 2009), *Rhododendron* (Milne *et al.* 1999), *Serapias* (Sardaro *et al.* 2012), *Saxifraga* (Mas de Xaxars *et al.* 2015) and *Narcisssus* (Marques *et al.* 2010), or among the same species in disjunct parts of their range, *e.g.* in *Phlomis* (Albaladejo *et al.* 2005; Georgescu *et al.* 2016), *Helichrysum* (Galbany-Casals *et al.* 2012) and *Narcisssus* (Marques *et al.* 2010). Several of the examples we report illustrate the importance of hybridisation as species ranges have contracted and expanded and brought congeners into reproductive contact, *e.g. Primula* (Casazza

et al. 2013), *Abies* (Bella *et al.* 2015) and *Narcissus* (Marques *et al.* 2007).

DISCUSSION

In this study we illustrate the replicated occurrence of hybridisation between *Cyclamen repandum* subsp. *repandum* and *C. balearicum* on the Mediterranean islands of Corsica and Sardinia. Patterns of floral morph frequencies and flower size indicate that the hybrid populations on the two islands may be at different stages in the evolutionary process and that hybrid vigour may contribute to the dynamics of the process. We set these results into the context of the conservation significance of hybrid populations in Mediterranean plants.

Table 3. Evidence for (column '1') the role of homoploid hybridisation (H) and allopolyploidy (P) (column 2) in species diversification (D) or population level variability due to hybridisation that is potentially on-going (O) in Mediterranean species and their populations.

