

**Hybridisation and detection of a hybrid zone between mesic and desert ragworts  
(*Senecio*) across an aridity gradient in the eastern Mediterranean**

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## **Abstract**

**Background:** Hybrid zones provide excellent opportunities for studying plant adaptation and speciation.

**Aims:** We tested whether two herbaceous species of *Senecio*, *S. vernalis* and *S. glaucus*, hybridise in the eastern Mediterranean region and form a hybrid zone across an aridity gradient in the Jordan Rift Valley.

**Methods:** Allozyme variation surveyed across both species was analysed by the programme STRUCTURE to assign individuals to genetic groups and determine levels of admixture. Populations in the Jordan Rift Valley were subsequently subjected to a cline analysis.

**Results:** STRUCTURE showed that interspecific hybrids were produced at low frequency along the Israeli coastal plain where *S. glaucus* is represented by ssp. *glaucus*. In contrast, hybrids were more commonly produced in central populations of the Jordan Rift Valley. Here the two species form a hybrid zone with *S. vernalis* occurring in mesic sites to the north and *S. glaucus* (ssp. *coronopifolius*) in arid sites to the south. Cline analysis showed that the hybrid zone is centred towards the northern end of the Dead Sea, but failed to distinguish how it is maintained.

**Conclusions:** Future detailed genetic and ecological analysis of the *Senecio* hybrid zone should improve our understanding of plant adaptation and speciation across aridity gradients.

**Keywords:** aridity gradient; cline analysis; gene flow; hybridisation; hybrid zone; Mediterranean; reproductive isolation; *Senecio*; speciation

## Introduction

Hybridisation between plant species is a relatively common phenomenon though restricted to certain families and genera (Ellstrand et al. 1996; Whitney et al. 2010; Marques et al. 2018). The frequency of unique hybrids per non-hybrid plant species is estimated to vary from ca. 4 to 33%, according to geographical region (Ellstrand et al. 1996; Whitney et al. 2010; Guo 2014; Stace et al. 2015; Marques et al. 2018). Some of these estimates may increase as floras are studied more intensively. For example, based on records available to Ellstrand et al. (1996) hybrid frequency in the flora of the British Isles was estimated to be ca. 22% (642 unique hybrids among 2950 known species comprising the flora). This estimate rose to about 26% by 2010 (Whitney et al. 2010) based on an updated edition of the *New Flora of the British Isles* (Stace 1997), and recently to ca. 33% following publication of the *Hybrid Flora of the British Isles* (Stace et al. 2015). For the Mediterranean region, Marques et al. (2018) estimated a hybrid frequency of ca. 6%, but cautioned on the reliability of this estimate due to limited information available from the eastern Mediterranean and along the whole of the North African coast. For the Iberian Peninsula, for which much greater and more detailed information was available, the estimated hybrid frequency increased to ca. 13% (Marques et al. 2018).

Though records of spontaneous plant hybrids in the wild are plentiful, they are usually based on morphological assessment and drawn from species accounts published in regional floras (Whitney et al. 2010; Marques et al. 2018). Hence, for the vast majority of hybrids, there is an absence of population information and genetic analysis. Although, genetic analysis of putative hybrids usually confirms that such plants are hybrids, this is not always the case (see for example, Durka et al. 2017). Moreover, an absence of population information and particularly population genetic

analyses of hybrids and their parent species means that for most reported hybrids there is no information on the genetic structure of populations and hybrid zones containing hybrids, and factors, such as gene flow, selection and genetic drift, determining their structure.

In a recent review of the literature, Abbott (2017) listed 137 pairs or groups of native plant taxa (subspecies and species) of equivalent ploidy known to have formed hybrid zones that have been genetically analysed to some degree. According to Harrison (1993), hybrid zones occur at locations where “genetically distinct individuals meet and mate, resulting in at least some offspring of mixed ancestry”. Most of the 137 hybrid zones listed by Abbott (2017) occur in North and Central America, Europe and Asia (85%) with very few reported from other parts of the world (eight in Australia, six in each of South America and the Pacific islands, three in the Canary Islands and one in Africa). The current low number of genetically studied plant hybrid zones undoubtedly reflects, in part, the scant human, scientific and financial resources available for undertaking such work. However, it is also possible that hybrid zones comprising populations in which hybrids are present may be rarer than indicated from the known ability of plants to hybridise in the wild (Abbott 2017). Many plant taxa that occur in sympatry or parapatry might thus produce an occasional hybrid without forming a hybrid zone comprising one or more populations containing hybrids and their derivatives. Hewitt (1988) and Harrison (1990) have emphasised the value of studying hybrid zones referring to them as “natural laboratories for evolutionary studies” and “windows on evolutionary process”, respectively. Thus, from the standpoint of gaining an improved understanding of diverse aspects of plant evolution and speciation across the plant kingdom, there is value in detecting and

studying additional plant hybrid zones, particularly in regions where few or no hybrid zones have been reported previously.

The genus *Senecio* (Asteraceae) comprises about 1000 species (Pelser et al. 2010), some of which are well-known for their ability to hybridise with other species in the genus (Abbott and Lowe 1996; Prentis et al. 2007; Lowe and Abbott 2015; Osborne et al. 2016; Bog et al. 2017). Phylogeographic and phylogenomic analyses of Mediterranean *Senecio* (circumscribed by Alexander 1979) have indicated that interspecific hybridisation and gene flow commonly occur in this complex (Comes and Abbott 2001; Osborne et al. 2016). However, only one diploid *Senecio* hybrid zone has been reported and analysed genetically within this group, which is the hybrid zone between the high elevation species, *S. aethnensis* Jan. ex DC., and the low elevation species, *S. chrysanthemifolius* Poiret, that occurs on Mount Etna, Sicily (James and Abbott 2005; Brennan et al. 2009; Chapman et al. 2013; Filatov et al. 2016). Here we report the presence of a hybrid zone between *S. glaucus* L. and *S. vernalis* Waldst. & Kit. across an aridity gradient in the eastern Mediterranean.

