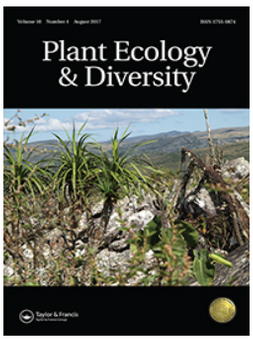

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Recurrent origin of peripheral, coastal (sub)species in Mediterranean *Senecio* (Asteraceae)

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Background: It is argued that coastal endemic taxa may evolve in parallel at the periphery of the distributional range of a widespread species.
Aims: We tested this hypothesis for the origins of three peripheral, coastal isolates of *Senecio, S. glaucus* ssp. *glaucus* (Israel), *S. g.* ssp. *coronopifolius* p.p. (Sicily), and *S. hesperidium* (Morocco), from widespread *S. glaucus* ssp. *coronopifolius*. We also determined the relative roles of selection vs. genetic drift in shaping phenotypic divergence in ssp. *glaucus* and *S. hesperidium*, using Lande's test of neutral morphological change.
Methods: We surveyed morphological and/or allozyme variation in the three peripheral isolates and mainly inland populations of *S. g.* ssp. *coronopifolius.*
Results: Genetic data supported independent origins of the coastal taxa from nearby populations of ssp. *coronopifolius*. These descendant and ancestral populations showed pronounced morphological but weak genetic differentiation. Phenotypic similarities between ssp. *glaucus* (Israel) and *S. hesperidium* (Morocco) in plant height and floral traits may have resulted from parallel divergent selection from ssp. *coronopifolius*, though drift remains an alternative cause in *S. hesperidium*.
Conclusions: Our results indicate parallel ecotype formation and (sub)speciation in *Senecio* in which primarily selective vs. neutral determinants promoted the recurrent origin of coastal types in, respectively, Israel and Morocco.

Keywords: allozyme diversity; Asteraceae; genetic drift; Mediterranean; morphometrics; natural selection; parallel speciation; *Senecio*

Introduction

The origin of incipient (neo-)species on the periphery of a species' range or at disjunct peripheral sites is a topic of long-standing interest in evolutionary biology (e.g. Mayr 1963; Eldredge and Gould 1972; Carson and Templeton 1984; Gould and Eldredge 1993; Levin 2000, 2001; Gaston 2003; Hardie and Hutchings 2010). Indeed, small populations isolated at species' range margins ('peripheral isolates' *sensu* Mayr 1963) may play an important role in population genetic divergence and the formation of species because of the increased efficiency of genetic drift, reduced gene flow, and/or intensified selective pressures (Soulé 1973; Lesica and Allendorf 1995; Bridle and Vines 2006; Hardie and Hutchings 2010). Likewise, it has been argued that peripheral isolates are formed 'on a regular basis' in many plant species (Stebbins 1974; Grant 1981; Levin 2000, 2001). Building on earlier empirical studies (e.g. Turesson 1922; Gregor 1939; Clausen et al. 1948), it is feasible therefore that peripherally isolated populations occurring in the same range of habitats within an ancestral species contribute to the recurrent formation of phenotypically similar ecotypes (varieties, subspecies, and perhaps even new species) via parallel adaptation to similar selective pressures (e.g. Schluter 2004; Rundle and Nosil 2005; Abbott and Comes 2007; reviewed in Ostevik et al. 2012).

However, proving such parallel ecological (incipient) speciation is a daunting task with four criteria needing to be satisfied (Schluter and Nagel 1995; Ostevik et al. 2012): (1) (phylo-)genetic evidence has to show that populations of the same ecotype from different areas are distinct (*independence* criterion), while those of different ecotypes from the same area are closest relatives; (2) and (3) populations of different ecotypes from the same area must be reproductively isolated while those of the same ecotype must be reproductively compatible (*isolation* vs. *compatibility*); and (4) the shared characteristics of the same ecotypes must be shown to have evolved through ecological divergent selection. In fact, Ostevik et al. (2012) concluded from their review that strong evidence for this type of speciation is unexpectedly scarce in plants "relative to the many well-characterized systems in animals" (e.g. sticklebacks: McKinnon and Rundle 2002; marine snails: Johannesson et al. 2010). This discrepancy may reflect less rigorous testing along the aforementioned criteria in plants, yet it would seem premature to state that "plants are less prone to parallel ecological (incipient) speciation than animals" (Ostevik et al. 2012). After all, numerous plant studies of ecotypic differentiation have demonstrated that various traits potentially associated

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with reproductive barriers have been assembled repeatedly, mostly likely through ecological divergent selection, with most of the available data relating to correlated changes in edaphic tolerances, reproductive phenotype (including flowering time) and/or mating system (e.g. Macnair and Gardner 1998; Vijverberg et al. 2000; Foster et al. 2007; Pérez 2011). Nonetheless, few studies have attempted to trace such parallelism in multiple traits in coastal environments as, for example, in *Lasthenia californica* (Rajakaruna et al. 2003a, 2003b) and *Senecio lautus* (Roda et al. 2013), despite widespread earlier evidence that such peripheral and supposedly 'stressful' habitats (Soulé 1973; Hardie and Hutchings 2010) constitute a premier setting for the recurrent origin and adaptive divergence of plant ecotypes (*Hieracium umbellatum*: Turesson 1922; *Plantago maritima*: Gregor 1939; *Agrostis stolonifera*: Hannon and Bradshaw 1968; Venables and Wilkins 1978).

Senecio glaucus L. and *S. hesperidium* Jahand., Maire and Weiller (sect. *Senecio*, Asteraceae) offer an interesting system to further unveil the role of peripherally isolated coastal populations in promoting parallel (incipient) speciation. Both species are diploid ($2n = 20$), entomophilous, annual herbs with showy, rayed capitula that produce pappus-bearing fruits (achenes), hence facilitating dispersal by wind, at least potentially, over long distances. Based on few morphological features, two subspecies have been recognised within *S. glaucus*: ssp. *glaucus*, sometimes treated as a separate species, *S. joppensis* Disnm. (e.g. Feinbrun-Dothan 1978; Danin 2006), and ssp. *coronopifolius* (Maire) Alexander. The latter is one of the most widespread members of the section, with a natural range from the Canary Islands through the southern Mediterranean Basin/North Africa to south-west Asia, the western Himalayas and the deserts of north-west China (Figure 1; Alexander 1979; R.J. Abbott, pers. obs.). It is found in a variety of naturally or man-disturbed habitats such as agrestral and ruderal sandy fields, river banks, stony and rock slopes and desert wadis, at elevations between 0 and 2370 m a.s.l. (Alexander 1979). By contrast, ssp. *glaucus* is endemic to the Mediterranean coastal plain of Israel, where it occurs on maritime sands or sandy dunes not far from the sea (Figure 1; Alexander 1979). It differs from ssp. *coronopifolius* in having a more robust habit, larger capitula, fleshy leaves, and distinctive trifurcate tips of the leaf lobes (Alexander 1979). Finally, *S. hesperidium* is a highly restricted south-west Moroccan endemic, known only from a few populations in the Souss region (Mam-3 *sensu* Rankou et al. 2013), where it occurs on colonised sand dunes and sandy slopes by the sea (Figure 1; Alexander 1979; R.J. Abbott, pers. obs.). Both,

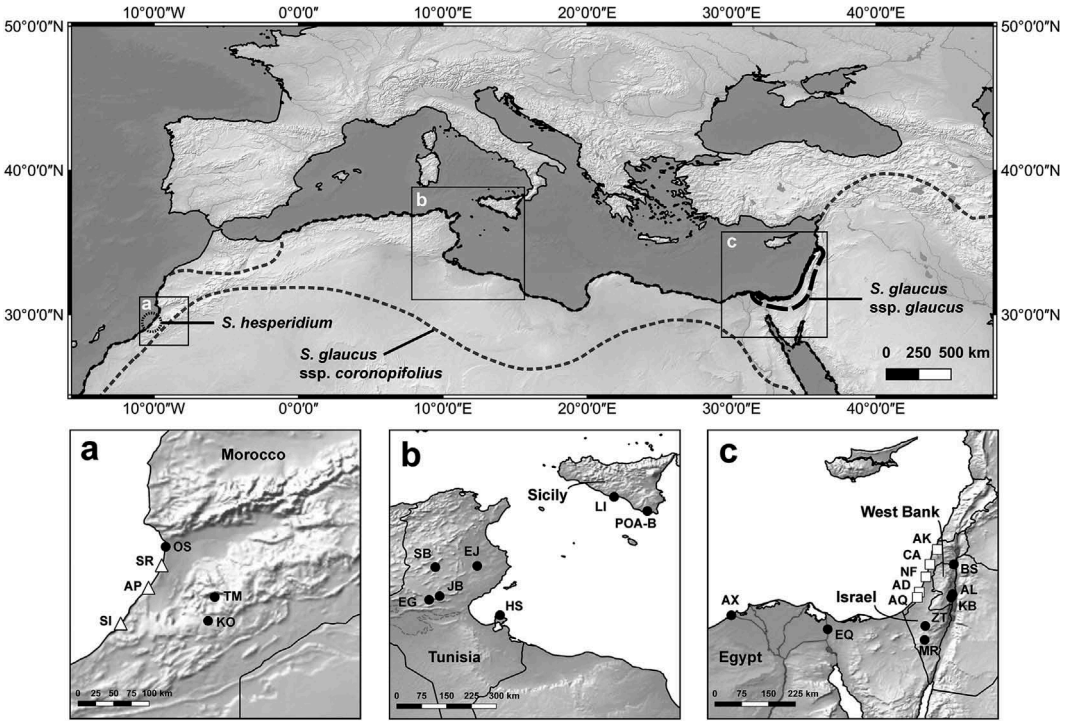

Figure 1. Geographic distribution of *Senecio hesperidium* (dotted circle), *S. glaucus* subspp. *glaucus* (bold long-dashed line) and *coronopifolius* (dashed line) in the southern Mediterranean Basin/North Africa (modified from Alexander 1979). Localities for the taxa sampled in (a) Morocco, (b) the Central Mediterranean (Tunisia, Sicily) and/or (c) the Near East (Israel, West Bank, Egypt) are indicated by symbols (filled white triangles, *S. hesperidium*; filled white squares, *S. g.* ssp. *glaucus*; solid circles, *S. g.* ssp. *coronopifolius*). Most populations sampled were analysed for both allozyme and morphometric variation, except population SI of *S. hesperidium* (only morphometrics) as well as one Sicilian (POB) and all Tunisian (EJ, SB, JB, EG, HS) populations of *S. g.* ssp. *coronopifolius* (only allozymes). Distribution maps were drawn using QGIS v.2.0.1 (QGIS Development Team 2013) and free vector and raster map data at NATURAL EARTH (http://www.naturalearthdata.com; last accessed 10 May 2017). Refer to Table 1 for locality abbreviations and further details.

