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Biogeography and evolution of seeder and resprouter forms of *Erica coccinea* (Ericaceae) in the fire-prone Cape *fynbos*

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Abstract The genus *Erica* represents the epitome of plant biodiversity in the South African Cape fynbos with over 700 species. This genus is composed of seeder and resprouter species, but both species diversity and endemism are strongly linked to the seeder habit and concentrated in the southwestern Cape Floristic Region (CFR). *Erica coccinea* is a relatively abundant and widespread fynbos species whose most remarkable morphological feature is the existence of distinct seeder and resprouter forms, frequently—but not always—in disjunct populations. Both higher within-population genetic diversity and among-population differentiation have been found in seeders, most likely as a consequence of the shorter generation times and faster population turnovers. Resprouters, despite being less diverse, are suspected to be ancestral. However, no solid evidence has yet been provided for the ancestrality of the resprouter form, or for the demographic processes that have determined the current distribution of genetic diversity in both regeneration forms. Here, we used microsatellites and sequences of the nuclear ribosomal internal transcribed spacers to describe the phylogeographic structure of seeder and resprouter *E. coccinea* populations and provide good evidence for the ancestral status of the resprouter form and the comparatively

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Electronic supplementary material

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high rates of molecular evolution in derived seeder populations. We also reveal that mixed populations, where both seeder and resprouter individuals co-occur, were originated by secondary contacts. This study highlights the role of fire in driving accelerated diversification in seeder lineages of highly speciose CFR fynbos taxa.

Keywords Fynbos biodiversity · Internal transcribed spacers · Life-history traits · Molecular evolutionary rates · Post-fire regeneration

Introduction

The geographical pattern of population genetic variation within a species, which reflects its demographic history (Avise 2000; Hewitt 1996, 2000), may also be influenced by other factors such as the contemporary ecological landscape or its own life-history traits (Knowles 2009, and references therein). Thus, understanding the role of extrinsic (e.g. climatic fluctuations, ecological gradients) and intrinsic (life-history) processes in shaping geographical patterns of population genetic structure is critical to exploring species' evolutionary history (Knowles 2009). In this sense, a high frequency of unique or private alleles in a population or group of populations of a given species is usually taken as indicative of biogeographic origin and/or isolated refugium for that species (e.g. Hewitt 1996; Ishida and Taylor 2007; Migliore et al. 2012). However, a high frequency of private alleles has also been suggested as a signature of accelerated evolution—i.e. increased rate of molecular change—in different taxa (e.g. Segarra-Moragues and Ojeda 2010; Jia et al. 2011).

The South African Cape *fynbos*, within the Cape Floristic Region (hereafter, CFR), is renowned for its high plant biodiversity at all spatial scales (Goldblatt and Manning 2002). A winter rainfall regime and a remarkable climatic and topographical stability since the Pliocene in the western CFR (Midgley et al. 2005; Cowling et al. 2015) have favoured high diversification and low extinction rates in fynbos taxa, thereby accounting for its outstanding biodiversity (Linder 2003; Cowling et al. 2009, 2015). Superimposed on this climatic stability is the role of fire (Cowling 1987; Schnitzler et al. 2011), which might have been a crucial component in the evolution of the pyrophytic fynbos flora since the Miocene (Bytebier et al. 2011). However, the mechanism by which a regime of recurrent fires promotes plant speciation in the CFR is poorly understood (Barraclough 2006; Schnitzler et al. 2011; Litsios et al. 2014). It could involve short generation times in fire-sensitive taxa (*seeders*), compared to fire-resistant ones (*resprouters*), resulting in high rates of molecular evolution and, thereby, diversification in seeder taxa (Wells 1969). Although the generation time effect on molecular evolution in plants has been under debate (e.g. Whittle and Johnston 2003; Verdú et al. 2007; Soria-Hernanz et al. 2008), in the last few years, some robust analyses of the phylogenies of numerous lineages have undoubtedly shown higher rates of molecular changes associated with shorter generation times (Smith and Donoghue 2008; Müller and Albach 2010; Yue et al. 2010).