Detailed descriptions of the taxonomy, biology and geographical distributions of *S. glaucus* and *S. vernalis*, which differ notably in leaf shape, are given in Comes and Abbott (1999). In summary, both are diploid ( $2n=20$ ), self-incompatible, entomophilous, annual herbs that are widely distributed in Eurasia. *Senecio vernalis* is more northerly distributed, occurring in relatively mesic habitats throughout central and eastern Europe, south-west Asia, southern Russia, and Afghanistan (Alexander 1979). In contrast, *S. glaucus* occupies more arid (desert and semi-desert) habitats from the Canary Islands, through North Africa and the Middle East, to the Himalayas and north-west China (Alexander 1979; RJ Abbott, personal observation). In the eastern Mediterranean, the distributions of the two species overlap in Israel, the West

Bank, Jordan and Syria (Alexander 1979; Comes and Abbott 1999) with *S. vernalis* occurring often as a ruderal of disturbed sites in the mesic Mediterranean life zone, while *S. glaucus* mainly occupies more natural, arid habitats in the Irano-Turanian and Saharo-Arabian zones (see figure 1 in Comes and Abbott, 1999). Inland in Israel and the West Bank, along the Jordan Rift Valley, *S. vernalis* is replaced by *S. glaucus* as conditions change from mesic in the north to arid in the south. However, along the Mediterranean coast both species occur and sometimes form sympatric populations, though are normally spatially separated with *S. glaucus* confined to more xeric, maritime-sand habitats from which *S. vernalis* is excluded (Comes and Abbott 1999). Because reciprocal crosses between the two species are known to produce good seed (Alexander 1979; Kadereit 1983; Goodwin 2005) and both species tend to flower at the same time in areas of overlap (Comes and Abbott, personal observation), hybridisation and the formation of hybrid zones might be expected in regions of contact.

A phylogenetic analysis of Mediterranean *Senecio*, based on sequence variation of the internal transcribed spacer (ITS) regions of nuclear ribosomal DNA has indicated that *S. glaucus* and *S. vernalis* belong to two different well-supported clades that diverged from a common ancestor between 2.31 and 4.97 Mya (Comes and Abbott 2001). Within *S. glaucus*, a Mediterranean form endemic to the coastal plain of Israel is recognised as ssp. *glaucus*; this differs from the widespread and mainly inland form, ssp. *coronopifolius* (Maire) Alexander, in having a more robust habit, larger capitula, fleshy leaves and distinctive trifurcate tips of leaf lobes (Alexander 1979; Comes et al. 2017). A UPGMA dendrogram constructed from measures of genetic distance based on allozyme variation within and between *S. glaucus* and *S. vernalis* has clustered populations of the two species into two distinct

units and further showed that within *S. glaucus* populations of ssp. *glaucus* formed a discrete group in agreement with taxonomic separation based on morphology (Comes and Abbott 1999). *Senecio glaucus* and *S. vernalis* have been found not to be fixed for different alleles at the allozyme loci investigated and consequently hybrids were not readily identified. However, based on an estimated indirect measure of nuclear gene flow between the two species, it has been concluded that they were almost or fully isolated from each other in Israel and the West Bank, although sharing of chloroplast (cp) DNA haplotypes did not rule out the possibility that occasional hybridization occurs between the two species (Comes and Abbott 1999). At the time of conducting the analysis of the allozyme data set, the Bayesian clustering programme STRUCTURE (Pritchard 2000), now routinely used to assign individuals to groups and identify admixed (hybrid) individuals, was unavailable. Consequently, admixed individuals were not identified and hybrid zones could have been missed. To rectify this, we report here a reanalysis of the allozyme data collected by Comes and Abbott (1999), using STRUCTURE to detect the number of genetic groups in the total data set, the occurrence of admixture between genetic groups, the possible existence of hybrid zones, and the number of genetic groups in *S. glaucus* and their correspondence to the two recognised subspecies (subsp. *glaucus* and subsp. *coronopifolius*) within this taxon. STRUCTURE detected admixed individuals (hybrids) between *S. vernalis* and *S. glaucus* in some populations and a clear hybrid zone between the two species across the mesic-xeric aridity gradient that runs north to south in the Jordan Rift Valley of the eastern Mediterranean. We subjected this hybrid zone to further statistical analysis that included tests of hybrid index distributions, tests of linkage disequilibrium, and tests that included additional climatic data with the aim of determining how endogenous and exogenous selection might maintain a

cline in hybrid index from *S. vernalis* to *S. glaucus* moving south along the Jordan Rift Valley.

## **Materials and methods**

### *Plant material*

Sampling of material is described in detail in Comes and Abbott (1999). In summary, seed was collected from about 30 individuals from each of 11 populations of *S. vernalis* in Israel (total number of individuals, N = 331) and 14 populations of *S. glaucus*, 11 in Israel/the West Bank and three in northern Egypt (N = 423) (Table 1, Figure 1 reproduced from Comes and Abbott 1999). According to morphology, populations of *S. glaucus* along the Mediterranean coast of Israel (at AK, C, NOF, AD and AQ) represent *ssp. glaucus*, whereas populations from elsewhere represent *ssp. coronopifolius* (Table 1). At AK, the two species occurred in sympatry and sampling was conducted such as to avoid collecting seed from intermediate plants considered to be hybrids. Although in retrospect this action was unfortunate, sampling at this site was conducted primarily for a phylogeographic comparison of the two species and it was decided to avoid sampling hybrids at the time. At two other sites (BS and MAS) plants were considered to represent *S. glaucus ssp. coronopifolius*, but some had atypical morphology for this taxon and were possibly hybrids, showing intermediate expression of some leaf shape traits relative to *S. glaucus ssp. coronopifolius* and *S. vernalis*, respectively. However, at both of these sites and at all other sites except the one at AK, seeds were collected from a random sample of plants.

### *Allozyme variation survey*



Allozyme variation was surveyed across eight loci (*Aat-3*,  *$\beta$ -Est-3*, *Idh-1*, *G3pd-1*, *Pgi-1* and *-2*, *Pgm-1* and *-2*) using starch gel electrophoresis on leaf protein extracts taken from plants raised from seed (one per mother plant) in a glasshouse (Comes and Abbott 1999). Loci *Pgi-1* and *Pgm-1* were monomorphic across all individuals surveyed and omitted from further analysis. A total of 31 and 36 alleles were detected in *S. vernalis* and *S. glaucus*, respectively, across the six polymorphic loci.