genus	ecological and evolutionary context of hybridisation	1	2	reference
Abies	*A. x borisii-regis* populations with a restricted distribution in the southern Balkan Peninsula result from hybridisation between *A. cephalonica* and *A. alba*	H	D	Bella *et al.* (2015)
Acrolophus	The origin and diversification of section *Willkommia* is linked to hybridisation	H	D	Suárez-Santiago *et al.* (2007)
Alyssum	Allopolyploid speciation accompanied by ecological specialisation and geographic isolation in the *Alyssum montanum* complex	P	D	Zozomová-Lihová *et al.* (2014)
Anacamptis	Hybridisation between widespread *A. fragrans* and Mallorcan endemic *A. robusta* has produced hybrid *Anacamptis x albuferensis* that is expanding in sympatry with the latter	H	O	Ren *et al.* (2014)
Anacamptis	Hybrid zones of *Orchis laxiflora* and *O. palustris* with a low frequency of hybrids	H	O	Arduino *et al.* (1996)
Anthemis	Hybridisation in the *A. secundiramea* group in both the eastern (*A. arvensis* and *A. peregrina*) and western (*A. maritima* and *A. pedunculata*) Mediterranean	H	D	Lo Presti & Oberprieler (2011)
Anthoxanthum	Recurrent hybridisation (and/or polyploidy) from the Miocene to the Late Pleistocene	H	D	Pimentel *et al.* (2013)
Antirrhinum	Recurrent hybridisation and species divergence	H	D	Vargas *et al.* (2009)
Antirrhinum	A cline in flower colour across a hybrid zone (*A. majus pseudomajus* and *A. majus striatum*)	H	O	Whibley *et al.* (2006)
Arenaria	Widespread species (*A. erinacea*, *A. tetraquetra* and *A. querioides*) have gene sequences scattered through an ITS tree due to hybridisation and/or incomplete lineage sorting	H	D	Valcárcel *et al.* (2006)
Armeria	Gene sequence data for several species and samples of disjunct subspecies of *A. villosa* indicate frequent hybridisation	H	D	Fuertes *et al.* (1999)
Armeria	*A. villosa*, *A. splendens* and *A. filicaulis* in distinct altitudinal belts share haplotypes as a result of altitudinal range extensions and (secondary) contact among taxa	H	O	Gutiérrez *et al.* (2002)
Avena	Historical evolution of crop ancestors	P	D	Liu *et al.* 2017
Bellevalia	Allopolyploid origin of Cretan endemic *B. sitiaca*	P	D	Bareka *et al.* (2012)
Brachypodium	Allopolyploid origin of *B. distachyon* in the southern part of the Iberian peninsula	P	D	Hammami *et al.* (2011)
Campanula	Evolution and ecological specialisation of narrow endemic *C. specularioides* in rocky limestone habitats in the mountains of the Betic Serrania de Ronda	H	D	Cano-Maqueda *et al.* (2008)
Cardamine	Hybridisation in the *C. maritima* group in coastal areas of the Balkans	H	D	Kučera *et al.* (2010)
Centaurea	The absence of conspecific groups in the *Acrolophus* subgroup due to hybridisation	H	D	Suárez-Santiago *et al.* (2007)
Centaurea	Hybridisation between two narrow endemic species (*C. horrida* and *C. filiformis*) on the island of Tavolora (northeast Sardinia)	H	D	Pisanu *et al.* (2011)
Centaurea	Evolution of the Iberian endemic *C. podospermifolia*	H	D	López-Pujol *et al.* (2012)
Centaurium	Allopolyploid speciation of endemic *C. bianoris* on Majorca (salmon-coloured corolla) from *C. maritimum* (yellow corolla) and *C. tenuiflorum* subsp *acutiflorum* (pink corolla)	P	D	Zeltner (1978); Mansion *et al.* (2005); Guggisberg *et al.* (2006)
Cyclamen	The evolution of intermediate floral phenotypes and genetic introgression in mixed populations of *C. balearicum* and *C. repandum* on Corsica	H	O	Thompson *et al.* (2010)
Dianthus	Rapid diversification of allopolyploid *Dianthus broteri*	P	D	Balao *et al.* (2010)
Elatine	An incongruent phylogeny of Mediterranean species attests ancient hybridisation	H	D	Sramkó *et al.* (2016)
Gagea	30% of taxa thought to have evolved *via* hybridisation in sect. *Didymobulbos*	H	D	Peterson *et al.* (2009); Tison *et al.* (2013)
Helichrysum	Hybridisation between *H. orientale* and *H. stoechas* at different sites (Crete and Rhodes)	H	O	Galbany-Casals *et al.* (2012)
Helichrysum	Hybridisation among lineages of the *H. pendulum* complex in the western Mediterranean	H	D	Herrando Moraira (2014)
Limonium	Levels of intra-individual (*L. delicatulum*) and intra-population (*L. furfuraceum*) divergence similar to that among other *Limonium* species	H	O	Palacios *et al.* (2000)
Limonium	Patterns of morphological variation suggest multiple hybridisation in sympatric sites of *L. humile* with *L. vulgare* and *L. vulgare* with *L. narbonense*	H	O	Cortinhas *et al.* (2015)
Limonium	Patterns of genetic variation in *L. dufourii* best explained by multiple hybridisation events and isolation between populations	H	O	Palop-Esteban *et al.* (2007)
Linaria	Ten out of 17 individuals in *Linaria* subsection *Supinae* with incongruent positions in gene trees appear to be due to hybridisation	H	D	Blanco-Pastor *et al.* (2012)
Narcissus	Hybridisation (maternal *N. cavanillesii* with paternal *N. serotinus* or *N. miniatus)* has produced two hybrids (*Narcissus x alentejanus* and *N. perezlarae*, respectively) in different sites	H	D	Marques *et al.* (2010)
Narcissus	Allopolyploid origin of *N. obsoletus* in Spain	P	D	Lifante *et al.* (2009)
Narcissus	Variable rates of hybridisation between narrow endemic *N. cavanillesii* and widespread *N. serotinus* and variable frequency of hybrid plants in different populations	H	O	Marques *et al.* (2007)
Orchis	Hybridisation between *O. mascula* subsp. *ichnusae* and *O. provincialis* in Sardinia (but not in continental sites) and the hybrid status *O. x penzigiana*	H	O	Pellegrino *et al.* (2005)

(continued)

Table 3. (Continued)