S. g. ssp. *glaucus* and *S. hesperidium* have large capitula and fleshy leaves, although the latter generally lacks trifurcate leaf lobes (M. Coleman, pers. obs.).

Alexander (1975) suggested that these latter two coastal taxa could have been derived from nearby populations of *S. g*. ssp. *coronopifolius* (see also Kadereit 1984). If so, parallel selection under similar environmental conditions may have caused convergence among these descendant taxa in terms of certain gross morphological characters (Levin 2001; Schluter 2004; Rundle and Nosil 2005; Ostevik et al. 2012). A different scenario suggests that the overall similarities between *S. hesperidium* and *S. g*. ssp. *glaucus* truly reflect common ancestry, in which case their disjunct distribution might have arisen via long-distance dispersal. Phylogenetic analyses in Mediterranean species of sect. *Senecio* (Comes and Abbott 2001), based on nuclear ribosomal DNA (ITS), do not allow distinguishing unambiguously between these alternatives due to shallow levels of sequence variation.

A further question concerns the relationship between disjunct populations of *S. g*. ssp. *coronopifolius* in Sicily and Tunisia, isolated by a sea strait (Figure 1). We are aware of only few populations of this subspecies in Sicily, where it is apparently restricted to maritime sand dune systems and marshland in the southern part of the island (near Licata/Pozzallo; Alexander 1979; Chapman and Abbott 2005; R.J. Abbott and H.P. Comes, pers. obs.). There is some evidence from chloroplast (cp) DNA indicating that *S. glaucus* has repeatedly colonised Sicily from Tunisia (Chapman and Abbott 2005), yet data from nuclear markers are currently lacking to support this scenario. Although morphological examination of material from Sicily and Tunisia has revealed some consistent differences in various floral and leaf traits (Chapman and Abbott 2005), this has not been recognised taxonomically (e.g. Euro+Med 2006–).

In this paper, we report the results of a survey of genetic (allozyme) and morphological variation in peripheral vs. non-peripheral (interior) populations of *Senecio* by means of a hierarchical sampling design in three Mediterranean/North African regions: west (Morocco: *S. hesperidium* vs. *S. glaucus* ssp. *coronopifolius*), central (ssp. *coronopifolius* from Sicily vs. Tunisia), and east [ssp. *glaucus* from Israel vs. ssp. *coronopifolius* from Israel/West Bank/Egypt (henceforth 'Near East')]. One specific goal of this work was to clarify the genetic and morphological relationships between *S. hesperidium* and *S. g*. ssp. *glaucus*, and to obtain evidence for or against the hypothesis of an independent origin of these coastal endemics from the widespread ssp. *coronopifolius* via parallel adaptation to similar environments. Our general hypothesis is that each of the three peripheral isolates originated independently from nearby populations of the latter taxon, and that the isolates' genetic and/or phenotypic divergence varies among regions, potentially reflecting various stages in the process of parallel (sub) speciation in different areas of the Mediterranean/North African range margin of ssp. *coronopifolius*. For example, based on prior taxonomic and morphometric evidence (Alexander 1979; Chapman and Abbott 2005), one may expect an increasing level of divergence in the order: ssp. *coronopifolius* (p.p., Sicilian type) < ssp. *glaucus* < *S. hesperidium*. Furthermore, we are unaware of any studies of parallel (sub)speciation that have determined whether genetic drift could be a cause of phenotypic divergence, and we attempted to do this in the current analysis for two of the peripheral isolates examined (i.e. *S. hesperidium* and *S. g*. ssp. *glaucus*), using Lande's (1976) test of neutral morphological change. Overall, this study should contribute to a more thorough understanding of how genetic and phenotypic variation is partitioned among peripheral and more centrally located plant populations, and the extent to which this system conforms to expectations of parallel ecological (incipient) speciation (Ostevik et al. 2012).

Materials and methods

Plant material

All plant material of *S. hesperidium* and *S. glaucus* was grown from wild collected seed from a total of 26 populations in the southern Mediterranean. These populations were chosen from three different regions (west: Morocco; central: Sicily, Tunisia; and east: Israel, West Bank, Egypt; Table 1 and Figure 1). As detailed in Table 1, all *S. hesperidium* and the great majority of *S. g*. ssp. *coronopifolius* populations sampled in Morocco, Tunisia, and the Near East experience desert or semi-arid climates, whereas those in Sicily (ssp. *coronopifolius*) and coastal Israel (ssp. *glaucus*) are characterised by a Mediterranean climate (as also applies to a single Tunisian population, TM). However, all coastal populations (including those of *S. hesperidium*) are also expected to share particular edaphic conditions, especially water shortage and high salinity (e.g. Comes and Abbott 1999; Al-Taisan 2010; Busoms et al. 2015). Twenty-five populations were included in the allozyme study, 12 of which (from the Near East) had been analysed previously (Comes and Abbott 1999; see below), and 20 in the morphometric analysis. Nineteen populations were common to both studies. The major gap in our morphometric study involved *S. glaucus* from Tunisia although this region was represented in the allozyme study with five populations (Table 1).

Electrophoretic procedure

Allozyme variation was newly analysed in two populations of *S. hesperidium*, and 11 populations of *S. glaucus* ssp. *coronopifolius* from Morocco (three sites), Sicily (three sites), and Tunisia (five sites) (Table 1). Individual genotypes were assessed at seven presumptive allozyme loci by means of horizontal starch gel electrophoresis following Comes and Abbott (1999; and references therein). These loci code for aspartate aminotransferase (E.C. 2.6.1.1; locus *Aat-3*); NADP-dependent isocitrate dehydrogenase (E.C. 1.1.1.42; locus *Idh-1*); glyceraldehyde-3-phosphate dehydrogenase (E.C. 1.2.1.12; locus *G3pd-1*); phosphoglucoisomerase (E.C. 5.3.1.9; loci *Pgi-1* and *Pgi-2*); and phosphoglucomutase (E.C. 5.4.2.2; loci

Table 1. Locality information of sampled populations of *Senecio hesperidium* and intraspecific taxa/(sub)regional samples of *S. glaucus* from the western, central, and eastern Mediterranean region.

Region/taxon/ population code	Country, locality	Latitude	Longitude	Elev (m)	Map (mm)	Mat (°C)	Climate type[a]	*n* (allo)	*n* (morph)	Voucher[b]
West										
S. hesperidium										
SI*	Morocco, Sidi Ifni, roadside above port on compacted stony ground	29°23′N	10°11′W	0	133	19.2	BWh	NA	10	MC & RJA 13/99 (E)
AP*	Morocco, Aglou Plage, sandy shore	29°50′N	09°50′W	0	163	19.6	BWh	31	10	MC & RJA 67-2 (E)
SR*	Morocco, Sidi Rabat, sand dunes	30°07′N	09°40′W	0	201	19.1	BSh	30	10	DGF cult. 66-8 (STA)
S. g. ssp. *coronopifolius*										
KO	Morocco, Khmess Ait Ouafqa (Tiffermit), roadside adjacent to cultivated land.	29°25′N	09°05′W	1358	244	15.3	BSk	33	10	DGF cult. 69-1 (STA)
TM	Morocco, ca. 5 km S of Tizi Mlil, open woodland valley on rocky, sandy substrate	29°43′N	09°00′W	1246	331	13.9	Csa	31	10	DGF cult. 71–1 (STA)
OS	Morocco, Oued Sous, mouth of river Sous/Inezgane, growing along river	30°21′N	09°37′W	0	234	19.1	BSh	31	10	MC & RJA 11/99 (E)
Central										
Ssp. *coronopifolius*										
LI*	Italy, Sicily, 8 km E of Licata, sand dunes nr. Castello di Falconara	37°07′N	13°57′E	11	405	17.7	Csa	30	10	DGF cult. s.n. (STA)
POA*	Italy, Sicily, Pozzallo, open dune system	36°44′N	14°51′E	15	440	17.8	Csa	30	10	DGF cult. s.n. (STA)
POB*	Italy, Sicily, E of Pozzallo, marshland	36°44′N	14°51′E	15	440	17.8	Csa	25	NA	DGF cult. s.n. (STA)
EJ	Tunisia, El Jem, cultivated olive grove	35°15′N	10°15′E	74	274	18.9	BSh	11	NA	MC & RJA s.n. (E)
SB	Tunisia, Sbeitla, weed of flower beds and semi-natural vegetation	35°13′N	09°07′E	536	274	17.2	BSk	12	NA	MC & RJA s.n. (E)
JB	Tunisia, Djebel Biada (Jabal al Bayadah), nr. Saket, on limestone in sparse scrub	34°26′N	09°14′E	1041	193	18.0	BWh	14	NA	MC & RJA s.n. (E)
EG	Tunisia, El Guettar, roadside and adjacent irrigated land in wadi	34°20′N	08°57′E	233	152	19.3	BWh	19	NA	MC & RJA s.n. (E)
HS	Tunisia, Houmt-Souk, sandy shore and dunes	33°53′N	10°52′E	0	212	19.8	BSh	17	NA	MC & RJA s.n. (E)
East										
Ssp. *glaucus*										
AK*	Israel, Haifa Bay Area, ca. 8 km S of Akko, along Mediterranean coastal road	32°52′N	35°05′E	10	558	20.3	Csa	30	10	DGF cult. s.n. (STA)
CA*	Israel, Northern Sharon Coast, Caesarea, coastal sand dunes	32°30′N	34°54′E	14	557	20.2	Csa	30	10	DGF cult. 24-1 (STA)
NF*	Israel, Southern Sharon Coast, Nof Yam/Herzliya, developed entrance to beach	32°11′N	34°48′E	3	567	19.9	Csa	32	10	DGF cult. 25-1 (STA)
AD*	Israel, Judean Coast, Ashdod, coastal sand dunes	31°48′N	34°38′E	8	479	20.0	Csa	30	10	DGF cult. s.n. (STA)
AQ*	Israel, Judean Coast, Ashquelon, coastal sand dunes	31°40′N	34°35′E	33	421	19.9	Csa	30	10	DGF cult. 27-2 (STA)
Ssp. *coronopifolius*										
BS	Israel, Upper Jordan Valley, Bet-She`an, ruderal site	32°30′N	35°30′E	−132	344	21.8	BSh	36	10	DGF cult. 13-1 (STA)

(*Continued*)

Table 1. (*Continued*).