With ca. 700 species, the genus *Erica* represents the epitome of plant biodiversity in the CFR (Goldblatt and Manning 2002; Pirie et al. 2011). Most CFR *Erica* species occur in fynbos, associated with the recurrent presence of fire (Oliver 1991). This genus is composed of seeder and resprouter species, but the seeder habit is preponderant (ca. 90 % of the species; Ojeda 1998). Exceptionally, there are also 'mixed' species that include both seeder and resprouter individuals (Ojeda 1998). Species diversity and endemism in this genus are strongly linked to the seeder habit and the geographic pattern of this biodiversity is not random, but concentrated in the southwestern CFR (Ojeda 1998; Linder 2003).

Erica coccinea L. (Ericaceae) is a relatively abundant and widespread fynbos species in the western, winter rainfall half of the CFR (Oliver and Oliver 2002). It is one of the few 'mixed' *Erica* species, with distinct seeder and resprouter forms (Ojeda 1998; Bell and Ojeda 1999), which occur frequently—but not always—in allopatry. These two phenotypic forms have a genetic determinism (Verdaguer and Ojeda 2002, 2005), and ontogenetic evidence indicates that the seeder form is derived from the resprouter form (Verdaguer and Ojeda 2005). Both seeder and resprouter populations of *E. coccinea* are common in fynbos communities of coastal mountains and hills of the southwestern CFR, whereas seeder populations are scarce to absent in more inland mountains (Fig. 1). A climatic-geographic gradient of rainfall seasonality seems to account for this apparent north–south latitudinal partitioning of seeder and resprouter populations (Ojeda et al. 2005), since

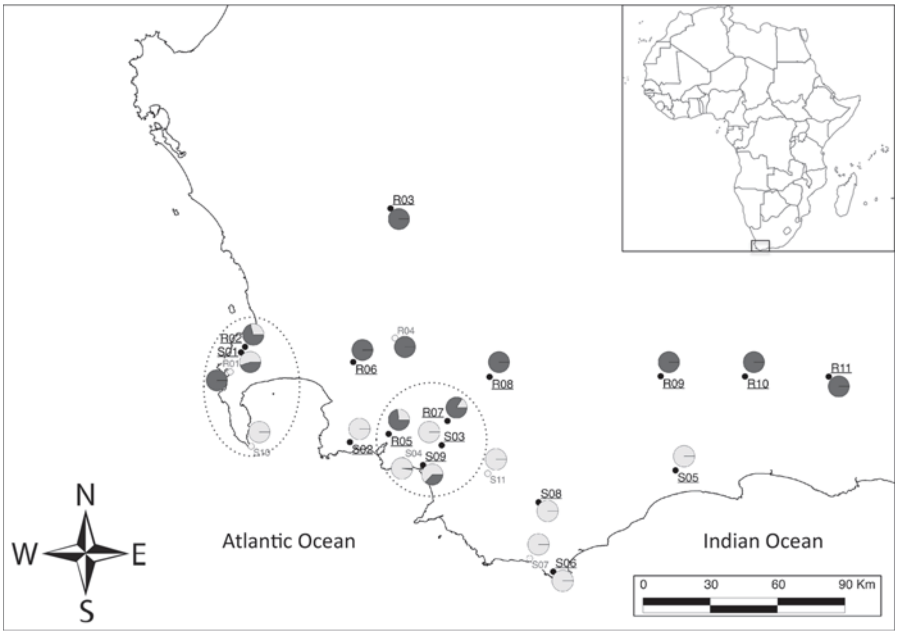

Fig. 1 Geographic distribution of *Erica coccinea* populations used in this study indicating the proportion of seeder (*light grey*) and resprouter (*dark grey*) regeneration forms. The Cape Peninsula (*left*) and Caledon (*right*) regions are enclosed in *dotted lines*. Populations used for the ITS phylogeographic analysis are marked with larger and *underlined labels*. Note that, for the ITS analysis, only seeder individuals were sampled from mixed populations S01 and S09, and only resprouters from R02 and R07, while both seeders and resprouters were sampled from R05 (labelled as R05s and R05r in Fig. 2). Both seeder and resprouter individuals from the five mixed populations sampled were used for the ABC simulations. For a more detailed location of all populations (except S11, described in the main text), see Table 1 in Segarra-Moragues and Ojeda (2010)