genus	ecological and evolutionary context of hybridisation	1	2	reference
Orchis	Leaf surface area is less in parental *Orchis simia* and *O. anthropophora* compared to adult progeny produced by hybridisation (*O. x bergonii*) suggesting a heterosis effect	H	O	Schatz *et al.* (2010)
Orchis	Hybrid zones in sympatric populations of *O. laxiflora* and *O. palustris* with low frequencies of partially fertile hybrids	H	O	Arduino *et al.* (1996)
Ophrys	Hybridisation among sympatric *O. lutea* and *O. fusca* in North Africa	H	O	Stebbins & Ferlan (1956)
Origanum	Hybrid zone containing hybrid *O. x intercendens* and its parental species *O. onites* and *O. vulgare* subsp. *hirtum* on Crete	H	O	Bariotakis *et al.* (2016)
Paeonia	In *P. coriacea* (Spain, Corsica, Sardinia and North Africa), *P. officinalis* (South and Central Europe) and *P. mascula* subsp. *Arietina* (Southeast Europe to Turkey), polyploids are more widespread than diploids	P	D	Sang *et al.* (1997); Ferguson & Sang (2001)
Phlomis	The genus *Phlomis* is represented on Crete by *P. cretica*, *P. fruticosa*, the island endemic *P. lanata* and three hybrids: *P. x cytherea* (*P. cretica* x *P. fruticosa*), *P. x commixta* (*P. cretica* x *P. lanata*) and *P. x sieberi* (*P. fruticosa* x *P. lanata*)	H	D	Georgescu *et al.* (2016)
Phlomis	Isozyme evidence for natural hybridisation and hybrid origin of endemic *P. margaritae*	H	D	Aparicio *et al.* (2000)
Phlomis	Maintenance of a hybrid complex between western Mediterranean *P. crinite and P. lychnitis* where the species overlap in Spain	H	O	Albaladejo *et al.* (2005); Albaladejo & Aparicio (2007)
Primula	Allopatric divergence of *P. allioni* in Pleistocene refugia in the Maritime Alps and introgression with *P. marginata*	P	D	Casazza *et al.* (2013)
Quercus	Evidence of ancient and on-going introgressive hybridisation between *Q. suber* and *Q. ilex* in Morocco and western Mediterranean Europe	H	O	Belahbib *et al.* (2001); Jiménez *et al.* (2004); Lumaret & Jabbour-Zahab (2009)
Ranunculus	Allopolyploid origin of *R. wettsteinii* from *R. montenegrinus* and *R. parnassiifolius*	P	D	Cires & Fernández Prieto (2014)
Rhododendron	Of six possible hybrid combinations of *Rhododendron* with sympatric localities in Turkey, five show evidence of hybrid formation	H	O	Milne *et al.* (1999)
Salvia	Hybridisation of *S. fruticosa* with *S. officinalis* along with the abandonment of grazing contribute to the marked expansion of the former	H	O	Radosavljević *et al.* (2015)
Saxifraga	Species of *Saxifraga* subsect. *Triplinervium* endemic to the northeastern Iberian Peninsula may result from recurrent isolation and hybridisation	H	D	Mas de Xaxars *et al.* (2015)
Saxifraga	The narrow endemic *S. callosa* var *lantoscana* in the Maritime and Ligurian Alps is a natural hybrid between *S. callosa* and *S. cochlearis*	H	D	Grassi *et al.* (2006)
Senecio	*S. flavus* and *S. glaucus* have parapatric distributions in the southeastern Mediterranean where genetically 'glaucus-like' *S. flavus* subsp. *breviflorus* has an allopolyploid origin from *S. flavus* subsp. *flavus* and *S. glaucus*	P	D	Comes & Abbott (2001)
Senecio	Hybrid swarms and parental Sicilian endemics *S. aethnensis* and *S. chrysanthemifolius* on Mt Etna	H	O	Abbott *et al.* (2000)
Serapias	Italian and Greek populations of *S. politisii* have two clades each with one parental species and an exclusive haplotype fixed in all populations on the Salento peninsula	H	D	Bellusci *et al.* (2008, 2010)
Serapias	Genetic relationships among 36 populations of eight *Serapias* species and nine populations of putative hybrids illustrate frequent hybridisation	H	O	Sardaro *et al.* (2012)
Thymus	Multiple cases of hybridisation among Iberian species	P	D	Morales (2002)
Ulex	Hybridisation between diploid species and subsequent polyploidisation, may have produced two tetraploids (*U. congestus* and *U. borgiae*) that have colonised new habitats	P	D	Cubas *et al.* (2005)
Vella	Allopolyploid origin of narrow endemic *V. pseudocytisus* subsp. *paui* in eastern Spain	P	D	Pérez-Collazos & Catalán (2006)
Veronica	Phylogenetic incongruence due to hybridisation in *Veronica* section *Beccabunga*	P	D	Ellmouni *et al.* (2017)
Veronica	Multiple origins of allopolyploid *V. cymbalaria*: one lineage likely originated in southern Turkey (parental *V. panormitana*), the other on the western coast of Turkey (parental *V. trichadena*), and both lineages spread across the Mediterranean	P	D	Albach (2007)
Viola	Hybridisation between *V. jaubertiana* (endemic to the Balearic Islands) and widespread *V. alba* subsp. *dehnhardtii* is unidirectional (*V. jaubertiana* is the unique ovule donor) and hybrids occur only in natural rupicolous habitats	H	O	Conesa *et al.* (2008)