### *Population structure*

A matrix of genotypes across all loci for all individuals in all populations surveyed was constructed (Table S1) and analysed to obtain the optimal number of genetic clusters ( $K$ ) in the total data set using a Bayesian clustering approach implemented in the programme STRUCTURE v2.3.4 (Pritchard 2000). The admixture model was employed with allele frequencies correlated among populations for  $K$  values from 1 to 10 with ten runs for each  $K$ . In each run a burn-in of 100,000 iterations followed by 100,000 Markov chain Monte Carlo (MCMC) iterations were used. STRUCTURE output files were uploaded to POPHELPER Structure Web App v1.0.10 (Francis 2017) to produce plots of the estimated log probability of data [ $\ln\text{Pr}(X|K)$ ] for each value of  $K$  [(Pritchard 2000, referred to as  $L(K)$  in Evanno et al. 2005)],  $\Delta K$ , denoting the rate of change of the log probability of data with respect to  $K$  (Evanno et al. 2005), and high definition barplots showing the assignment of individuals to genetic clusters and levels of admixture. STRUCTURE was also used in the same way (for  $K = 1-10$  with ten runs for each  $K$ ) to analyse a subset of data that included only individuals of *S. glaucus* to detect the optimal number of genetic clusters present in this species.

### *Cline analysis*

The 13 populations sampled along the Jordan Rift Valley, eight sampled as *S. vernalis* (numbers 1–6, 8) and six as *S. glaucus* ssp. *coronopifolius* (numbers 7, 10–14; Table 1), were treated as part of a transect crossing from the distribution range of *S. vernalis* into that of *S. glaucus* with a potential hybridising contact zone in between. The transect line was defined as the linear regression of these sample points against distance. Samples were mapped onto the transect line and the spatial distances between each point calculated using the `nearestPointOnSegment` and `spDistsN1` functions of the `MAPTOOLS` R package (Bivand et al. 2017). An alternative transect line and set of sample population distances that excluded the BS population site (number 7), a northern outlier of *S. glaucus* ssp. *coronopifolius* located ca. 50 km north of the northern boundary of the main distribution of the taxon in the rift valley (see figure 1 in Comes and Abbott 1999), was also calculated using these methods.

Hybrid index values based on allozyme genotypes were estimated for each individual in populations along the transect using the `est.h` function of the `INTROGRESS` R package (Gompert and Buerkle 2010). This measurement sums the weights of each allele at each locus according to the observed frequency difference between two samples representing each species so that hybrid index values scale between zero (in this case genetically similar to *S. vernalis*) and one (genetically similar to *S. glaucus*). Intermediate hybrid index values indicate hybrid individuals on a quantitative scale of relative genetic contributions from each species. The two most northerly populations (M'AS and ER) and the two most southerly populations (ZT and MR) sampled at each end of the transect were chosen as representative of each species in this case. Hybrid index measured in this way is preferable for hybrid cline analysis compared to other admixture measures such as  $Q$  (the posterior probability of assignment to a particular genetic group), generated by `STRUCTURE`, because it

summarises allele frequencies more directly upon which hybrid cline theory is based (Gompert and Buerkle 2009). Sigmoid models with estimated minimum and maximum allele frequencies were fitted to hybrid index population means along the transect using the default Metropolis–Hastings MCMC `hzar.doFit` function implemented by the HZAR R package (Derryberry et al. 2013). Two independent MCMC chains were run, linked by the `hzar.next.fitRequest` function. This analysis outputs log-likelihood confidence distributions for the cline centre and cline width. The significance of the cline was evaluated by analysis of variance (ANOVA) comparing residual variance from the null model of no change in mean population hybrid index along the transect line (null model) against a linear model of hybrid index explained by fitted clinal hybrid index values (clinal model). The influence of each of three environmental variables - mean annual precipitation, mean annual temperature, and elevation - in shaping the hybrid index cline was tested similarly by comparing residual variance from the linear model of mean population hybrid index explained by fitted clinal hybrid index values (clinal model) against a linear model of mean population hybrid index explained by fitted clinal hybrid index and each environmental variable in turn (clinal + environment models). Population-level values were used for these tests to match the population-level analysis that was performed to fit the original hybrid index cline.

#### *Linkage disequilibrium along the cline*

Selection against hybrids in the contact zone between two species can be detected as an elevation in linkage disequilibrium (LD) between molecular markers when their alleles have been scored according to parental frequency. This is because LD is initially high in F1 hybrids and declines in later hybrid generations. Selection against

hybrids reduces the persistence of later generation hybrids and therefore leads to elevated LD within the contact zone (Barton and Gale 1993; Arnold 1997). The allozyme data were re-scored as biallelic according to the relative frequency of each allele in the two sets of populations at each end of the transect used to represent each species for hybrid index estimation. Missing data were assigned where alleles were observed to be equally frequent in the samples of both species. LD between each pair of polymorphic biallelic loci in each population was measured as gametic disequilibrium correlation coefficients not assuming Hardy-Weinberg equilibrium ( $cr$ ) using GenAlEx v6.5 (Peakall and Smouse 2012). These  $cr$  values were further corrected for allele sharing between the species by multiplying these values by the allele frequency difference between species (Barton and Gale 1993). Elevated LD at the hybrid cline centre was tested by fitting a Gaussian (normal) curve to corrected  $cr$  values along the transect line. The significance of the fitted model was evaluated by comparing residual variance from the null model of no change in corrected  $cr$  values along the transect line against a linear model of fitted corrected  $cr$  values. An alternative way to test for selection against hybrids within populations is to examine the distribution of hybrid index values for a deficit of intermediate values. Therefore, the distribution of hybrid index values within each sample population was tested for multi-modality employing Hartigan's dip tests using the DIPTEST R package (Maechler 2015).

## **Results**

### *Number of genetic groups and levels of admixture across all populations*

Plots of mean  $L(K)$  and  $\Delta K$  for  $K=1-10$  indicated that the most likely number of genetic groups in the total data set was two ( $K=2$ ) (Figure S1 A and D). Mean  $L(K)$

showed a sharp increase between  $K=1$  and  $K=2$  and much smaller increases above  $K=2$ , while  $\Delta K$  fell to zero or close to zero at  $K$  values above  $K=2$ . The barplot indicating the assignment of individuals to groups when  $K=2$  (Figure 2) showed that one genetic group equated to *S. vernalis* and the other to *S. glaucus*. Although most individuals sampled from *S. vernalis* populations were assigned to one genetic group, while those from *S. glaucus* populations were assigned to the other group, the progeny from some populations were clearly admixed with posterior probability threshold ( $Q$ ) values  $< 0.9$ . Following Vähä and Primmer (2006), we regarded these individuals as hybrids in contrast to individuals with  $Q$  values  $> 0.9$  that were considered representatives of one or other of the two parent species.