other environmental conditions apart from rainfall seasonality (e.g. soil, mean annual rainfall, fire regime) are similar across the CFR mesic mountain fynbos (Campbell 1986; Ojeda 1998). Seeder and resprouter plants have a similar flower shape and share the orange-breasted sunbird (*Anthobaphes violacea*) as the main pollinator. However, their flowering phenologies hardly overlap: flowering spans from December to early June in resprouter plants (peak in January–February), and from late May to October (peak in June–July) in seeders (Malan 2013). Although both seeder and resprouter forms in this species seem to be pyrogenic recruiters, effective post-fire seedling recruitment in resprouter populations is much lower (Ojeda et al. 2005; Ojeda, personal field observations). However, since resprouter adult plants survive recurrent fires by resprouting, they may experience successive post-fire recruitment episodes before they eventually die. By contrast, recruitment in seeder plants occurs as a single episode, since it is preceded by the death of adults to fire. Hence, populations of seeder *E. coccinea* plants are expected to have shorter generation times and faster population turnover than those of resprouters.

Segarra-Moragues and Ojeda (2010) analysed the genetic structure of seeder and resprouter populations of *E. coccinea* by means of microsatellite markers and found both higher within-population genetic diversity and higher among-population differentiation in seeders. Taking into account that both regeneration forms produce small, oval seeds and have similar, short range means of seed dispersal, these results were interpreted as a consequence of the shorter generation times and faster population turnover in seeders, resulting in higher rates of molecular change and, hence, differentiation in seeder populations (Segarra-Moragues and Ojeda 2010). This microevolutionary (within species) approach thus illustrates how a regime of recurrent fires can promote diversification in seeder lineages of the genus *Erica*. However, Segarra-Moragues and Ojeda (2010) were not able to provide genetic evidence for the suspected ancestrality (based on ontogenetic characters; Verdaguer and Ojeda 2005) of the resprouter form, nor shed light on the demographic processes that have determined the current distribution of genetic diversity in both regeneration forms. Mixed populations, including both seeder and resprouter individuals, occur in the southwestern CFR (Fig. 1); whether they are dimorphic populations or the result of secondary contact between seeder and resprouter populations is also unknown.

Here, we used nuclear ITS sequences to explore the geographical pattern of genetic variation and phylogenetic relationships in seeder and resprouter populations of *E. coccinea* in the western half of the CFR, which represents most of its geographic range (Oliver and Oliver 2002). We also applied approximate Bayesian computation (ABC) approaches to nuclear microsatellites—complementing microsatellite data of Segarra-Moragues and Ojeda's (2010) with additional populations—to infer the evolutionary history of seeder and resprouter populations. Specifically, we aimed to (i) describe the phylogeographic structure of seeder and resprouter *E. coccinea* populations; (ii) provide further evidence for the ancestral status of the resprouter life form in this dimorphic species; (iii) assess the evolutionary origin of mixed populations, where both seeder and resprouter individuals co-occur; and (iv) illustrate changes in the rate of molecular evolution linked to post-fire regeneration forms. Our study provides new microevolutionary insights adding to those by Ojeda et al. (2005) and Segarra-Moragues and Ojeda (2010) to help understand the evolutionary history of the genus *Erica* in the CFR. Also, although extrapolation from microevolution to macroevolution must be taken with caution (e.g. Simons 2002), it emphasizes the role of fire in driving accelerated diversification in seeder lineages of highly speciose CFR fynbos taxa.