Allopatric hybrid populations at different stages in their evolution

As we have shown in Table 3, the recurrent origin of hybrids has been documented in several Mediterranean genera and species. Here we provide a new example of recurrent homoploid hybridisation between two Mediterranean species but on different islands, as reported for *Helichrysum orientale* and *H. stoechas* on Crete and Rhodes (Galbany-Casals *et al.* 2012). The same floral types that occur in three mixed populations of

Cyclamen repandum subsp. *repandum* and *C. balearicum* on Corsica also occur in a range of populations on the island of Sardinia, indicative of a recurrent origin of hybridisation on the two islands. Mixed populations occur in limestone woodlands and scrub that constitute the optimal ecological situation for *C. balearicum* but an ecologically marginal situation for *C. repandum* subsp. *repandum* (Debussche & Thompson 2003). Although the same six floral types coexist in all populations, their relative frequency and relative flower size and morphology show contrasting patterns of variation among populations on the two islands.

Three different hybrid floral types occur in the mixed populations, namely two floral types with bicoloured flowers with either a wide or narrow dark-pink basal ring in the corolla and a pale-pink flowered floral type. Both the bicoloured floral types have intermediate size and flower colour compared to the parental floral types, while pale flowers are smaller than all other floral types, even the small white-flowered floral type on Sardinia. Other than in one population on Sardinia, the pale-pink floral type is among the least frequent floral types in most populations as witnessed by its overall population frequency relative to other floral types. When its population-level frequency (surrogate for the number of individuals in a population) is compared with its mean frequency in small quadrats in a given population, the latter is significantly lower than expected, as if this floral type produces smaller numbers of flowers per plant than other floral types. The pale-pink floral type may suffer from hybrid depression or function similarly to floral types akin to parental *C. balearicum* that have similar (variation in) frequencies.

In contrast, several pieces of information point to hybrid vigour in the bicoloured floral types and in a way that attests to the fact that the evolutionary process of hybridisation may be at different stages on Corsica and Sardinia. First the bicoloured floral types have an intermediate flower size in populations on Corsica, whereas on Sardinia, where both bicoloured floral types have significantly larger flowers, those with wide basal pink corolla ring have flowers of a size that makes them resemble parental *C. repandum*. This shift in flower size may be a marker of more advanced introgression in Sardinian populations. Second, whereas on Corsica the parental floral type of *C. repandum* is the most frequent floral type in all three populations, in several populations on Sardinia the bicoloured floral types are either as frequent or more frequent than the parental floral type typical of *C. repandum*. In addition, in the comparison of flower number per quadrat (surrogate for the relative flower number per plant of each floral type) with flower number in the random flower count in a population (surrogate for the relative number of individuals of each floral type), the bicoloured flowers with a wide basal corolla ring are significantly more abundant than expected in quadrats, as if they produced relatively more flowers per individual than other floral types, particularly in Sardinian populations.

The genetic variability of the hybrid populations on Corsica is higher than in allopatric parental species populations in terms of both the presence of higher proportion of polymorphic loci and enhanced heterozygosity (Thompson *et al.* 2010). Hence, mixed populations have a basis on which to show hybrid vigour, as observed in wild *Narcissus* hybrids (Marques *et al.* 2010) where it may provide an initial advantage for the establishment of hybrids. This phenomenon can facilitate the increased frequency of hybrids in natural populations observed here in Sardinia, and can also confer evolutionary potential to natural hybrids. This has been observed in mixed populations of *Narcissus* species in the western Iberian Peninsula where the parental species show marked overlap in flowering phenology (Marques *et al.* 2011).