When offspring of populations were examined from the transect running approximately north to south in the Jordan Rift Valley and into the Negev Hills and northern Egypt, i.e., which included populations M'AS (1), ER (2), HU (3), Q (4), RAM (5), NY (6), BS (7), S (8), AL (10), KB (11), MAS (12), ZT (13), MR (14), ALEX (15), EQ (16) and CAI (17) (Figure 1, Table 1), it became apparent that a hybrid zone existed at middle latitudes between *S. vernalis* in the north and *S. glaucus* in the south. Thus, individuals in populations at BS, AL, KB and MAS, contained many hybrid progeny, while those from the population at S contained a few (Figure 2). Interestingly, the first four of these populations were recorded as *S. glaucus* ssp. *coronopifolius* at the time of sampling, although two (BS and MAS) were noted to contain some individuals with atypical leaf morphology (possibly hybrid), while individuals at AL and KB were at an advanced life-stage and difficult to type taxonomically. In contrast, the population at S was recorded as *S. vernalis*. Very few hybrids were found in the progeny of individuals sampled from other populations along this transect. Thus, one hybrid was recovered from the *S. vernalis* population at

M'AS, none were recovered from those at ER, HU, RAM and NY, and none were recovered from the *S. glaucus* populations at ZT, MR, ALEX, EQ and CAI.

Along the Mediterranean coastal plain where *S. glaucus* is represented by ssp. *glaucus* rather than ssp. *coronopifolius*, very few admixed individuals (hybrids) were present among the progeny of *S. glaucus* and *S. vernalis* populations surveyed (Figure 2). Thus, at AK where both species occurred in sympatry, two hybrids were detected among progeny of *S. vernalis* (most probably backcrosses to *S. vernalis*), while none were present among progeny of *S. glaucus* individuals. Similarly, a few hybrids were present among *S. vernalis* progeny surveyed from ZY, while none was present in those from the nearby *S. glaucus* population at C, and one hybrid was identified among offspring from both the *S. vernalis* population at TA and the *S. glaucus* population at NOF. No hybrids were recorded among progeny examined from either of the two most southern populations of *S. glaucus* ssp. *glaucus* at AD and AQ where no populations of *S. vernalis* were located nearby.

#### *Number of genetic groups within S. glaucus*

Although the analysis of the total data set by STRUCTURE indicated very strongly that only two genetic groups were present (Figure S1), and that these equated to *S. vernalis* and *S. glaucus*, respectively (Figure 2), a subset of the data comprising only *S. glaucus* populations (including those in which hybrids were produced) was also analysed using STRUCTURE to determine if further genetic subdivision might exist that differentiates ssp. *glaucus* from ssp. *coronopifolius*. Janes et al. (2017) have recently reemphasised the value of using STRUCTURE in a hierarchical manner to examine additional subgroups within a data set, even when  $K=2$  is identified as the top level of hierarchical structure.

Plots of mean  $L(K)$  and  $\Delta K$  for  $K=1-10$  for the *S. glaucus* data set indicated that two genetic groups were present ( $K=2$ ) (Figure S2 A and D). However, it is evident from the barplot (Figure 3) that the two subspecies are not easily distinguished by this division. Thus, although the majority of *ssp. glaucus* individuals examined owe most of their ancestry to one group, this is not the case for *ssp. coronopifolius* individuals, some of which show an affinity to the same genetic group as do the majority of *ssp. glaucus* individuals, while others have a closer affinity to a second genetic group, and many individuals are not clearly assigned to either group. Thus, individuals representing the two subspecies of *S. glaucus* could not be clearly assigned to two different genetic groups in this data subset.

#### *Cline analysis of the hybrid zone between S. vernalis and S. glaucus in the Jordan Rift Valley*

A sigmoid cline in hybrid index values of offspring was fitted to the 13 populations along the Jordan Rift Valley (Figure 4) that was effective at explaining hybrid index variation along the transect (Null vs. Cline ANOVA,  $F = 27.47_{12,11}$ ,  $P < 0.001$ ). The maximum likelihood cline centre was estimated to be 127 km (114 – 142 km 2 log-likelihood interval) from the northern end of the transect, and was located close to sample site S (8) towards the southern end of the part of the rift valley between the Sea of Galilee and the Dead Sea, i.e. the Lower Jordan Valley. The maximum likelihood cline width was estimated to be 174 km (144 – 213 km 2 log-likelihood interval). Minimum and maximum hybrid index was estimated to be 0.0081 and 0.9831 beyond the edge of each side of the hybrid cline. Removing the BS (7) population, a northern outlier of the distribution of *S. glaucus ssp. coronopifolius*, had a considerable impact on the fitted hybrid cline (Figure S3). The cline became steeper

[(cline width = 118 km (78 – 156 km 2 log-likelihood interval)] and its centre moved further southwards to 152 km (140 – 164 km 2 log-likelihood interval), closer to populations AL (10) and KB (11) near the northern end of the Dead Sea.

Of the three environmental variables examined, only mean annual precipitation showed significant ( $P = 0.001$ ) change along the transect, although mean annual temperature appeared to peak in the region towards the centre of the cline (Figure 5). Similarly, only mean annual precipitation showed a significant relationship ( $P = 0.003$ ) with hybrid index along the transect (Figure 5). The same patterns of change were evident when the BS (7) site was excluded from analysis (results not shown). However, ANOVA tests comparing models of the fitted hybrid cline only against models of the hybrid cline with each environmental variable indicated that none of the three environmental variables in combination with the fitted hybrid clines explained significantly more hybrid index variance than did the hybrid cline model alone either with the BS (7) population included or excluded (Table 2).

#### *Linkage disequilibrium (LD)*

Mean LD was not significantly elevated in populations with intermediate hybrid index values (Null vs. Gaussian ANOVA,  $F = 2.20_{12,11}$ ,  $P = 0.166$ ; Figure 6), suggesting weak selection against hybrids, such that hybrid lineages might persist long enough for hybrid LD to dissipate. Only for two populations was the corrected LD ( $cr$ ) value relatively high. In the *S. vernalis* population from M'AS (1) this was due to the presence of a single hybrid individual exhibiting a high level of *S. glaucus* ancestry. Removal of this individual reduced the corrected  $cr$  value from 0.146 to 0.046, i.e. to a value similar to those estimated for the other *S. vernalis* populations examined. In the other population, MAS (12) the relatively high  $cr$  value (0.155) apparently reflects



the presence of *S. glaucus*, *S. vernalis* and a mixture of different hybrid types (based on  $Q$  values) within the population (Figure 2). Tests of hybrid index distributions within population samples found no evidence for multi-modality that would suggest a deficit of intermediate hybrid index values and endogenous or exogenous selection against hybrids (Figure S4). Instead, most populations along the cline were unimodal for high or low hybrid index values with a few populations towards the centre of the transect [(BS (7), S (8), AL (10), KB (11), MAS (12)] showing broader distributions indicating the more frequent presence of hybrids.