Methods

Population sampling for ITS phylogeography and microsatellite-based ABC analysis

Sixteen *E. coccinea* populations from those analysed by Segarra-Moragues and Ojeda (2010), seven seeder (S01, S02, S03, S05, S06, S08 and S09), eight resprouter (R02, R03 and R06 to R11) and one mixed (R05r/s in Segarra-Moragues and Ojeda 2010), were used for the phylogeographic analysis (Fig. 1). Two of the sampled seeder (S01 and S09) and two of the resprouter populations (R02 and R07) analysed by Segarra-Moragues and Ojeda (2010) were later found to include both resprouter and seeder individuals. However, only seeder or resprouter samples, respectively, were used from them for the ITS analysis, as it was completed before that finding was made.

For the microsatellite-based ABC analysis, we used the microsatellite data from the 21 populations sampled by Segarra-Moragues and Ojeda (2010) to which we added microsatellite genotyping data of 30 seeder individuals each from R02 and R07, respectively, and of 20 and 30 resprouter individuals from S01 and S09, respectively. We also added a new seeder population (S11, Salmonsdam Nature Reserve, 34°25′22″S–19°38′09″E, $N = 36$). Therefore, eight resprouters (R01, R03, R04, R06, and R08–R11), nine seeders (S02–S08, S10 and S11) and five mixed populations (S01, S09, R02, R05 and R07) were considered in the ABC analyses (Fig. 1). Detailed location data for all populations (except S11) can be obtained from Segarra-Moragues and Ojeda (2010: Table 1).

DNA extraction, ITS sequencing and microsatellite data

Fresh leaves were collected in the field and dried in silica gel. DNA was extracted using the SpeedTools plant DNA extraction kit (Biotools, Madrid, Spain). Three plastid regions (*rbcL*, *trnH-psbA* and *trnC-petN1*, a total of 2489 bp) and the nuclear ribosomal internal transcribed spacers (ITS1-5.8s-ITS2 region, 727 bp) were initially screened for polymorphism in a panel of 15–18 samples following standard PCR protocols, as described in Shaw et al. (2005) for plastid regions and in Dick and Heuertz (2008) for ITSs. After amplification, PCR products were purified on filter columns (QIAquick96 kit, Qiagen, Hilden, Germany) and quantified on agarose gels (1 %). Sequencing reactions were performed in both directions using BigDye v.3.1 chemistry (Applied Biosystems, Lennik, Belgium) on an ABI3730 sequencer (Applied Biosystems). Sequences were edited and aligned in CodonCode Aligner 4.2.3. (CodonCode Corporation, Dedham, MA, USA). Only one polymorphism was found in plastid DNA (involving a single individual with *trnH-psbA*), and thus, only highly polymorphic ITS sequences were generated for the total sample.

Table 1 Number of ribotypes (nr), ribotype diversity (rd) and private ribotypes (number of private ribotypes and frequency in the population) for resprouter and seeder populations of *Erica coccinea* (see Fig. 1 and Segarra-Moragues and Ojeda 2010 for geographic location)

Population code	*N*	nr	rd	Private ribotypes	
				Number	Frequency
Resprouters					
R02	5	2	0.356	1	0.20
R03	4	2	0.250	0	0.00
R06	5	1	0.000	0	0.00
R07	5	3	0.733	1	0.40
R08	4	1	0.000	0	0.00
R09	5	2	0.200	0	0.00
R10	6	2	0.167	1	0.08
R11	5	2	0.356	0	0.00
R05r	9	3	0.582	1	0.06
Average	5.33	2	0.294	0.44	0.08
Seeders					
S01	5	2	0.200	0	0.00
S02	4	3	0.607	3	1.00
S03	5	3	0.622	2	0.40
S05	5	3	0.711	2	0.60
S06	4	2	0.250	2	1.00
S08	5	2	0.556	0	0.00
S09	5	2	0.200	0	0.00
R05s	10	3	0.542	1	0.65
Average	5.37	2.5	0.461	1.25	0.45

Averages by life form are also provided

Five individuals were sequenced in each population, except in the mixed R05r/s population, where 10 seeder and 10 resprouter individuals were examined. Thus, ITSs were sequenced in a total of 45 seeder and 50 resprouter individuals. Careful visual inspection of sequence chromatograms revealed that some polymorphisms contained additive polymorphic sites (APS), as expected in nuclear genes that have not undergone complete concerted evolution (Aguilar and Nieto Feliner 2003; Álvarez and Wendel 2003). Phased haploid sequences were successfully obtained using PHASE version 2.1 (Stephens and Donnelly 2003) with default parameters (see "Results"), suggesting that the ITS dataset could be interpreted in terms of biparental allelic inheritance.