Region/taxon/ population code	Country, locality	Latitude	Longitude	Elev (m)	Map (mm)	Mat (°C)	Climate type[a]	*n* (allo)	*n* (morph)	Voucher[b]
AL	West Bank, Dead Sea, Almog area, roadside	31°45′N	35°28′E	−355	100	24.0	BWh	28	10	DGF cult. 6-1 (STA)
KB	West Bank, Dead Sea, Khirbet Mezin, alluvial fan/delta built by a wadi mouth	31°40′N	35°26′E	−376	90	25.0	BWh	32	10	DGF cult. 4-1 (STA)
ZT	Israel, Be`er Sheva Basin, ca. 4 km S of Zomet Telalim	30°57′N	34°47′E	502	229	19.3	BSh	30	10	DGF cult. 8-1 (STA)
MR	Israel, Central Negev Hills, below Mizpe Ramon, roadside	30°36′N	34°46′E	850	183	16.9	BSk	30	10	DGF cult. 10-1 (STA)
AX	Egypt, Coastal Area, Alexandria, environs of El Silsila Fort	31°13′N	29°55′E	0	183	20.6	BWh	31	10	DGF cult. 31-5 (STA)
EQ	Egypt, Sinai/Suez Canal, El Quantara, surroundings of mooring	30°52′N	32°20′E	6	50	21.6	BWh	30	10	DGF cult. s.n. (STA)

Populations marked by an asterisk (*) are considered peripheral isolates. *n* refers to the number of individuals surveyed in the allozyme (allo) and morphometric (morph) analyses. Note that the 12 populations from the Near East were previously analysed for allozyme variation (Comes and Abbott 1999) and are included for comparative purposes.
Elev: elevation (in metres above or below sea level; derived from www.geoplaner.com); Map and Mat: mean annual precipitation and temperature, respectively [derived from www.climate-data.org, except for sites AL and KB (Holzapfel et al. 1995)]; NA: not analysed.
[a] Climate type according to the Köppen–Geiger classification (Peel et al. 2007): BSh and BSk: hot and cold semi-arid climate, respectively; BWh: hot desert climate; Csa: hot-summer Mediterranean climate.
[b] Vouchers deposited by M. Coleman and R.J. Abbott (MC & RJA) or David G. Forbes (DGF) at the herbaria of the Royal Botanic Garden, Edinburgh (E) or the University of St Andrews (STA).

Pgm-1 and *Pgm-2*). Genetic interpretations of enzyme systems AAT and PGI in *Senecio* have been described elsewhere (Abbott et al. 1992; Ashton and Abbott 1992). Interpretation of the other systems (G3PD, IDH, PGM) was based on the generally conserved enzyme substructure, subcellular location, and number of allozyme loci in higher plants (Weeden and Wendel 1989). Three to four standards were run within each gel to allow for comparison of allozyme loci and allelic data (and their designations) with previous studies. Accordingly, we also used the individual genotypic data set of 12 *S. glaucus* populations from the Near East provided by Comes and Abbott (1999), extracting only information provided by the above five enzyme systems. The final data set used comprised genotypic information at seven loci for 683 individuals from 25 populations, with 11–36 individuals per site (mean ± sd: 27.3 ± 6.88; sample sizes are given in Table 1). The single-individual genotype data matrix generated for this study can be found in Appendix 1 (supplemental data).

Statistical analysis of allozyme data

To first clarify genetic relationships among all populations, we used BIOSYS-1 v.1.7 software (Swofford and Selander 1989) to calculate pairwise chord distances (D_c; Cavalli-Sforza and Edwards 1967) based on allele frequencies at all seven loci surveyed. Similar to our previous studies (Comes and Abbott 1999), we used the chord distance because it is relatively insensitive to variation in evolutionary rates among loci and lineages (Swofford et al. 1996). The resulting distance matrix was subjected to the unweighted pair-group method with arithmetic averages (UPGMA), as implemented in DendroUPGMA (http://genomes.urv.cat/UPGMA/; Garcia-Vallve et al. 1999). The obtained dendrogram output (in Newick format) was visualised and edited using POPTREEW (Takezaki et al. 2014).

The following parameters of genetic diversity were calculated over all allozyme loci for each population, using BIOSYS-1: the percentage of loci polymorphic at the 5% level ($P_{5\%}$), the mean number of alleles observed per locus (A_O), the observed (direct count) heterozygosity (H_O), and Nei's (1987) unbiased estimate of heterozygosity, viz. gene diversity expected under Hardy–Weinberg equilibrium (H_E). Taking variation in sample size into account, we also estimated the total allelic richness (R_S) over all loci per population, using the rarefaction method implemented in FSTAT v.2.9.3.2. (Goudet 2001). Estimation of R_S was adjusted for a minimum sample size of 11 individuals surveyed in population EJ.

To test for departures from random mating, we calculated the inbreeding coefficient f (F_{IS}) across all polymorphic loci for each population according to Weir and Cockerham (1984) using FSTAT. In addition, Hardy–Weinberg equilibrium was tested at each locus in each population and across loci in all populations using the GENEPOP v.3.3 (Raymond and Rousset 1995) probability test option (Fisher's method).

To assess whether population diversity differed between subregions within each of the three Mediterranean regions, mean values of $P_{5\%}$, R_S, and H_E

were calculated over populations within each subregion. Differences in estimates of R_S and H_E between subregions were tested using a permutation procedure implemented in FSTAT, with populations allocated at random to the two alternative subregions within each region.

We also tested whether peripheral populations have passed through a severe and recent genetic bottleneck, as potentially evidenced by a transient excess of observed heterozygotes relative to allele numbers at neutral loci (Luikart et al. 1998; Harper et al. 2003). The significance of departure from mutation-drift equilibrium expectations was calculated for each population using both a sign test, and a one-tailed Wilcoxon signed-rank test as implemented in BOTTLENECK v.1.2.02 (Cornuet and Luikart 1996; Piry et al. 1999). In these analyses, data were pooled across loci, and all loci were assumed to fit an infinite allele model of mutation.

Hierarchical analyses of molecular variance (AMOVAs) were carried out in ARLEQUIN v.3.5 (Excoffier and Lischer 2010) for each Mediterranean region separately (i.e. west: Morocco; central: Sicily/Tunisia; east: Israel/West Bank/Egypt) to quantify the partitioning of allozyme variation at all seven loci between peripheral and non-peripheral groups of populations/taxa (F_{CT}), and among populations within such groups (F_{SC}). Significance levels of variance components were evaluated by a permutation approach (10,100 replicates).

Finally, as the population genetic analyses of this study assume marker neutrality, we also used ARLEQUIN to carry out the Ewens–Watterson–Slatkin exact test of selective neutrality at each polymorphic locus within each population under the infinite allele model. This test compares the observed homozygosity with the expected neutral-equilibrium homozygosity, given the observed number of alleles. Significance was assessed by Slatkin's (1996) exact *P*-test, using 1,000 permutations per test (99 tests in total).

Morphometric analysis

Ten plants (in nearly all cases one offspring per mother plant) were grown from each of 20 populations (Table 1): 6 from Morocco (three of *S. hesperidium* and three of *S. glaucus* ssp. *coronopifolius*); 2 of ssp. *coronopifolius* from Sicily; and 12 populations from the Near East (five of ssp. *glaucus* and seven of ssp. *coronopifolius*). The 200 plants were grown from seed to maturity as single individuals in pots of 13 cm in diameter containing a 1:1 mix of Levington's M2 compost and gravel. Plants were arranged in a fully randomised block design and grown in a greenhouse with natural light supplemented by 400 W mercury vapour lamps with a 16-h day length. At full anthesis of the apical capitulum, each plant was measured for 13 quantitative characters. Ten of those were descriptors of the capitulum, while the remaining three characters were plant height, inflorescence length, and leaf lobe number. Full details of the quantitative measurements made are provided in Table A1 of Appendix 2 (supplemental data). In addition, the presence vs. absence of trifurcate leaf lobes was recorded and frequencies were calculated. This character was recorded as present if any of the lobes of the fifth leaf to emerge displayed this character.

Statistical analysis of morphometric data

For principal components analysis (PCA), character means were calculated for each population and the resulting 20 population × 13 character data matrix was standardised by characters, i.e. subtracting the character mean of the total sample from each populational value, and then dividing by the character standard deviation (program STAND of NTSYS-PC v.2.02g; Rohlf 1998). The standardised matrix was exported to PAST v.1.23 (Hammer et al. 2001) and used to create a character × character correlation matrix as well as a population × population Euclidean distance matrix. The PCA was conducted on the correlation matrix and the populations were projected in two dimensions. A minimum-length spanning tree (MST) was constructed from the distance matrix and superimposed onto the PCA plot to identify similarities among populations when all dimensions were taken into account.

The full 200 individuals × 13 character matrix was also subjected to univariate analyses of variance (ANOVAs) and Tukey's HSD (honestly significant difference) post-hoc test using SPSS v.12.0 (SPSS Inc., Chicago, IL, USA) to detect significant inequality of character means among groups of populations. In these analyses, *S. hesperidium, S. glaucus* ssp. *glaucus*, and the three regional samples of ssp. *coronopifolius* (Morocco, Sicily, Near East) were treated as separate groups. Phyllary number (PN) and mean ray floret number (MRN) were found to deviate strongly from normality, and thus were natural log-transformed to meet assumptions of ANOVA. Adopting a multivariate approach, overall differences in morphology among groups were tested pairwise using a permutation procedure based on Mahalanobis' squared distances (with 2,000 replicates) as implemented in PAST. This permutation procedure does not depend on the assumption of multivariate normal distributions and equal covariance matrices (Hammer et al. 2001). Differences in overall morphology between adjacent groups per region (only Morocco and the Near East) were also determined with a nested multivariate analysis of variance (MANOVA) using JMP™v.13.2.0 (SAS Institute Inc., Cary, NC, USA). 'Groups' and 'populations nested within groups' were used as effects in the model, and the response design was specified to give a single value (JMP option 'Sum'). Variables PN and MRN were transformed prior to analysis as indicated above.

Genetic drift as a possible cause of morphological change

We tried to assess whether random genetic drift could be a contributing cause of significant differences in morphology between those peripheral (assumed 'derived') and non-peripheral (assumed 'ancestral') taxa for which both genetic and morphological data were available, namely *S. hesperidium* and *S. glaucus* ssp. *coronopifolius* from

Morocco, and subspp. *glaucus* and *coronopifolius* from the Near East. To this aim, we employed Lande's (1976) statistical test for the null hypothesis of phenotypic evolution by drift using the formula $N^* = [(1.96)^2\ h^2\ t]/(z/s)^2$, where N^* is the population size at which the observed trait difference is at the 95% confidence limit (or above which drift would be unlikely at $P \leq 0.05$), h^2 is the narrow-sense heritability of the trait, t is time measured in generations after population divergence, z is the mean trait difference between populations, and s is the pooled standard deviation for the trait. Thus, if the observed effective population size (N_e) is much larger than N^*, then this neutral hypothesis should be rejected with 95% confidence (see also Rasner et al. 2004; Yeh 2004).