## **Discussion**

### *Hybridisation and detection of a hybrid zone between S. vernalis and S. glaucus*

Analysis of an allozyme data set obtained by Comes and Abbott (1999) using the computer programme STRUCTURE (Pritchard et al. 2000) showed that admixed individuals representing hybrids between *S. vernalis* and *S. glaucus* occur among progeny of both species in areas where they are in contact in the eastern Mediterranean region (Israel and the West Bank). Though hybrids were rare or absent in the progeny of individuals of both species sampled along the Israeli coastal plain, where *S. glaucus* is represented by ssp. *glaucus*, they were more common among offspring of the two species in a region of contact in the Jordan Rift Valley where *S. glaucus* is represented by ssp. *coronopifolius*. Here a hybrid zone is present between the two species across an aridity gradient running north to south along the rift valley with *S. vernalis* restricted to mesic habitats mainly in the north and *S. glaucus* to xeric ones in the south.

From a population genetic analysis of the same data set conducted before the release of STRUCTURE, Comes and Abbott (1999) concluded that ‘... *S. vernalis*

and *S. glaucus* form a zone of secondary contact in the Near East, accompanied by an almost complete interspecific barrier to nuclear gene flow ...'. Reanalysis of the data set using STRUCTURE shows the latter is clearly not the case, though interspecific nuclear gene flow in the Jordan Rift Valley hybrid zone is limited mainly to central populations located in the Lower Jordan Valley and northern Dead Sea area with minor evidence of it occurring outside the hybrid zone in populations located approximately 100 km north or south of these central populations. Comes and Abbott (1999) have concluded that hybridisation was absent or very infrequent between the two species largely because populations clustered according to taxonomy in an allozyme-derived UPGMA genetic distance tree. If, instead, a taxonomic incongruent placement of populations had been observed, introgressive hybridisation as a possible cause would have been indicated and likely stimulated an analysis of hybrids within populations using, for example, the maximum likelihood method of Nason et al. (1992). The present finding from the STRUCTURE analysis that hybridisation occurs between *S. vernalis* and *S. glaucus* also helps to explain the previously reported widespread cpDNA haplotype sharing between them (Comes and Abbott 1999), although as pointed out at that time, such sharing of haplotypes across the species boundary is erratic, i.e. without clear delimitation of areas/zones of cytoplasmic gene exchange.

The greater frequency of hybrids formed between *S. vernalis* and *S. glaucus* ssp. *coronopifolius* in the Jordan Rift Valley than between *S. vernalis* and *S. glaucus* ssp. *glaucus* along the Israeli coast might reflect a stronger reproductive isolating barrier between the two species when ssp. *glaucus* is involved. However, at the time of sampling seed from plants at the AK coastal site, where the species were sympatric, we observed a large number of individuals of intermediate leaf morphology that were

assumed to be F1 hybrids. Seed was not collected from these individuals, but instead only from plants that morphologically represented one or other of the two parental species. Among the progeny raised from this seed, no hybrids were recovered from *S. glaucus* ssp. *glaucus* and only a few from *S. vernalis*. This indicates that a relatively strong prezygotic barrier exists between the two taxa, in contrast to what was expected given the many putative hybrids present at the AK site at the time of sampling. It is possible that conditions may sometimes favour hybridisation between these two taxa, but at other times prezygotic barriers are effective in reducing hybridisation to a low level. In addition, the apparent absence of post-F1 hybrids at AK at the time of sampling, might suggest that a strong postzygotic barrier exists between these two taxa preventing post-F1 hybrids establishing at the site. Further research is required to establish the nature and strength of pre- and postzygotic reproductive isolating barriers that exist between *S. vernalis* and *S. glaucus*, as conducted on a number of other plant species pairs that form hybrid zones in the wild (e.g., Kay 2006; Sambatti et al. 2012; Scopece et al. 2013; Briscoe Runquist et al. 2014; Kenney and Sweigart 2016).

A further point of interest to emerge from the STRUCTURE analysis is that hybridisation between the two species may be asymmetric with the direction of gene flow depending on the subspecies of *S. glaucus* involved. Thus, along the Israeli coast where hybridisation between the two species was rare, more hybrids were recovered from progeny of *S. vernalis* than *S. glaucus* ssp. *glaucus*, indicating that gene flow occurs more commonly into *S. vernalis* than in the reverse direction. In contrast, in central populations of the Jordan Rift Valley hybrids were more common in progeny of *S. glaucus* ssp. *coronopifolius*, suggesting that gene flow occurs predominantly into this subspecies. Asymmetrical gene flow between plant taxa that form hybrid zones is

often reported or inferred (Abbott 2017) and can be caused by a number of different factors, e.g. differential abundance of parental lineages, differential gamete production and fertilisation, and differential embryo development and offspring survival (Tiffin et al. 2001; Turelli and Moyle 2007; Lowry et al. 2008). Further research is required to confirm that gene flow between the two species is asymmetric and dependent on the subspecies of *S. glaucus* involved and, if so, what the causes of this might be.

### *Population structure*

Analysis by STRUCTURE of the entire data set resolved two genetic groups corresponding to *S. glaucus* and *S. vernalis*, respectively. The majority of populations based on progeny tests were mainly monospecific containing individuals assigned to one or other group. However, as already emphasised, some populations of both species contained admixed individuals representing interspecific hybrids. Although STRUCTURE strongly indicated the occurrence of only two genetic groups, a separate analysis of *S. glaucus* data indicated the presence of two genetic groups within this species. Whereas many offspring of *S. glaucus* ssp. *glaucus* were assigned to one of these groups, and there was a tendency for progeny of ssp. *coronopifolius* (from some populations) to be assigned predominantly to the other group, the correspondence between subspecies of *S. glaucus* and genetic group was far from complete. It is possible that a division within *S. glaucus* into two genetic groups, corresponding to the two subspecies respectively, may become more evident should a genome-wide analysis of nuclear variation be undertaken involving thousands of genetic markers.