Finally, nuclear microsatellite data (eight microsatellite loci) were retrieved from Segarra-Moragues and Ojeda (2010) plus the new microsatellite data from the four mixed populations and the new seeder population (see above). Microsatellite genotyping was performed as described by Segarra-Moragues and Ojeda (2010). The whole dataset included diploid genotypes for a total of 725 individuals (373 seeder and 352 resprouter) from 22 populations.

Phylogeographic analysis

A number of ribotypes (nr) and ribotypic diversity (rd) were computed for each population using DnaSP version 5.10 (Librado and Rozas 2009) and averaged for seeders and resprouters. Phased ITS sequences were used to construct a median-joining network using NETWORK version 4.6 (Fluxus Technology Ltd, Suffolk, UK; Bandelt et al. 1999). Finally, the geographical distribution of ITS variants was plotted using ArcMap9.3.1 (ArcGIS 9, ESRI, Redlands, CA, USA).

Inference of ancestral life form

Phylogenetic analysis

We conducted a phylogenetic analysis on ITS sequences to explore (i) whether seeder and resprouter life forms in *E. coccinea* correspond to distinct ITS entities, providing supporting evidence for two taxa, and (ii) whether it could help elucidate the ancestral life form. In a previous study by Segarra-Moragues and Ojeda (2010) based on microsatellite markers, seeder and resprouter forms could not be genetically segregated. We extracted ITS data from Genbank selecting one or two sequences per major clade of the *Erica* genus (clades defined in Pirie et al. 2011) and including all available sequences of the "F" clade to which *E. coccinea* belongs. We used MUSCLE ver. 3.8 software (Edgar 2004) to compute a multiple alignment of downloaded sequences and one sequence for each phased ribotype discovered in this study. For ribotypes VII, XII, XVII and XVIII, no full length ITS sequences were available, so that these ribotypes were excluded from the analysis. A first phylogenetic tree was constructed including only the phased ribotypes. In addition, a second phylogenetic tree was constructed from all unphased and phased *E. coccinea* sequences produced in this study and including three *Erica* species closely related to *E. coccinea* (*E. cumuliflora*, *E. penicilliformis*, and *E. palliflora*) for rooting purposes. Alignment curation and maximum likelihood phylogenetic analysis was then conducted using the Phylogeny.fr online pipeline (Dereeper et al. 2008) with default parameters.

ABC simulations

ABC was used to compare alternative demographic scenarios based on the nuclear microsatellite data obtained for the 22 populations (nine seeders, eight resprouters and five mixed). Coalescent simulations were used applying two sets of models using the ABC framework in DIYABC v1.0.4.46beta (Cornuet et al. 2008; available at http://www1.montpellier.inra.fr/CBGP/diyabc). ABC implementation in DIYABC allows to define the ancestral and derived branches for each splitting event, and thus, by comparison of models with different assumed ancestral populations, DIYABC can potentially provide information on ancestrality of life forms. The first set of simulations ('two-groups' models, see Fig. S1 in Supplementary Information) were then run grouping individuals by life form, to determine whether the seeder or the resprouter form was ancestral (by comparing scenarios 1 and 2 in Fig. S1A) and to compare simple models without change in effective population size with models that implemented population size changes in seeders (scenario 3 in Fig. S1A). This set of ABC simulations also included models where resprouters were grouped into western (R03, R04, R06) or eastern (R09, R10, R11) populations (see Segarra-Moragues and Ojeda 2010; Fig. S1B). A second set of simulations ('three-groups' models, see Fig. S2 in Supplementary Information) was designed to discern the origin of mixed populations from the Cape Peninsula (S01 and R02) and the Caledon region (S09, R05 and R07; see Fig. 1). In this latter case, we compared alternative demographic scenarios where mixed populations were ancestral with others where mixed populations were originated by secondary contact of distinct seeder and resprouter lineages. These simulations were run separately for the two regions with mixed populations (i.e. Cape Peninsula and Caledon regions). All priors had uniform distributions, except for mutation rates (log-uniform). Priors and summary statistics are presented in Table S1, provided as Supplementary Material. Each run consisted on 3 million simulations, distributed equally among demographic scenarios.