Assuming that heterozygosity (H_E) decreases approximately at a rate of $1/(2N_e)$ per generation (Crow and Kimura 1970), we calculated N_e over time (viz. generations) from our allele frequency data as $N_e = 1/[2\ (1-H^{1/t})]$, where H is the ratio of H_E in derived vs. ancestral populations (see also Rasner et al. 2004). Estimates of N^* were necessarily restricted to those traits for which estimates of h^2 had previously been obtained from crossing experiments and parent-offspring (F_2/F_3) regressions in annual *Senecio* (*S. vulgaris* subspp.; Comes 1994), namely plant height (PH), capitulum width (CW), calyculus bract number (CN), and disc floret number (DN; see Table 6). In addition, we assumed an allozyme mutation rate (μ) of 10^{-6} per locus per generation (Ouborg et al. 1999), and a generation time of 1 year, for translating mean estimates of genetic distance (D_c) to approximate estimates of divergence time, following the equation $t \approx D_c/2\mu$ (Nei et al. 1983; Kalinowski 2002).

Caveats in our analyses

We note in advance that the results of our analyses must be interpreted with some caution mainly because of (1) a geographically biased sampling scheme with focus on three study regions; (2) a limited set of allozyme loci with potentially low population-genetic resolution and little phylogenetic information; (3) a bias towards capitulum traits measured; and (4) probably unrealistic assumptions underlying Lande's (1976) method (e.g. long-term constancy in heritability and population size). Moreover, due to differences in species' biology and/or experimental design, our estimates of h^2 obtained for *S. vulgaris* may not necessarily predict those in *S. hesperidium* and *S. glaucus* (e.g. Geber and Griffin 2003; Ashman and Majetic 2006), even though heritabilities, when measured for the same trait or trait type, "tend to be roughly similar not only across populations but even across species" (cf. Hill 2010). Nonetheless, despite these caveats and limitations, our study is the first to examine a possible example of recurrent and marginal (sub)species formation in Mediterranean sect. *Senecio*, and thus should contribute to current debates over the frequency of parallel speciation in plants (Ostevik et al. 2012).

Results

Allozyme analysis

Across the 683 individuals of *S. hesperidium* and *S. glaucus* scored, five polymorphic loci were identified (*Aat-3, G3pd-1, Idh-1, Pgi-2, Pgm-2*), and a total of 24 alleles were recorded. Two loci (*Pgi-1, Pgm-1*) were invariant in all 25 populations studied. Allele frequencies per locus, pooled over populations within *S. hesperidium* and the five intraspecific/(sub)regional samples of *S. glaucus* are detailed in Table A2 of Appendix 2.

Based on the exact test for neutrality, only allozyme frequencies in five populations (POA, POB, BS, KB, AX) at the *Aat-3* locus and in one population (KB) at the *G3pd*-1 locus showed significant deviations from the neutral equilibrium model (P = 0.01–0.04). Otherwise the allozyme frequencies at any locus within each population could be explained by chance alone (P = 0.06–1.00). Hence, as traditionally assumed for plant allozymes (e.g. Gottlieb 1981; Crawford et al. 1985; Weeden and Wendel 1989), variation in the great majority of loci and populations surveyed herein seems to conform to the expectations of the neutral theory (e.g. Nei and Graur 1984; Merilä and Crnokrak 2001; Rowe et al. 2017; but for exceptions see, e.g. Storz and Nachman 2003; Malherbe et al. 2005).

The UPGMA analysis of chord distances (D_c; see Table A3 of Appendix 2) separated populations into four main clusters (Figure 2). The most basal cluster comprised all populations of *S. glaucus* ssp. *coronopifolius* from Tunisia, with the three disjunct Sicilian populations nested therein. The second division in the dendrogram separated all inland populations of ssp. *coronopifolius* from Morocco, with the two coastal *S. hesperidium* populations from Morocco nested therein, from the remaining material of *S. glaucus* from the Near East. Within this latter group, two subclusters were evident, one of which comprised all coastal populations of ssp. *glaucus* from Israel, while the other consisted of all populations of ssp. *coronopifolius* from this region. In summary, the UPGMA tree topology (1) identified *S. hesperidium* as peripheral derivative of *S. glaucus* ssp. *coronopifolius* from Morocco; (2) recognised ssp. *coronopifolius* from Tunisia as likely progenitor of con(sub)specific populations in Sicily; and (3) supported ssp. *glaucus* as being most closely allied to ssp. *coronopifolius* from the Near East.

A comparison of mean D_c values among populations between nearest-neighbouring peripheral and non-peripheral samples (Table 2) showed that the genetic distance between *S. hesperidium* and Moroccan *S. glaucus* ssp. *coronopifolius* was low (D_c = 0.177) and of similar magnitude as the one between ssp. *coronopifolius* from Tunisia and Sicily (D_c = 0.163). By contrast, a greater genetic distance was evident between subspp. *glaucus* and *coronopifolius* from the Near East (D_c = 0.245). In fact, this level of differentiation fell within the range of values observed between the widely disjunct accessions of ssp. *coronopifolius* from Morocco, Tunisia, and the Near East

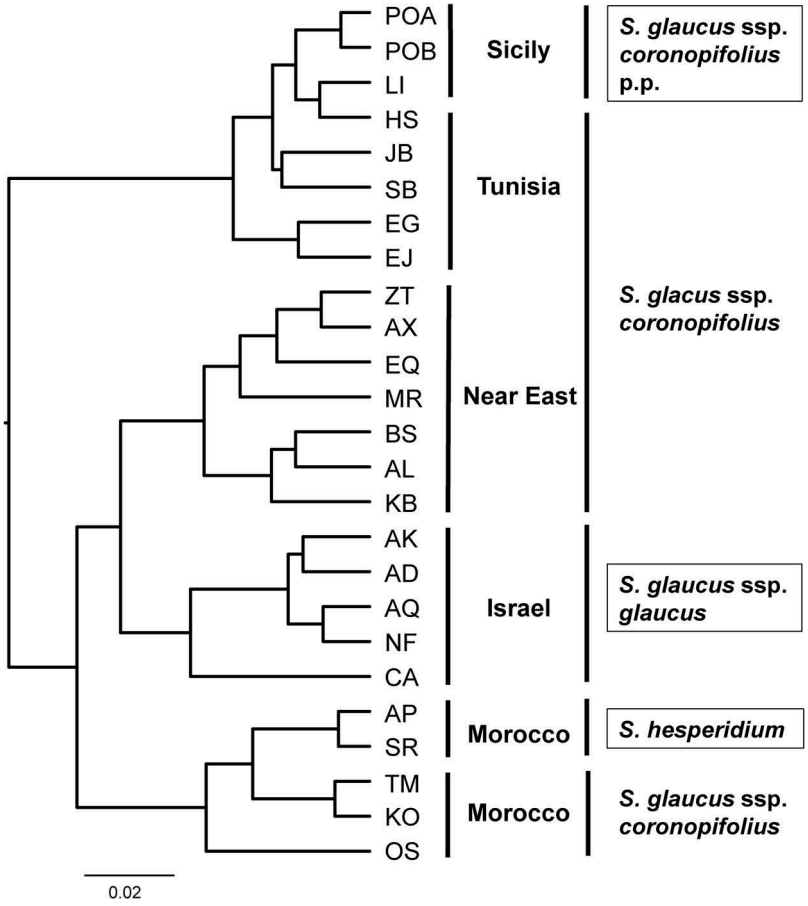

Figure 2. UPGMA dendrogram depicting allozyme-derived cord distances (D_c; Cavalli-Sforza and Edwards 1967) between populations of *S. hesperidium* and *S. glaucus* subspp. *glaucus* and *coronopifolius*. Populations are labelled according to their geographic origin. The scale represents genetic distance. Population codes are identified in Table 1.

Table 2. Mean genetic chord distances (D_c; Cavalli-Sforza and Edwards 1967) based on allele frequencies at all seven allozyme loci among populations within and between *Senecio hesperidium* and intraspecific taxa/(sub)regional samples of *S. glaucus*.

		S. glaucus				
	S. hesperidium (Morocco)*	Ssp. *coronopifolius* (Morocco)	Ssp. *coronopifolius* (Sicily)*	Ssp. *coronopifolius* (Tunisia)	Ssp. *glaucus* (Israel)*	Ssp. *coronopifolius* (Near East)
S. hesperidium (Morocco)*	0.088 (0.088–0.088)					
S. g. ssp. *coro.* (Morocco)	**0.177** (0.154–0.197)	0.171 (0.093–0.222)				
S. g. ssp. *coro.* (Sicily)*	0.373 (0.335–0.419)	0.327 (0.254–0.373)	0.129 (0.084–0.160)			
S. g. ssp. *coro.* (Tunisia)	0.326 (0.294–0.363)	0.293 (0.238–0.350)	**0.163** (0.111–0.238)	0.159 (0.121–0.211)		
S. g. ssp. *glaucus* (Israel)*	.384 (0.360–0.404)	0.315 (0.277–0.364)	0.280 (0.223–0.383)	0.262 (0.174–0.365)	0.163 (0.109–0.259)	
S. g. ssp. *coro.* (Near East)	0.297 (0.232–0.364)	0.264 (0.190–0.331)	0.241 (0.184–0.282)	0.208 (0.156–0.271)	**0.245** (0.169–0.347)	0.181 (0.109–0.242)

Ranges are in parentheses. Comparisons between geographically nearest-neighbouring peripheral (*) and non-peripheral samples are marked bold.

(D_c = 0.208–0.293). The values of average D_c between populations within peripheral samples ranged from 0.088 to 0.163 and partly overlapped with those of the non-peripheral ones (D_c = 0.159–0.181; Table 2).

On the basis of the population genetic relationships identified above, the distribution of alleles at several loci displayed interesting geographical patterns. At the *Pgm-2* locus, all peripheral samples, i.e. *S. hesperidium, S.*

glaucus ssp. *coronopifolius* from Sicily, and ssp. *glaucus*, possessed only a subset of alleles present in their geographically proximal neighbours, namely ssp. *coronopifolius* from Morocco, Tunisia, and the Near East, respectively (see Table A2 in Appendix 2), and the same applied to the *Aat-3* locus, except for the Moroccan contrast. A similar picture emerged for *S. hesperidium* and *S. glaucus* ssp. *glaucus* at the *Pgi-2* locus, as for Sicilian ssp. *coronopifolius* at the *G3pd-1* locus. In contrast, *S. hesperidium* possessed three rare alleles (*Aat-3d, G3pd-1a, Idh-1a*) not observed in *S. glaucus* from Morocco, but otherwise present at low to moderately high frequencies in both the central and eastern Mediterranean.