### *Clinical analysis of the Jordan Rift Valley hybrid zone*

A sigmoid cline of hybrid index against distance provided a highly significant fit for the 13 populations distributed north to south along the Jordan Rift Valley and into the Negev Hills beyond. The width of this cline was estimated to be 174 km with its centre located close to population S (8) in the Lower Jordan Valley, 127 km from the most northerly *S. vernalis* populations examined. Whereas *S. vernalis* occurs commonly and at high density at the northern end of the Jordan Rift Valley with many populations present, the distribution of *S. glaucus* is more fragmented and sparse at the southern end (Comes and Abbott, personal observations). The central part of the cline where hybrids are most common covers an area to the north of and along the western banks of the Dead Sea where populations were also sparsely distributed (HP Comes and RJ Abbott, personal observations) and coincides with the highest mean annual temperature along the transect (Figure 5).

The shape and width of the cline in hybrid index is expected to be dependent on levels of nuclear gene flow (seed and pollen) between populations and the strength of selection for and against dispersed genes along the transect. In the Jordan Rift Valley levels of dispersal and selection will be affected by both anthropogenic and natural factors. For example, because a main road runs along the length of the valley, gene dispersal is likely to be affected by traffic (mainly aiding seed dispersal) in addition to natural factors such as wind and insects (mainly aiding seed and pollen dispersal, respectively). It is feasible that human-induced, long-distance seed dispersal was responsible for the establishment of the *S. glaucus* ssp. *coronopifolius* population at the BS (7) site >50 km north of the northern boundary of the main distribution of this taxon in the rift valley. The effect of removing this population from the analysis was to reduce the width of the cline from 174 km to 118 km, and move the cline

centre further to the south closer to populations AL (10) and KB (11), i.e., 152 km from the most northerly *S. vernalis* populations examined. It is also feasible that human-induced long distance dispersal from the BS site (or *S. glaucus* populations further south) was responsible for the occurrence of one admixed progeny in the most northerly population of *S. vernalis* (M'AS) located in the Golan Heights (Figure 2).

An indication of whether selection across the cline is largely determined by (i) external environmental effects (exogenous selection) with intermediate hybrid phenotypes favoured in intermediate habitats, or (ii) by a balance of gene dispersal between populations and selection (exogenous or endogenous) against ill-fit hybrids, was obtained first by examining the influence of three extrinsic environmental variables - mean annual precipitation, mean annual temperature, and elevation - in shaping the cline. Only mean annual precipitation changed significantly along the transect, being high in the north and low in the south, and was significantly related to hybrid index across the cline. However, this variable in combination with the fitted hybrid cline failed to explain significantly more allele frequency variance than did the hybrid cline model alone either with or without the BS (7) population included (Table 2). Consequently, although annual mean precipitation is likely to be of major importance with regard to the adaptation of *S. vernalis* and *S. glaucus* to their respective mesic and xeric habitats, our results do not support the hypothesis that it determines the change in hybrid index across the cline, favouring hybrids at intermediate levels of annual mean precipitation.

If, alternatively, the hybrid index cline is determined by a balance of gene dispersal between populations and selection against ill-fit hybrids, it is expected that linkage disequilibrium (LD) for the loci investigated should be elevated at the centre of the cline due to endogenous and/or exogenous selection against hybrids, and an

absence, therefore, of advanced generation hybrids in which a breakdown of LD has occurred due to recombination. Contrary to this, we found no consistent evidence for elevated LD in populations towards the centre of the cline (Figure 6), although slightly elevated LD was evident in one central population at MAS (12). Furthermore, although tests of modality of hybrid index distributions within populations indicated that no population exhibited a multi-modal distribution, central populations were characterized by a broader distribution of hybrid index values indicating the presence of some advanced (non-F1) generation hybrids in these populations (Figure S4). In summary, hybrid individuals were relatively rare in this hybrid zone despite little genetic evidence for selection against hybrids. However, it is possible that our analysis failed to detect elevated LD and a general absence of advanced generation hybrids in central populations because of the very low number of loci employed. Recently it has been shown that the accuracy of results obtained from this form of analysis may depend on the number and genomic distribution of genetic markers used (Christe et al. 2016). Thus, studies of hybrid zones between two poplar (*Populus*) species using a relatively low number of genetic markers have initially indicated that hybrids were later generation recombinants (Lexer et al. 2005, 2010; Lindtke et al. 2012). However, when several hundred thousands of SNPs were later employed in the analysis, it became clear that most hybrids were in fact F1s and that strong selection against post-F1 hybrids likely occurred in these hybrid zones (Lindtke et al. 2014; Christe et al. 2016).

### **Conclusions and future directions**

Reanalysis of the allozyme data set of Comes and Abbott (1999) using the programme STRUCTURE (Pritchard 2000) clearly demonstrated that hybrids form between *S.*

*vernalis* and *S. glaucus* in the eastern Mediterranean and that a hybrid zone is present between the two species in the Jordan Rift Valley. This hybrid zone is the second to be detected among Mediterranean ragworts and emphasises further the value of this ragwort complex in the analysis of plant speciation and hybridisation (Osborne et al. 2016). To our knowledge, this hybrid zone is also the first to be reported between plant species in the Jordan Rift Valley, although genetic differentiation for traits likely to be adaptive is known to occur within plant species distributed across aridity gradients in Israel (Volis 2007) and for one species of *Eruca* (Brassicaceae) a hybrid zone was reported in the northern part of the Jordan Rift Valley between two putative ecotypes (Westberg et al. 2013).

A clinal analysis of hybrid index across the hybrid zone showed that a relatively broad sigmoid cline, centred towards the northern end of the Dead Sea, provided the best fit. However, analysis of this cline failed to indicate how it is maintained. Thus, our analysis suggested that hybrids were neither at an advantage in environments intermediate to those occupied by the parental species, as expected if the hybrid zone was a ‘bounded hybrid superiority hybrid zone’ (after Moore 1977), nor indicated that they exhibited lower extrinsic and/or intrinsic fitness across all environments relative to the parent species, as expected for a ‘tension zone’ (after Barton and Hewitt 1985). More detailed genetic analysis of the hybrid zone involving more populations and a large number of genetic markers is now required to examine LD across the nuclear genome and whether early or late generation hybrids prevail in central populations. In addition, it will be of interest to determine which forms of reproductive isolation (pre- or post-zygotic) act to maintain *S. vernalis* and *S. glaucus* as distinct species, despite their ability to hybridise, and whether reproductive



isolation is stronger between *S. vernalis* and *S. glaucus* ssp. *glaucus* than between *S. vernalis* and *S. glaucus* ssp. *coronopifolius*.