Results

High-quality forward and reverse ITS sequences were obtained and assembled for 91 (out of 95 sampled) individuals for an ITS region of 732 bp (Genbank accession numbers KT898981-KT899071). Within this region, we found 14 single nucleotide polymorphisms (SNPs) and 3 indels (GT/- in position 283–284 bp, *T*/- in position 298 bp and *A*/- in position 682 bp), which combined into 18 phased ITS ribotypes (Fig. 2; Fig. S3 and Table S2 in Supplementary Information). Most ribotypes were found in only one of the life forms, with 10 ribotypes appearing only in seeders and four only in resprouters (Fig. 2). From the remaining four ribotypes shared between both life forms (III, IV, V and IX), ribotype III was widespread but found mostly in resprouters, whereas ribotype V was present mostly in seeders. Ribotype IX was found

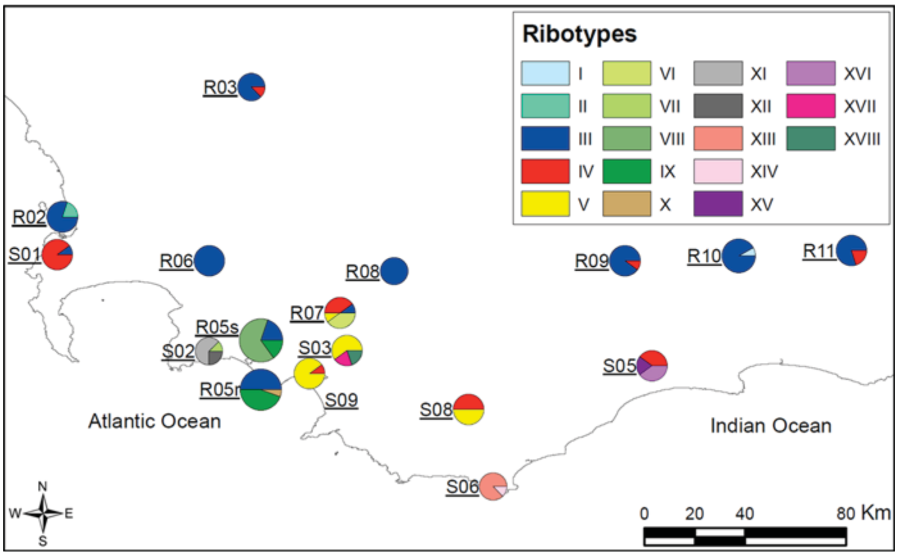

Fig. 2 Geographic distribution of 18 ITS ribotypes found in *E. coccinea*. Pie charts are proportional to sample size. Notice that a prefix 'S' indicates a seeder population and a prefix 'R' a resprouter population, except for R05 where both seeders (R05s) and resprouters (R05r) were sampled

in the two life forms, but only in the R05 mixed population, where both seeders and resprouters were sampled. Finally, Ribotype IV (red in Fig. 2) was the only widespread ribotype that was also widely shared by the two life forms. It must be noted that, with the exception of this latter ribotype, most ribotype sharing was restricted to the Cape Peninsula and the Caledon regions, where mixed populations are found (see Fig. 1). Ribotypic diversity was higher in seeders than in resprouters (Table 1).