The estimates of allozyme diversity varied greatly across populations of *S. hesperidium* and *S. glaucus* (Table 3). The least polymorphic populations were from Morocco (SR), Sicily (LI, POB), and Tunisia (HS; all $P_{5\%}$ = 14.3%) while two populations from the Near East (AQ, AL) were most polymorphic (both $P_{5\%}$ = 71.4%). The mean numbers of alleles observed per locus (A_O) ranged from 1.6 in Sicily (LI, POB) to 3.0 in the Near East (ZT). Lowest estimates of both allelic richness (R_S) and Nei's gene diversity (H_E) were observed in populations SR (R_S = 1.42; H_E = 0.046) and LI (R_S = 1.33; H_E = 0.081), while population AL from the Near East was the most diverse (R_S = 2.42; H_E = 0.285)

Comparison of non-peripheral and peripheral samples (i.e. subregions) within each of the three Mediterranean regions surveyed indicated apparently reduced levels of allozyme diversity in *S. hesperidium* from Morocco and Sicilian *S. glaucus* ssp. *coronopifolius* in terms of $P_{5\%}$, R_S, and H_E (Table 4). However, the permutation tests (conducted for R_S and H_E only) revealed no significance in each case, although in the central Mediterranean the estimator of allelic richness (R_S) was close to significance (one-sided P = 0.055). Moreover, no reduced levels of allozyme diversity were evident in the Near East, where all three estimators were markedly similar between subspp. *glaucus* and *coronopifolius* (Table 4).

As indicated in Table 3, the mean coefficient of inbreeding ($f = F_{IS}$, calculated across all polymorphic loci) within populations was generally low and close to zero for both *S. hesperidium* (–0.011) and *S. glaucus* (0.038). Significant departures from random mating were detected only in two populations each from the Near East (AQ, NF) and Tunisia (EJ, HS), and this was mostly due to a slight deficit of heterozygotes (Table 3). Overall, these results confirm that most of these populations are highly outcrossing, with neither peripheral (or non-peripheral) populations showing signs of marked inbreeding. BOTTLENECK failed to detect any evidence of a transient excess of heterozygotes in any of the populations analysed (Table 3). Hence, in none of the peripheral populations from Morocco (*S. hesperidium*), Sicily (*S. glaucus* ssp. *coronopifolius*), and Israel (ssp. *glaucus*) was a severe and recent bottleneck indicated.

The hierarchical AMOVAs estimated for each region separately (Table 5) revealed low but significant eco-geographical differentiation between peripheral *S. glaucus* ssp. *glaucus* and non-peripheral ssp. *coronopifolius* in the Near East (F_{CT} = 0.059, $P < 0.001$), and the same was true for differentiation among populations within those groups (F_{SC} = 0.084, $P < 0.001$). By contrast, no significant differentiation was detected between either *S. hesperidium* and Moroccan *S. g*. ssp. *coronopifolius* (F_{CT} = 0.016, P = 0.206) or Sicilian and Tunisian ssp. *coronopifolius* (F_{CT} = 0.014, P = 0.248), while low but significant population differentiation was found within each subregion (west: F_{SC} = 0.073; central: F_{SC} = 0.047; both $P < 0.001$).

Morphometric analysis

The first two components of the PCA plot (Figure 3) accounted for 51.66% and 19.72% of the total variance, respectively. The first component (PC1) separated most of the *S. glaucus* populations from the Near East into two high- and low-score clusters, corresponding to subspp. *glaucus* and *coronopifolius*, respectively. However, there was no clear break between these two nominal groups, as was evident in the allozyme dendrogram (Figure 2). The connection between these two groups was through population AX, as shown by the MST (i.e. based on the full dimensional space). The second component (PC2) emphasised the phenotypic differentiation between Moroccan ssp. *coronopifolius* and all *S. glaucus* populations from the Near East. The two Sicilian populations of *S. g*. subsp. *coronopifolius* (LI, POA) were connected by the MST to those from Morocco, despite one of them (LI) showing affinities to those from the Near East (BS, ZT) in two-dimensional space. All three populations of *S. hesperidium* (AP, SI, SR) were clearly separated from Sicilian/Moroccan *S. g*. ssp. *coronopifolius* along PC1 and less so along PC2; they had their MST connection via population AP to ssp. *glaucus* (CA).

As seen in Table A1 of Appendix 2, the first component had positive and very high (≈ 0.9) correlation coefficients (loadings) for several capitulum characters, including PL, MRN, DN, and CW. By contrast, one vegetative character, leaf lobe number (LLN), as well as CN and ray floret shape (RS) dominated the second component, albeit with moderately high loadings (≈ 0.65–0.70). An examination of group means for each character by means of Tukey's HSD test (Table A4 in Appendix 2) revealed that both *S. hesperidium* and *S. g*. ssp. *glaucus* were characterised by relatively large-sized plants (PH) that, in contrast to ssp. *coronopifolius* from Morocco and the Near East, respectively, produced significantly broader capitula (CW) with more numerous calyculus bracts (CN), phyllaries (PN), and disc florets (DN), as well as longer and broader ray florets (MRL, MRW; see also Figure A1 in Appendix 3 for box plots of traits). However, there were also significant differences between *S. hesperidium* and *S. g*. ssp. *glaucus* in seven of the 13 characters examined

Table 3. Estimates of allozyme diversity ($P_{5\%}$, A_O, R_S, H_O, H_E) and Weir and Cockerham's (1984) coefficient of inbreeding (f) within populations of *Senecio hesperidium* and *S. glaucus*.

Region/taxon/ population code	$P_{5\%}$	A_O	R_S	H_O	H_E	f	HW[a] χ^2	HW[a] P	P Sign test	P Wilcoxon signed-rank test
West										
S. hesperidium										
AP*	28.6	1.9	1.56	0.097 (0.060)	0.095 (0.060)	−0.014	0.0	1.000	0.087	1.000
SR*	14.3	1.7	1.42	0.048 (0.023)	0.046 (0.022)	−0.032	3.1	0.544	0.095	1.000
Mean						−0.011				
95% CI						−0.002 to −0.063				
S. g. ssp. *coronopifolius*										
KO	28.6	2.1	1.81	0.139 (0.071)	0.143 (0.068)	0.029	15.1	0.056	0.229	0.953
TM	42.9	2.0	1.77	0.147 (0.092)	0.140 (0.080)	−0.054	4.4	0.821	0.376	0.938
OS	42.9	2.1	1.85	0.157 (0.072)	0.177 (0.088)	0.115	6.1	0.637	0.671	0.906
Central										
Ssp. *coronopifolius*										
LI*	14.3	1.6	1.33	0.086 (0.080)	0.081 (0.075)	−0.061	4.3	0.639	0.741	0.875
POA*	42.9	1.9	1.74	0.129 (0.096)	0.146 (0.095)	0.122	10.1	0.261	0.369	0.844
POB*	14.3	1.6	1.53	0.114 (0.102)	0.117 (0.104)	0.021	3.4	0.501	0.754	0.250
EJ	28.6	1.9	1.86	0.104 (0.061)	0.160 (0.095)	0.363	12.8	0.012*	0.346	0.938
SB	28.6	1.9	1.84	0.143 (0.107)	0.155 (0.107)	0.083	8.1	0.088	0.655	0.250
JB	42.9	1.9	1.84	0.143 (0.099)	0.149 (0.106)	0.045	1.8	0.937	0.455	0.813
EG	57.1	2.4	2.20	0.180 (0.089)	0.193 (0.105)	0.069	5.0	0.753	0.199	0.922
HS	14.3	1.9	1.69	0.101 (0.082)	0.120 (0.101)	0.165	19.2	0.038* [b]	0.518	0.938
East										
Ssp. *glaucus*										
AK*	42.9	2.0	1.88	0.238 (0.113)	0.219 (0.104)	−0.088	6.7	0.352	0.565	0.150
CA*	42.9	2.1	1.94	0.248 (0.124)	0.241 (0.112)	−0.028	9.8	0.135	0.331	0.156
NF*	42.9	2.6	2.00	0.192 (0.088)	0.200 (0.093)	0.043	14.7	0.023*	0.493	0.953
AD*	57.1	2.4	2.14	0.290 (0.116)	0.277 (0.110)	−0.049	3.1	0.927	0.522	0.313
AQ*	71.4	2.6	2.18	0.243 (0.100)	0.274 (0.091)	0.117	24.8	0.006**	0.530	0.500
Ssp. *coronopifolius*										
BS	57.1	2.6	2.34	0.246 (0.100)	0.251 (0.104)	0.021	3.0	0.981	0.517	0.500
AL	71.4	2.9	2.42	0.276 (0.104)	0.285 (0.111)	0.034	7.8	0.651	0.537	0.406
KB	42.9	2.4	2.16	0.295 (0.143)	0.258 (0.127)	−0.145	10.2	0.116	0.433	0.313
ZT	57.1	3.0	2.41	0.271 (0.124)	0.261 (0.118)	−0.041	2.7	0.953	0.595	0.563
MR	57.1	2.9	2.39	0.200 (0.080)	0.250 (0.106)	0.204	14.7	0.143	0.459	0.891
AX	42.9	2.3	2.08	0.203 (0.111)	0.225 (0.117)	0.100	4.4	0.621	0.575	0.125
EQ	42.9	2.3	1.81	0.176 (0.084)	0.200 (0.093)	0.120	6.0	0.418	0.291	0.906
Mean (*S. glaucus*)						0.038				
95% CI						−0.010 to 0.076				

Mean estimates of f for each species were obtained by jackknifing over loci. Ninety-five per cent confidence intervals (CI) around these means were obtained by bootstrapping over loci. Tests of Hardy–Weinberg (HW) equilibrium across loci within populations (Fisher's method) are indicated. Also shown are results of the BOTTLENECK analysis with P values for both a sign test and a one-tailed Wilcoxon signed-rank test. Standard deviations are shown in parentheses. Populations marked by an asterisk (*) are considered peripheral isolates.

$P_{5\%}$: percentage of polymorphic loci (5% criterion); A_O, mean number of alleles observed per locus; R_S, mean number of alleles per locus calculated after rarefaction method (allelic richness); H_O, mean observed (direct count) heterozygosity; H_E, mean expected heterozygosity, viz. Nei's (1987) unbiased gene diversity.

[a] Hardy–Weinberg equilibrium rejected at * $P < 0.05$, and ** $P < 0.01$.