Comes and Abbott (1999) have considered that the eastern Mediterranean represents a region of secondary contact between *S. vernalis* and *S. glaucus*. Consequently, the hybrid zone detected between these two species in the Jordan Rift Valley will have formed as a result of secondary contact between the two species after divergence in allopatry, rather than by primary intergradation due to divergence of an ancestral species across an environmental gradient in the face of gene flow (Abbott 2017). In support of the secondary contact model, phylogenetic analysis (Comes and Abbott 2001) has revealed that the two species are not sister species and instead belong to two different well-supported clades, each containing other *Senecio* species, that diverged from a common ancestor between 2.31 and 4.97 Mya. Detailed analysis and long-term monitoring of this hybrid zone will provide an opportunity to determine how adaptive divergence and the identity of these two species are maintained across an aridity gradient in the face of gene flow, and how the hybrid zone might respond to future climate change and human-mediated disturbance.

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No potential conflict of interest was reported by the authors.

## Supplemental data

Supplemental data for this article can be accessed here. [link to data]

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Table 1. Locality information of sampled populations of *Senecio vernalis*, *S. glaucus* ssp. *coronopifolius* (*cor.*) and ssp. *glaucus* (*gl.*) from Israel, the West Bank and Egypt as previously analysed for allozyme variation (Comes and Abbott 1999). \* Denotes populations included in the cline analysis.

Taxon/(Population number)	Population code	Country, locality	Latitude	Longitude	Elev (m)	Map (mm)	Mat (°C)	Climate type <sup>a</sup>	
<b>Israel (inland) and West Bank</b>									
<i>S. vernalis</i>	(1)	M'AS*	Israel, Golan, ca. 2 km W of Mas`ada, roadside.	33°14'N	35°43' E	604	817	14.2	Csa
<i>S. vernalis</i>	(2)	ER*	Israel, Golan, near Zomet El Rom, abandoned military base	33°09'N	35°47'E	973	755	14.8	Csa
<i>S. vernalis</i>	(3)	HU*	Israel, Hule Plain, ca. 4 km S of Zomet Gonen, roadside	33°08'N	35°34'E	128	585	20.4	Csa
<i>S. vernalis</i>	(4)	Q*	Israel, Golan, near Qazrin, entrance Zawitan Valley, roadside	32°59'N	35°43'E	363	511	19.5	Csa
<i>S. vernalis</i>	(5)	RAM*	Israel, Western Galilee, Rama, orchard on terra-rossa, next to Rd. No. 85	32°56'N	35°22'E	350	676	19.5	Csa
<i>S. vernalis</i>	(6)	NY*	Israel, Kinnereth, south entrance of Nahal Yehudiyya, amidst basaltic rocks	32°53'N	35°39'E	-199	376	22.1	BSh
<i>S. glaucus</i> ssp. <i>cor.</i>	(7)	BS*	Israel, Upper Jordan Valley, Bet-She`an, ruderal site	32°30'N	35°30'E	-132	344	21.8	BSh
<i>S. vernalis</i>	(8)	S*	West Bank, Lower Jordan Valley, picnic area near "The Memorial", south of Tel Sartaba	32°03'N	35°27'E	-274	219	23.4	Bwh
<i>S. vernalis</i>	(9)	J	Israel, Judean Hills, Jerusalem, building site	31°47'N	35°13'E	803	477	17.2	CSa
<i>S. glaucus</i> ssp. <i>cor.</i>	(10)	AL*	West Bank, Dead Sea, Almog area, roadside	31°45'N	35°28'E	-355	100	24.0	BWh
<i>S. glaucus</i> ssp. <i>cor.</i>	(11)	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
<i>S. glaucus</i> ssp. <i>cor.</i>	(12)	MAS*	Israel, Dead Sea, S of Massada, opposite Lissan Peninsula, brackish playa	31°19'N	35°22'E	-362	107	23.9	BWh

<i>S. glaucus</i> ssp. <i>cor.</i>	(13)	ZT*	Israel, Be`er Sheva Basin, ca. 4 km S of Zomet Telalim	30°57'N	34°47'E	502	229	19.3	BSh
<i>S. glaucus</i> ssp. <i>cor.</i>	(14)	MR*	Israel, Central Negev Hills, below Mizpe Ramon, roadside	30°36'N	34°46'E	850	183	16.9	BSk
<b>Egypt</b>									
<i>S. glaucus</i> ssp. <i>cor.</i>	(15)	ALEX	Egypt, Coastal Area, Alexandria, environs of El Silsila Fort	31°13'N	29°55'E	0	183	20.6	BWh
<i>S. glaucus</i> ssp. <i>cor.</i>	(16)	EQ	Egypt, Sinai/Suez Canal, El Quantara, surroundings of mooring	30°52'N	32°20'E	6	50	21.6	BWh
<i>S. glaucus</i> ssp. <i>cor.</i>	(17)	CAI	Egypt, Cairo, Ain-Shams University, Botanic Garden	30°03'N	31°15'E	25	18	21.3	BWh
<b>Israel (coastal plain)</b>									
<i>S. vernalis</i>	(18)	AK	Israel, Haifa Bay Area, ca. 8 km S of Akko, along Mediterranean coastal road	32°52'N	35°04'E	10	558	20.3	Csa
<i>S. glaucus</i> ssp. <i>gl.</i>	(19)	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
<i>S. vernalis</i>	(20)	ZY	Israel, Carmel, Zikhron Ya`aqov, wasteground	32°34'N	34°57'E	136	611	19.8	Csa
<i>S. glaucus</i> ssp. <i>gl.</i>	(21)	C	Israel, Northern Sharon Coast, Caesarea, coastal sand dunes	32°30'N	34°54'E	14	557	20.2	Csa
<i>S. vernalis</i>	(22)	TA	Israel, Coastal Plain, Tel Aviv, ruderal site at "Jabotinsky Roundabout"	32°05'N	34°46'E	5	562	20.2	CSa
<i>S. glaucus</i> ssp. <i>gl.</i>	(23)	NOF	Israel, Southern Sharon Coast, Nof Yam/Herzliya, developed entrance to beach	32°11'N	34°48'E	3	567	19.9	Csa
<i>S. glaucus</i> ssp. <i>gl.</i>	(24)	AD	Israel, Judean Coast, Ashdod, coastal sand dunes	31°48'N	34°38'E	8	479	20.0	Csa
<i>S. glaucus</i> ssp. <i>gl.</i>	(25)	AQ	Israel, Judean Coast, Ashqelon, coastal sand dunes	31°40'N	34°35'E	33	421	19.9	Csa

Abbreviations: Elev, elevation (in metres above or below sea level; derived from [www.geoplaner.com](http://www.geoplaner.com)); Map and Mat, mean annual precipitation and temperature, respectively [derived from [www.climate-data.org](http://www.climate-data.org), except for sites S ([www.worldclim.org/current](http://www.worldclim.org/current)) and AL and KB (Holzapfel et al. 1995)].