Variation in frequency and size of white flowers also fits with the idea of a difference in the evolutionary stage of hybridisation on the two islands. *Cyclamen balearicum* has never been reported from Sardinia, which could result from a gap in floristic knowledge, but could also illustrate that, in terms of the true morphology of *C. balearicum* (small white flowers slightly tinged with reddish veins, and silvery grey patches to their dark green leaves), this species has become more completely introgressed than on Corsica. Our personal observations indicate a similar trend for leaf morphology, and we found no leaves typical of *C. balearicum* on Sardinia. On Corsica, the floral phenotype that resembles parental *C. balearicum* is however still present despite genetic introgression (Thompson *et al.* 2010), evidence that selection may be acting to maintain the ancestral floral phenotype, perhaps as a result of selection for autonomous self-pollination (Affre & Thompson 1999). In this study we found that on Corsica there is a significant distinction between 'repandum-like' white flowers with an exerted stigma that are significantly larger than 'balearicum-like' white flowers with an inserted stigma. On Sardinia this distinction in size is less apparent, as if the *C. balearicum* phenotype with an inserted stigma has evolved larger flowers due to a longer history of introgression by the *C. repandum* genome. On Sardinia, white flowers are extremely rare in several populations, particularly the form with an exerted stigma, which becomes increasingly rare (relative to the form with an inserted stigma) as the total frequency of white flowers declines. All in all, it would appear that white flowers with an inserted stigma (typical of parental *C. balearicum*) have been maintained in more populations (perhaps by selection for autonomous selfing) than white flowers with an exerted stigma that may have been more frequently introgressed by pink-flowered forms.

These differences in floral type frequencies and flower size fit with the idea of a more advanced status of the hybridisation process in the Sardinian populations than in the Corsican populations. This may be a question of age, *i.e.* an earlier hybridisation event on Sardinia, or because of the greater spatial extent and number of mixed limestone populations on Sardinia. Assuming that each hybrid zone on the two islands has arisen from contact between previously separate species, the observed variation in floral traits and relative frequency in floral types may represent recent contact with a neutral mixing of genes of the two species, or a longer history of contact, and/or floral type variation subject to selection maintaining differences (*e.g.* see Whibley *et al.* 2006).

In addition to age, the differences in hybrid floral type frequencies could be due to an initially greater size in the initial *C. balearicum* population, which may have been spread over a larger geographical area on Sardinia. The typical habitat of *C. balearicum*, but not *C. repandum* subsp. *repandum*, is limestone woodland (Debussche & Thompson 2003), which is much more spatially extensive in northwest Sardinia than on Corsica. Indeed we were able to sample mixed populations in a larger geographic area on Sardinia than on Corsica. The abundance of hybrid floral types on Sardinia may thus have been

favoured by a greater potential for continued hybridisation on Sardinia due to initially higher abundance of the parental *C. balearicum*.

Our results open two interesting perspectives for future work. First, information on the production of fruit and viable seeds in the different floral types in mixed populations on the two islands, along with observations of pollinator visitation rates to the different floral types represent interesting perspectives for future work here. This would help validate any potential hybrid vigour, particularly on Sardinia, and would shed light on the underlying processes that facilitate the evolution of the frequency of hybrid floral types. In addition, it would be interesting to confirm observations of occasional occurrence of flower colour variation outside of the limestone massifs studied on the two islands to assess the potential for the spread of multi-coloured and white floral types toward the rest of the *C. repandum* distribution on the two islands. This would be congruent with the geological history of Sardinia where the northwestern limestone areas were probably isolated from the rest of the island in the Miocene (Carmignani *et al.* 2016 and references therein).

In the Mediterranean region, hybridisation represents an evolutionary process generating diversity as species ranges have contracted and expanded and brought congeners into reproductive contact (Thompson 2005; Table 3). Although further studies will be required to better assess the relationships between hybridisation and specific paleo-climatic events, hybrid speciation has probably played a major role where the climatic niche of parental species has shifted either following sea level variation due to the Messinian salinity crisis (Gargani & Rigollet 2007; Mongelli *et al.* 2012) or Quaternary glaciations (Mikolajewicz 2011). Several examples in Table 3 attest to the evolution of hybrid species in climatic refugia or zones of contact that occur as a result of range expansion (Casazza *et al.* 2013; Ren *et al.* 2014; Bella *et al.* 2015; Mas de Xaxars *et al.* 2015). This provides evidence that hybridisation has been a key process underlying the temporal phasing of the evolutionary dynamics of Mediterranean plants with geological and climate history, and thus merits interest from conservation scientists (Thompson 2005).