[b] Analysed for only a single locus (i.e. *Aat-3*) due to almost complete fixation at the other loci surveyed, using the Hardy–Weinberg chi-square test option in BIOSYS-1 (Swofford and Selander 1989).

(Table A4). In four of those traits (IL, CN, MRN, MRW), ssp. *glaucus* displayed higher and more extreme values, not (or only rarely) observed in any other group analysed, whereas *S. hesperidium* had distinctly broader capitula (CW) and exceptionally longer, but also narrower ray florets (MRL, RS). In addition, trifurcate leaf lobes were absent in *S. hesperidium*, as in all western and central material of *S. g.* ssp. *coronopifolius* studied. In contrast, this character was present in nearly all populations of *S. glaucus* from the Near East (except MR), and significantly associated with ssp. *glaucus* (94%) rather than ssp. *coronopifolius* (43%; $\chi^2 = 33.2$, df = 1, $P < 0.001$).

The multivariate permutation tests based on Mahalanobis' distances showed that all pairwise comparisons of overall morphology among the five groups studied were highly significant (all P values < 0.001;

Table 4. Mean estimates of allozyme diversity ($P_{5\%}$, R_S, H_E) calculated over all seven loci and populations within *Senecio hesperidium* and intraspecific/(sub)regional samples of *S. glaucus*.

	West Mediterranean			Central Mediterranean			East Mediterranean		
	S. hesperidium (Morocco)* $N = 2$	*S. g.* ssp. *coronopifolius* (Morocco) $N = 3$	P	*S. g.* ssp. *coronopifolius* (Sicily)* $N = 3$	*S. g.* ssp. *coronopifolius* (Tunisia) $N = 5$	P	*S. g.* ssp. *glaucus* (Israel)* $N = 5$	*S. g.* ssp. *coronopifolius* (Near East) $N = 7$	P
$P_{5\%}$	21.5	38.1	NA	23.8	34.3	NA	51.4	53.1	NA
R_S	1.49	1.81	0.130	1.54	1.89	0.055	2.03	2.23	0.129
H_E	0.071	0.153	0.113	0.115	0.157	0.208	0.242	0.247	0.448

Peripheral samples are marked by asterisks (*). Probability values for differences between non-peripheral (G_1) and peripheral (G_2) samples within each Mediterranean region are given for one-sided tests ($G_1 > G_2$), after 10,000 permutations, using FSTAT (Goudet 2001).
N: number of populations surveyed; NA: not analysed; $P_{5\%}$: percentage of polymorphic loci (5% criterion); R_S: mean number of alleles per locus calculated after rarefaction method (allelic richness); H_E: mean expected heterozygosity, viz. Nei's (1987) unbiased gene diversity.

Table 5. Hierarchical analyses of molecular variance (AMOVAs) for allozyme variation at seven loci within *S. hesperidium* and *S. glaucus* (subspp. *glaucus* and *coronopifolius*) sampled from three Mediterranean regions (i.e. west: Morocco; central: Sicily/Tunisia; east: Israel/West Bank/Egypt), and based on the partitioning of populations into peripheral and non-peripheral subregions (see footnote).

Region/source of variation	Degrees of freedom	Sum of squares	Variance components	Percentage of variation	*F*-statistics	*P*
West[a]						
Among subregions	1	3.60	0.008	1.60	$F_{CT} = 0.016$	0.206
Among populations	3	7.55	0.034	7.21	$F_{SC} = 0.073$	<0.001
Within populations	307	130.00	0.424	91.19	$F_{ST} = 0.088$	<0.001
Central[b]						
Among subregions	1	2.56	0.007	1.40	$F_{CT} = 0.014$	0.248
Among populations	6	8.10	0.023	4.65	$F_{SC} = 0.047$	<0.001
Within populations	308	144.20	0.468	93.95	$F_{ST} = 0.061$	<0.001
East[c]						
Among subregions	1	27.05	0.059	5.95	$F_{CT} = 0.059$	<0.001
Among populations	10	56.70	0.078	7.88	$F_{SC} = 0.084$	<0.001
Within populations	726	622.23	0.857	86.17	$F_{ST} = 0.138$	<0.010

Significance values (*P*) obtained for variance components are indicated based on 10,100 permutations.
[a] *S. hesperidium* (AP, SR) vs. *S. glaucus* ssp. *coronopifolius* Morocco (KO, TM, OS).
[b] Ssp. *coronopifolius* Sicily (LI, POA, POB) vs. Tunisia (EJ, SB, JB, EG, HS).
[c] Ssp. *glaucus* Israel (AK, CA, NF, AD, AQ) vs. ssp. *coronopifolius* Israel/West Bank/Egypt (BS, ZT, MR; AL, KB; AX, EQ).
Population codes are identified in Table 1.

Table A5 in the Appendix 2). The group effect from the nested MANOVA confirmed that *S. hesperidium* and *S. g.* ssp. *coronopifolius* from Morocco differed significantly in overall phenotype (exact *F* test: $F_{1,\ 54} = 28.171$, $P < 0.001$), and the same applied to *S. g.* subspp. *glaucus* and *coronopifolius* from the Near East ($F_{1,\ 108} = 34.524$, $P < 0.001$); however, the population within group effect was only significant in the latter region ($F_{10,\ 108} = 5.895$, $P < 0.001$) but not in Morocco ($F_{4,\ 54} = 1.957$, $P = 0.114$). Overall, these results indicate that peripheral taxa diverged considerably from their nearest-neighbouring relatives in phenotype, in both the western and eastern Mediterranean.

Genetic drift as a possible cause of morphological change

Assuming an allozyme mutation rate (μ) of 10^{-6}, the mean genetic distances (D_c; Table 2) observed between *S. hesperidium* and *S. glaucus* ssp. *coronopifolius* from Morocco ($D_c = 0.177$), and between *S. g.* subspp. *glaucus* and *coronopifolius* from the Near East ($D_c = 0.245$), translated to divergence times of about 88,500 and 122,500 years, respectively. When combined with our estimates of H_E (Table 4), these timings suggest a faster and much stronger decrease in genetic diversity (54%) from Moroccan *S. g.* ssp. *coronopifolius* to *S. hesperidium* ($H_E = 0.153$ vs. 0.071) when compared to the situation in the Near East, where diversity decreased by only 2% from *S. g.* ssp. *coronopifolius* to ssp. *glaucus* (0.247 vs. 0.242) over an extended time period, thus implying much lower effective population sizes in the former taxon pair relative to the latter ($N_e = 5.8 \times 10^4$ vs. 3.0×10^6).

Table 6 shows estimates of the maximum effective population size (N^*; *sensu* Lande 1976) for one vegetative (PH) and three floral traits (CW, CN, DN; all with

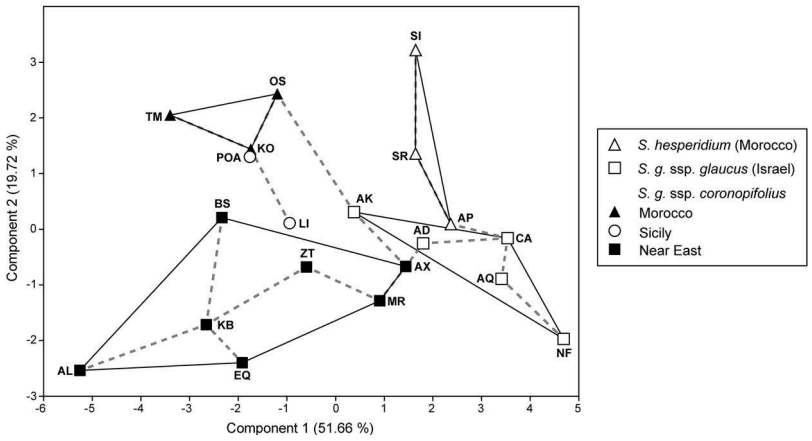

Figure 3. Principal components analysis (PCA) plot of the first two components based on 13 morphological variables in populations of *S. hesperidium* (Morocco) and *S. glaucus* subspp. *glaucus* (Israel) and *coronopifolius* (Morocco, Sicily, Near East), with a minimum-length spanning tree (MST) superimposed (dashed lines). Convex hulls (solid lines) depict the smallest area occupied by different groups. Percentages of total variance explained by each component are noted in parentheses. Note the lack of data for populations of *S. g.* ssp. *coronopifolius* from Tunisia. Refer to Table 1 for population codes.

Table 6. Results for maximum estimates of effective population sizes for rejection of the neutral hypothesis at the 95% level (N^*; *sensu* Lande 1976) for four trait means between (1) *S. hesperidium* and *S. glaucus* ssp. *coronopifolius* (Morocco); and (2) *S. g.* subspp. *glaucus* and *coronopifolius* (Near East), respectively.

		S. hesperidium vs. *S. glaucus* ssp. *coronopifolius* (Morocco) $t = 88.5$, $N_e = 5.8 \times 10^4$				*S. glaucus* ssp. *glaucus* vs. ssp. *coronopifolius* (Near East) $t = 122.5$, $N_e = 3.0 \times 10^6$			
Trait[a]	h^2	z	s	z/s	N^*	z	s	z/s	N^*
PH	0.43	52.9	67.75	0.781	2.4×10^5	57.3	96.71	0.592	5.8×10^5
CW	0.14	1.65	0.986	1.673	1.4×10^4	1.49	1.087	1.371	3.5×10^4
CN	0.65	2.53	1.831	1.382	1.2×10^5	1.29	2.234	0.577	9.2×10^5
DN	0.50	37.0	28.12	1.136	1.3×10^5	28.2	27.66	1.020	2.3×10^5

Calculations were restricted to traits with known heritability and showing significant mean differences at the 5% level (see Table A4 in Appendix 2).
t: divergence time in thousands of years ago (kya); N_e: observed effective population size (both estimated from allozyme data; see 'Materials and methods'); h^2: narrow-sense heritability (taken from Comes 1994); z: mean difference in phenotype; s: pooled standard deviation; z/s: mean difference in phenotype in units of standard deviations; N^*: population size above which genetic drift would be unlikely ($P \leq 0.05$) to have led to the observed differences in trait means.
[a]Trait abbreviations: PH: plant height; CW: capitulum width; CN: calyculus bract number; DN: disc floret number (see Table A1 in Appendix 2 for details).

known heritabilities) that differed significantly at the 5% level between the two taxa from each region (see Table A4 in Appendix 2). For the Moroccan pair, estimates of N^* for PH, CN and DN ($1.2–2.4 \times 10^5$) were one order of magnitude larger than the inferred N_e (5.8×10^4; see above), while for CW the estimate of N^* was somewhat lower (1.4×10^4). Hence, for the three former traits, we cannot reject the null hypothesis of drift causing their increase from *S. g.* ssp. *coronopifolius* to *S. hesperidium*, whereas this hypothesis is formally rejected (at the 5% significance level) for an increase in capitulum width (CW). By contrast, for the two subspecies of *S. glaucus* in the Near East, estimates of N^* for all four traits were at least one (PH, CN, DN: $2.3–9.2 \times 10^5$) or even two (CW: 3.5×10^4) orders of magnitude smaller than the inferred N_e (3.0×10^6), suggesting that genetic drift would be unlikely ($P \leq 0.05$) to have led to the observed increases for all those traits in ssp. *glaucus* relative to ssp. *coronopifolius*.