Climate data for ER, HU, NY and MAS were obtained for the nearest locations for which data were available, i.e. Majdal Shams, Kirjat Schmona, Ein Gev and Ein Gedi, respectively.

<sup>a</sup> 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.

Table 2. Model comparison tests of the relative importance of environmental variables influencing clinal variation in hybrid index across the Jordan Rift Valley transect. Analysis of (a) all 13 populations, and (b) with population 7 (BS) excluded. Bold text indicates that the model explains significantly more variance at a >95% confidence level. Subscript numbers are degrees of freedom for *F* test.

(a)

Environmental variable	Null vs. environment <i>F</i> test	Cline vs. cline + env. <i>F</i> test
Elevation	0.38 <sub>12,11</sub>	0.02 <sub>11,10</sub>
Mean annual precipitation	<b>14.63</b> <sub>12,11</sub>	0.22 <sub>11,10</sub>
Mean annual temperature	1.29 <sub>12,11</sub>	0.01 <sub>11,10</sub>

(b)

Environmental variable	Null vs. environment <i>F</i> test	Cline vs. cline + env. <i>F</i> test
Elevation	0.18 <sub>11,10</sub>	<0.01 <sub>10,9</sub>
Mean annual precipitation	<b>15.96</b> <sub>11,10</sub>	0.03 <sub>10,9</sub>
Mean annual temperature	1.01 <sub>11,10</sub>	0.01 <sub>10,9</sub>

## Figure Legends

Figure 1. Localities of populations of *Senecio vernalis* (squares) and *S. glaucus* (triangles) sampled in Israel, the West Bank and Egypt. The dashed lines mark four climatic regions (I-IV) according to the Thornthwaite Moisture Index (Thornthwaite 1948) as (I) humid, (II) subhumid, (III) semiarid and (IV) arid (Sadan and Pohoryles 1979). Areas at 500-m elevation or higher are indicated by stippling. Locality abbreviations are described in Table 1 with ASHQ and ASHD referred to as AQ and AD, respectively. (Reproduced with permission from Comes and Abbott 1999).

Figure 2. Results of genetic assignment of progeny sampled from populations of *Senecio vernalis* and *S. glaucus* using STRUCTURE when  $K = 2$ . Each individual is represented by a vertical bar displaying the individual's probability ( $Q$  score) of belonging to one or other of two clusters (genetic groups), indicated in blue and red, respectively. Vertical dotted white lines separate groups of individuals according to sampling locality. Population codes are described in Table 1 with *S. vernalis* and *S. glaucus* populations along the Israeli coastal plain denoted with an added V and G, respectively.

Figure 3. Results of genetic assignment of progeny sampled from populations of *Senecio glaucus* ssp. *glaucus* and ssp. *coronopifolius* using STRUCTURE when  $K = 2$ . Each individual is represented by a vertical bar displaying the individual's probability ( $Q$  score) of belonging to one or other of two clusters (genetic groups), indicated in brown and green, respectively. Vertical dotted white lines separate groups of individuals according to sampling locality. Population codes are described in Table 1 with *S. glaucus* ssp. *glaucus* populations along the Israeli coastal plain denoted with

an added G.

Figure 4. Maximum likelihood cline for mean hybrid index against distance (km) for populations of *Senecio vernalis* and *S. glaucus* ssp. *coronopifolius* sampled along the Jordan Rift Valley transect. Numbers denote populations according to Table 1. The 95% credible cline interval is shown in grey. Vertical bars indicate 95% confidence intervals of hybrid indices.

Figure 5. Relationships between environmental variables (elevation, precipitation and temperature) and distance, and hybrid index and environmental variables, for populations across the Jordan Rift Valley transect. Open circles represent sampling localities.  $F$  and  $p$  values are from ANOVAs testing the significance of linear relationships. Dotted line indicates the fitted linear regression line if significant.

Figure 6. Relationship between corrected linkage disequilibrium ( $cr$ ) and hybrid index for populations sampled along the Jordan Rift Valley transect. The  $F$  value and probability ( $p$ ) for significance of the fitted Gaussian curve are shown at the top right of the figure. Population numbers denote populations according to Table 1.

### **Supplementary Figure Legends**

Figure S1. Plots for  $K=1-10$  of (A) mean log probability of  $K$  [ $L(K)$ ], (B) first derivative [ $L'(K)$ ], (C) second derivative [ $L''(K)$ ], and (D)  $\Delta K$ , generated by POPHELPER from outputs of STRUCTURE analyses conducted on all populations of *Senecio glaucus* and *S. vernalis* listed in Table 1.

Figure S2. Plots for  $K=1-10$  of (A) mean log probability of  $K$  [ $L(K)$ ], (B) first derivative [ $L'(K)$ ], (C) second derivative [ $L''(K)$ ], and (D)  $\Delta K$ , generated by POPHELPER from outputs of STRUCTURE analyses conducted on populations of *Senecio glaucus* ssp. *coronopifolius* and ssp. *glaucus* listed in Table 1.

Figure S3. Maximum likelihood cline for mean hybrid index against distance (km) for populations of *Senecio vernalis* and *S. glaucus* ssp. *coronopifolius*, excluding population 7 (BS), sampled across the Jordan Rift Valley transect. Numbers denote populations according to Table 1. The 95% credible cline interval is shown in grey. Vertical bars indicate 95% confidence intervals of hybrid indices.

Figure S4. Distribution of hybrid index values for individuals within each of the 13 populations sampled across the Jordan Rift Valley transect plus population 9 (J). The dip test statistic and probability for multi-modality is given for each distribution. Note that MAS1 and MAS2 refer to populations M'AS and MAS, respectively.

### **Supplementary Table legend**

Table S1. Genotypes of individuals surveyed from each population across six allozyme loci. Population codes are the same as in Table 1 except that *Senecio vernalis* and *S. glaucus* populations along the Israeli coastal plain are distinguished by adding a V or G to their codes, respectively.