Conservation value of hybrid populations

The evolutionary processes that maintain and promote diversity within and among species have been increasingly recognised as important elements for biodiversity conservation (Smith *et al.* 1993; Moritz 1994, 2002; Crandall *et al.* 2000; Stockwell *et al.* 2003; Ellstrand *et al.* 2010). Hybridisation represents a topic of particular interest in this respect due to its major role in plant evolution (Allendorf *et al.* 2001; Cozzolino *et al.* 2006; Ellstrand *et al.* 2010; Thompson *et al.* 2010; Stronen & Paquet 2013; Jackiw *et al.* 2015).

In this context, most authors distinguish between hybrids that have a natural or anthropogenic origin because hybridisation among introduced and native species may lead to genetic pollution of the latter. Thompson (2005) proposed that sites of potential and known hybridisation in the Mediterranean be identified in a database containing: (i) sites where endemic species are at risk from genetic introgression; (ii) sites of risk for the evolution of invasive species; and (iii) potential sites of future evolution in the native flora. We detected 42 clearly established cases of homoploid hybridisation; 50% of cases of homoploid hybridisation documented in the literature on Mediterranean plants involve examples akin to our study system of *Cyclamen* in which mixed populations occur and hybridisation bears a contemporary mark, *e.g.* in *Narcissus* (Marques *et al.* 2007, 2010), *Anacamptis* (Ren *et al.* 2014) and orchids (Cozzolino *et al.* 2006). Such cases often concern a rare or endemic species that hybridises with a more common or widespread species, *i.e.* situations that are conjointly of types 'a' and 'c' mentioned by Thompson (2005); *i.e.* an endemic species that is both at risk of genetic extinction but undergoes introgression that is a natural evolutionary process that enhances genetic and floral diversity. Hence the dilemma discussed by Cozzolino *et al.* (2006); Marques *et al.* (2007); Thompson *et al.* (2010) and López-Pujol *et al.* (2012) of whether or not we should conserve pure parental species or high diversity hybrid populations that may represent incipient species. The design of conservation management policy here greatly depends on balancing the need to safeguard the 'purity' of a species (of particular significance when placed on a protection list) *versus* the need to protect hybrid populations that may be the most 'viable' for contemporary evolution (*e.g.* Allendorf *et al.* 2001, 2004; Marques *et al.* 2007).

The genetic variability of the hybrid *Cyclamen* populations we studied on Corsica is higher than in parental species populations (Thompson *et al.* 2010). In addition, the floral variability detected in the St. Florent populations covers the entire range of floral types of different allopatric subspecies of *C. repandum* across the western Mediterranean (Debussche & Thompson 2002). We also detected that hybrid populations can show hybrid vigour and on-going evolution. Given their localisation on islands, the parental species are not genetically vulnerable to extinction from hybridisation that cannot occur in populations outside of Corsica and Sardinia due to the allopatric distribution of the two parental species. Thus, in situations such as ours, and probably several of the 21 examples we detected of contemporary hybridisation and evolution, we contend that there is a need for a conservation strategy for hybrid populations, at least until there is more information on their ecological persistence and adaptive capacity. The mixed populations we describe on Corsica and Sardinia may ultimately represent a novel form of "evolutionary significant units" (Moritz 1994) and are thus priority sites for future conservation policy whose objective is the protection of the processes that create diversity in natural populations (Forest *et al.* 2007).

Conservation objectives should thus target not only pure species and their populations, including species of hybrid origin, but also mixed populations with hybrid individuals, that represent sources of genetic variation for future evolution, in order to assure the conservation of evolutionary potential (Thompson *et al.* 2010; López-Pujol *et al.* 2012). However hybridisation and other evolutionary processes are rarely clearly identified in conservation plans. Our two study areas occur in areas that have conservation management plans that recognise that they have been historically associated with different traditional human uses of Mediterranean woodlands. Conservation plans for hybridisation sites should thus assess any effects of contemporary human intervention and woodland management on their viability and evolution. Indeed, the integration of our results into management guidelines is the next challenge.

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