Discussion

Population genetic relationships and levels of divergence

The allozyme data reported here suggest that coastal ecotypes have evolved multiple times independently from inland progenitors of *S. glaucus* ssp. *coronopifolius* at the periphery of its range in North Africa and the Near East, i.e. in south-west Morocco (*S. hesperidium*), southern Sicily (*S. g.* ssp. *coronopifolius* p.p.) and Israel (*S. g.* ssp. *glaucus*). Three lines of evidence support this hypothesis. First, in the UPGMA dendrogram of genetic (D_c) chord distances between populations (Figure 2), both *S. hesperidium* and Sicilian *S. g.* ssp. *coronopifolius* occupied different but nested, and thus potentially derived positions relative to adjacent populations of the latter subspecies from Morocco and Tunisia, respectively. Second, populations of ssp. *glaucus*, although forming a separate cluster, attached most closely to ssp. *coronopifolius* from the Near East (Figure 2), and were also most closely allied

to this latter material based on the D_c values recorded (Table 2). This supports the previous hypothesis (Alexander 1975) that ssp. *glaucus* originated from populations in this eastern region of ssp. *coronopifolius*. Finally, all peripheral isolates had no private alleles (in relation to the entire data set) and only a subset of those present in their nearest neighbours [i.e., generally at *Pgm-2*, but also, depending on the region, at *Aat-3* (Sicily, Israel), *G3pd-1* (Sicily) and *Pgi-2* (Morocco, Israel); Table A2], as would be expected from recently diverged progenitor-derivative pairs (e.g. Levin 2000; Crawford 2010; Lopéz et al. 2011).

We did not find a gradient of increasing allozyme divergence across the three descendant ecotypes as might have been expected from their taxonomic ranks (i.e. Sicilian ssp. *coronopifolius* p.p. < ssp. *glaucus* < *S. hesperidium*). Instead, the mean D_c values between ancestral and descendant populations within each Mediterranean region (west, central, east) were of similar magnitude and low, ranging from 0.163 to 0.245 (Table 2). Again, these values are typical for conspecific plant populations and/or congeneric species related as progenitor-derivative pairs (Gottlieb 1981; Crawford et al. 1985; Briggs and Walters 2016). In fact, hierarchical AMOVAs (Table 5) indicated non-significant levels of subdivision between those pairs in Morocco (*S. hesperidium* vs. *S. g*. ssp. *coronopifolius:* F_{CT} = 0.016, P = 0.206) and the central Mediterranean (Sicilian vs. Tunisian ssp. *coronopifolius:* F_{CT} = 0.014, P = 0.248), while there was a numerically low but significant level of differentiation detectable in the Near East (subspp. *glaucus* vs. *coronopifolius:* F_{CT} = 0.059, $P < 0.001$). Such low levels of genetic divergence between ancestral and descendant populations are generally taken as evidence for a relatively recent divergence history (Levin 2001; Ostevik et al. 2012), barring, of course, extensive post-divergence gene flow (see below).

Times and modes of divergence between ancestral and descendant populations

Molecular clock estimates from nuclear ribosomal DNA (Comes and Abbott 2001) suggest early-to-mid Pleistocene crown ages (ca.1.0–0.44 million years ago, Ma) for the relevant clade of sect. *Senecio* (termed 'A') containing *S. hesperidium, S. glaucus,* and other diploid Mediterranean species (e.g. *S. aethnensis* Jan. ex DC., *S. gallicus* Vill., *S. leucanthemifolius* Poiret.; see also Coleman et al. 2003 for similar time estimates). Assuming an allozyme mutation rate in the order of 10^{-6} (Ouborg et al. 1999), the similarly low mean D_c values between *S. hesperidium* and *S. g*. ssp. *coronopifolius* (0.177), and between Sicilian and Tunisian ssp. *coronopifolius* (0.163), correspond to the last glacial period [ca. 88.5 and 81.5 thousand years ago (ka), respectively], while the slightly higher D_c value between subspp. *glaucus* and *coronopifolius* from the Near East (0.245) suggests a divergence during the Last Interglacial (ca. 122.5 ka). It is also well recognised that the Pleistocene climatic fluctuations fragmented ranges and fostered plant evolution throughout the Mediterranean Basin and adjacent regions (Pons and Quézel 1985; Cheddadi et al. 2005; Médail and Diadema 2009; Nieto Feliner 2014). The dates of divergence obtained in this study therefore suggest a biogeographic scenario where the intensification of glacial/interglacial cycles from ca. 0.45 Ma onward (Cheddadi et al. 2005) may have provided new ecological opportunities for the establishment, isolation and divergence of peripheral populations of *S. g*. ssp. *coronopifolius*, giving rise to parallel, independent parapatric coastal taxa in Morocco (*S. hesperidium*) and Israel (*S. g*. ssp. *glaucus*; see also Alexander 1975; Kadereit 1984). This hypothesis gains further weight from the fact that the extant ranges of both descendant taxa correlate to some extent with putative glacial refugia ('Souss Valley/western Anti-Atlas' and 'Israel/Palestine', respectively) as described for various other plant and animal species (Médail and Diadema 2009; Blondel et al. 2010; Planas et al. 2014).

Considering the central Mediterranean, it is perhaps not surprising to find a close genetic link between populations of ssp. *coronopifolius* across the Strait of Sicily, given the high dispersal ability of *Senecio* through wind-borne achenes. It is noticeable, however, that the estimated date of divergence between Tunisian and Sicilian material falls into a glacial period (see above). If correct, this would support the hypothesis that glacial sea-level lowstands facilitated biotic exchange in the Sicilian insular system (Nieto Feliner 2014), as postulated for other coastal or low elevation plants (e.g., *Matthiola* spp.: Sanchéz et al. 2005; *Anthemis secundiramea*: Lo Presti and Oberprieler 2011; *Linaria* sect. *Versicolores*: Fernández-Mazuecos and Vargas 2011). Moreover, the separate genetic placement of the Sicilian populations (LI vs. POA/POB) within the Tunisian cluster (Figure 2) agrees with evidence from cpDNA, indicating that there have been recurrent introductions of *S. glaucus* to Sicily from North Africa (Chapman and Abbott 2005).

Levels of inbreeding and contrasting patterns of genetic diversity

As further based on the allozyme data, we found no evidence for strong signatures of inbreeding at the species and population levels (Table 3). That is, for both *S. hesperidium* and *S. glaucus*, the mean coefficient of inbreeding ($f = F_{IS}$) was close to zero, and the same was true for most populations sampled. Positive values of f and significant heterozygote deficiencies were only found in populations of ssp. *coronopifolius* from Morocco (EJ, HS) and ssp. *glaucus* (AQ, NF; Table 3). Such deviations from panmixia could have arisen from various site-specific factors promoting bi-parental inbreeding (e.g. restricted pollinator foraging regimes, clumping and/or high plant density; Comes and Abbott 1998, 1999). Alternatively, such deviations may indicate cryptic natural variation for pseudo-self-compatibility (Liu et al. 2007), but there is as yet no

experimental evidence for this phenomenon in *S. glaucus*. In any event, and despite these exceptions, the present results do not jeopardise the general perception that random mating is the norm in diploid and self-incompatible species of sect. *Senecio* (e.g. Abbott and Forbes 1993; Abbott et al. 2000).

More surprisingly, none of the populations of three descendant ecotypes, viz. peripheral isolates showed a transient excess of heterozygotes (Table 3), and neither were there significant differences in mean genetic diversity (in terms of R_S and H_E) between peripheral and non-peripheral (ancestral) samples within each of the three Mediterranean regions (Table 4). These non-significant results, however, could be due to the small sample sizes of populations and loci screened, hence limiting the statistical power of the bottleneck and permutation tests used (Peery et al. 2012; Yineger et al. 2014). Nevertheless, there was a drastic reduction of genetic diversity in *S. hesperidium* (with ~30% decrease in R_S and ~54% decrease in H_E) in comparison with Moroccan *S. g.* ssp. *coronopifolius*, and a similar but less pronounced decrease in Sicilian relative to Tunisian material of the latter subspecies (~19% in R_S; ~27% in H_E). The reduced genetic diversity in *S. hesperidium* may reflect a small number of individuals involved in its origin and/or consistently small population sizes (Levin 2000; Hardie and Hutchings 2010; Lopéz et al. 2011), while the decreased diversity in Sicilian *S. glaucus* concurs with the hypothesis of multiple founder effects during island colonisation and subsequent genetic drift (Chapman and Abbott 2005). By contrast, in the Near East, genetic diversity was only slightly reduced in ssp. *glaucus* relative to ssp. *coronopifolius* (~9% in R_S; ~2% in H_E; Table 4), suggesting relatively increased population sizes that buffered against the loss of genetic variation via drift (see also below).

Morphological divergence between ancestral and descendant populations

Much in contrast to the allozyme data, our PCA of morphometric measurements (Figure 3) revealed that the coastal ecotypes from both Morocco (*S. hesperidium*) and Israel (*S. g.* ssp. *glaucus*) have diverged strongly in phenotype from nearby ancestral populations of ssp. *coronopifolius*. Although such differentiation appeared to be more gradual and less pronounced in the Near East (see also Table A5), the among-group (or subregion) effect there was just as significant as in Morocco (both P values < 0.001 in nested MANOVAs). Notably, in each region, the main multivariate axes of divergence were in plant size and floral morphology (i.e. capitulum width, number of calyculus bracts, phyllaries and disc florets, ray floret length and width), with both coastal derivatives showing significantly larger values (Table A4). However, despite their resemblance in gross morphology, *S. hesperidium* and *S. g.* ssp. *glaucus* also differed significantly in seven of the 13 traits examined (Table A4). A similar morphometric study has previously shown (Chapman and Abbott 2005) that Sicilian ssp. *coronopifolius* significantly differs from Tunisian (mostly inland) source material in various floral and leaf traits, although in this case the coastal (island) derivative showed mostly reduced values (e.g. in leaf perimeter/dissection, inflorescence length, ray floret length). In the present study, lacking morphometric data from Tunisian material, the Sicilian type was morphologically intermediate to ssp. *coronopifolius* from Morocco and the Near East, and clearly distinguishable from both ssp. *glaucus* and *S. hesperidium* (Figure 3).

Taken together, these results have two major implications. First, the significant morphological differentiation between ancestral and descendant populations sharply contrasts with their weak differentiation at allozyme loci. We suppose that the very low genetic divergence between these populations more likely reflects shared ancestry rather than ongoing gene flow among them, as frequently invoked for organisms that are characterised by great differentiation in phenotype but not in genotype (e.g. Avise 2000; Rheindt et al. 2011; and further references therein). In turn, this inference of impeded post-divergence gene flow (viz. hybridisation) makes it unlikely that our dates of divergence are underestimates (see above). Nonetheless, it could be argued that our allozyme markers may have been too few (or too crude) to provide sufficient discrimination between such recently diverged population groups or taxa. This argument, however, is tempered with the observation of significant F_{SC} values within each study region (Table 5), suggesting that our markers at least provided sufficient population-genetic resolution.

Second, despite their broadly similar environments, the three independently evolved coastal ecotypes exhibit only partly similar and clearly non-identical phenotypes. Although little is known about the genetic bases of the (quantitative) traits involved, a significant fraction of this variation is likely heritable, given that comparisons were generally made under controlled glasshouse conditions (Chapman and Abbott 2005; this study). Overall, this would suggest that the three coastal derivatives follow their own evolutionary trajectories, perhaps reflecting the recruitment of different mutations at the quantitative trait loci controlling their divergent phenotypes (Ostevik et al. 2012; Martin and Orgogozo 2013; Roda et al. 2013; further references therein).

Reproductive isolation and the evolutionary forces shaping phenotypic divergence

As with any study of (incipient) speciation, a crucial question remains regarding the nature and extent of reproductive isolation between descendant and ancestral populations (Levin 2001; Coyne and Orr 2004; Ostevik et al. 2012). In general, however, all diploid taxa of Mediterranean *Senecio* (clade 'A' *sensu* Comes and Abbott 2001) produce vigorous and largely fertile artificial hybrids (Alexander 1979; Kadereit 1984; Abbott et al. 1995), notwithstanding some reduced pollen viability reported in an F_1 hybrid between *S. hesperidium* and *S. glaucus* (ca. 75% stainable pollen) relative to a typical inter- or intraspecific cross (usually above ca. 80–90%;

Alexander 1979). Thus, there is little evidence of strong intrinsic post-zygotic barriers to gene flow, indicating that reproductive isolation in our study system should be primarily extrinsic. Specifically, the divergence of Sicilian ssp. *coronopifolius* from its Tunisian source populations is most likely due to geographical isolation (with the Sicilian Strait acting as a present-day barrier to gene flow; Chapman and Abbott 2005), although selection against immigrants in the different environments (Mediterranean coastal vs. semi-arid/desert, mostly inland) may further maintain differences between these disjunct populations (Ostevik et al. 2012). By contrast, in Morocco and the Near East, it would seem that ecologically based reproductive barriers had a primary role in the origin of, respectively, *S. hesperidium* and *S. g*. ssp. *glaucus*, because these coastal taxa are apparently derived from mostly inland, semi-arid/desert populations of ssp. *coronopifolius*, i.e. despite opportunities for gene flow. Taken at face value, the latter two systems are prime candidates of parallel ecological (sub)speciation (*sensu* Ostevik et al. 2012), especially as the two descendant taxa share characteristics with potential consequences for fitness and extrinsic reproductive isolation, including an increase in plant height, greater numbers of disc florets (viz. broader capitula) as well as more 'showy' ray florets (see above). In general, such broadly consistent patterns of morphological divergence among unrelated taxa are viewed as indirect evidence for a non-random process in parallel, and hence divergent natural selection in generating adaptation to shared environments (e.g. Levin 2001; Schluter 2004; Ostevik et al. 2012). In our case, this would imply adaptation to shared coastal environments, although the possibility remains that both edaphic and climate conditions acted in concert driving local adaptation of ssp. *glaucus* to coastal-Mediterranean habitats in the Near East.

We attempted to explore this issue by applying Lande's (1976) statistical test for the hypothesis of neutral evolution to a subset of traits known to be heritable in *Senecio* (i.e. plant height, PH; capitulum width, CW; calyculus bract number, CN; and disc floret number, DN; Table 6). For *S. g*. ssp. *glaucus*, our data are consistent with the above expectation, suggesting that effective population size (N_e), as calculated from the allozyme data, was too large for genetic drift causing the observed change in all four traits ($N_e >> N^*$). For this case, therefore, it is feasible that divergent selection favoured larger coastal plants producing more disc florets in broader capitula (possibly also bearing more attractive ray florets). Along this adaptive scenario, such trait changes would have conveyed a higher reproductive output and served concurrently in assortative mating by attracting different pollinators, hence leading to pollinator isolation from ancestral inland populations of ssp. *coronopifolius* (e.g. Harder and Johnson 2009; Ostevik et al. 2012; Anderson et al. 2016). However, for *S. hesperidium*, effective population size was found to be sufficiently small for drift to account for the magnitude of change in three of the traits examined (PH, CN, DN; $N_e << N^*$) but this did not apply to CW ($N_e > N^*$; Table 6). Clearly, the former outcome does not mean that the null hypothesis of drift is 'true', but only that there is insufficient evidence to reject this hypothesis. It is still possible that the similarities between *S. hesperidium* and *S. g*. ssp. *glaucus*, in all four traits considered, have been shaped by selection in parallel, although drift remains an alternative cause of most of these changes in *S. hesperidium* (excepting CW).

There are at least two possible explanations for why different evolutionary forces could have been at play in shaping phenotypic variation in these latter taxa. First, nearly neutral theory (Ohta and Gillespie 1996) predicts that population dynamics become more similar to random genetic drift as N_e declines (e.g. at range edges due to high demographic or environmental stochasticity; Soulé 1973; Hardie and Hutchings 2010), whereas selection should be more efficient at large population sizes (see also Hodgins-Davis et al. 2015). Currently, for our system, we have only crude estimates of N_e, to say nothing about historical fluctuations in population size. However, by taking range size as an independent proxy of N_e, it should not be unexpected that stochastic/neutral processes had a larger role in driving phenotypic divergence in the rare and local Moroccan endemic *S. hesperidium* than in the more widespread and abundant *S. g*. ssp. *glaucus*, which has large and near continuous populations along the Mediterranean coast of Israel (Danin 2006; R.J. Abbott and H.P. Comes, pers. obs.). Second, it is widely recognised that steep ecological gradients are particularly permissive of divergent selection (e.g. Barton 1999; Bridle and Vines 2006; Evans et al. 2016). Such conditions are clearly borne out in the Near East, where sharp ecological (e.g. edaphic, climatic) clines occur across short distances from Mediterranean to semi-arid/desert ecosystems, and which coincide with the distributional margins of many organisms, including the two subspecies of *S. glaucus* (e.g. Yom-Tov and Tchernov 1988; Kadmon and Danin 1997; Kark et al. 1999; Danin 2006).

Conclusions and future directions

The present data suggest that *S. glaucus* ssp. *coronopifolius* gave rise to two coastal taxa with similar phenotypes at its range margins in Morocco (*S. hesperidium*) and Israel (*S. g*. ssp. *glaucus*), in addition to a phenotypically deviant but ecologically similar variant in Sicily, founded from Tunisia. Our estimates of divergence times from the allozyme data further suggest that these divergences occurred during the last glacial/interglacial cycle(s). We therefore propose that the pronounced morphological but weak genetic differentiation between these ancestral and descendant populations largely reflects recent divergence rather than high levels of hybridisation/post-divergence gene flow. Finally, based on our estimates of N_e and tests of neutral morphological change (Lande 1976), we hypothesise that ecological divergent selection drove the origin of ssp. *glaucus*, possibly via changes in plant size and floral morphology (potentially underlying

reproductive output and premating reproductive isolation). However, though selection may have brought about similar phenotypic changes in *S. hesperidium*, these tests largely failed to disprove drift as an alternative cause (with the exception of similarly broad capitula), perhaps reflecting the importance of stochastic-demographic processes in the evolution of this local endemic. Overall, these results point at an interesting case of parallel ecotype formation and (sub)speciation in *Senecio* in which either primarily selective (deterministic) or primarily neutral (stochastic) determinants promoted the recurrent origin of coastal types in, respectively, Israel and Morocco (and possibly Sicily). Concomitantly, our study highlights the importance to critically examine the common notion that broadly similar phenotypes among independently evolved ecotypes reflect parallel adaptation to shared environments.

However, in view of a number of caveats and assumptions included in our analyses (see 'Materials and methods'), future research should test the validity of the above inferences along multiple criteria as recently proposed for the study of 'parallel ecological speciation' in plants (Ostevik et al. 2012; see Introduction). For example, high-throughput genotype data from a larger number of populations of each study region (and ideally throughout the North African range of ssp. *coronopifolius*) together with coalescent-based approaches should give more conclusive information on ancestral-descendant relationships, divergence times, post-divergence gene flow, and historical N_e, despite challenges remaining to reliably infer such parameters from such recently diverged organisms (e.g. Barker 2011; Jónás et al. 2016). In addition, current and future genomic studies in Mediterranean *Senecio* (e.g. Brennan et al. 2016; Chapman et al. 2016) should facilitate the search for neutral vs. adaptive mechanisms underlying parallel isolation in our study system [e.g. comparisons of divergence in quantitative phenotypic traits attributable to additive genetic effects (measured as Q_{ST}) to divergence at supposedly neutral marker genes (F_{ST}); F_{ST} outlier loci screens; or even the detection of selective sweeps at loci underlying putative adaptive traits]. Moreover, field studies could be initially focussed on the Near East to test (e.g. via reciprocal transplants) whether the two diverging subspecies of *S. glaucus* are truly reproductively isolated by ecological divergent selection against immigrants or hybrids in the different parental environments (Ostevik et al. 2012; Richards and Ortiz-Barrientos 2016; Richards et al. 2016). Finally, for all these future directions, one has to bear in mind that ssp. *glaucus* and the two other descendants likely evolved specific changes not measured here, e.g. in germination, life history, reproductive phenotype (including flowering time) and/or particular edaphic tolerances (e.g. to soil salinity, salt-spray, water shortage, low organic matter), as shown for coastal variants in a variety of other species (e.g. Hannon and Bradshaw 1968; Comes 1995; Rajakaruna et al. 2003a, 2003b; Roda et al. 2013; Busoms et al. 2015).

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Supplemental data

Supplemental data for this article can be accessed here.

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