Seeder and resprouter ribotypes were intermingled in the median-joining network and separated by only few mutations (Fig. 3). Notably, one shared ribotype (Ribotype IV) and one resprouter ribotype (Ribotype VI, private to population R07) were central in the network. Ribotypes found in seeders, apart from being more abundant (see above), also displayed a greater evolutionary distance among each other, with a higher number of mutations between private and widespread or central ribotypes (average of 1.8 for seeders vs. 1 for resprouters). Moreover, seeder populations S02, S03, S05 and S06 were remarkable for having highly differentiated ribotypes (XII, XVIII, XVI and XIV, respectively; Fig. 3).

A phylogenetic analysis based on phased ribotypes revealed that all *E. coccinea* ribotypes clustered in the same clade, together with the two *E. coccinea* sequences available on Genbank, with unresolved relationships (Fig. S4 in Supplementary Information). A second phylogenetic analysis, including both phased and unphased ribotypes and only close outgroups, also showed unresolved phylogenetic relationships between seeders and resprouters; only two clades, one resprouter and one seeder, were distinguished with >90 % bootstrap support from a base population of both seeders and resprouters (Fig. S5 in Supplementary Information). Thus, ITS phylogenies failed to distinguish seeder and resprouter life forms as two distinct monophyletic clades, providing no support for the hypothesis that these life forms correspond to two separate taxa. It did not either resolve the ancestral life form, as the *E. coccinea* clade constituted a polytomy with derived ribotypes belonging to each of the two life forms in both phylogenetic trees.

The best ABC scenario for the 'two-groups' models, however, pointed to resprouters as the ancestral form, with seeders splitting from them some 3070 generations ago (95 % CIs: 774–7610 generations ago). The split of seeders from resprouters took place before western and eastern resprouter populations diverged, estimated at about 533 generations ago (95 % CIs: 108–1250 generations ago). Interestingly, estimates of effective population size were much higher in seeders (59,600, 95 % CIs: 19,500–97,400) than in resprouters (9580, 95 % CIs: 3480–14,700). Models including effective population size changes in

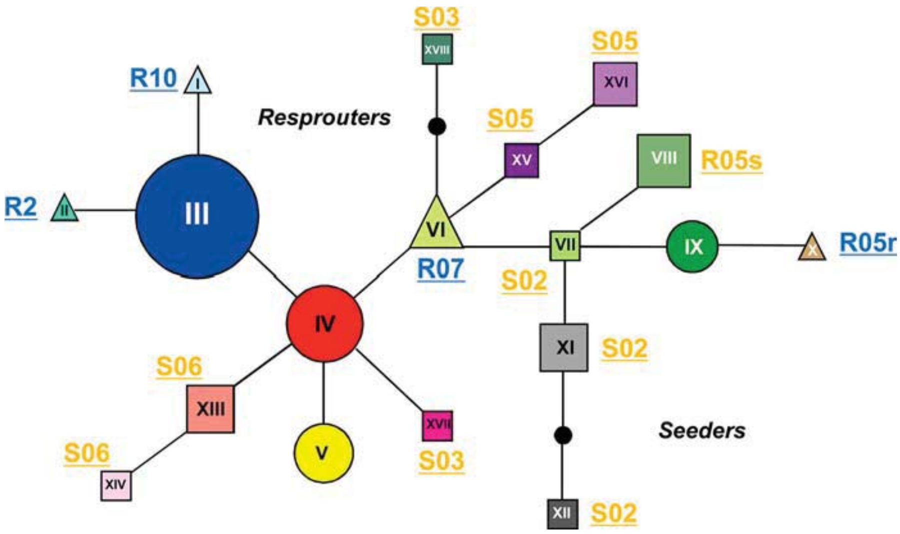

Fig. 3 Median-joining phylogenetic network. Ribotype colours and codes are the same as in Fig. 2. *Circles* indicate ribotypes shared between seeders and resprouters. The remaining 14 ribotypes were private to resprouter (*blue labels*) and seeder (*orange labels*) populations, in turn indicated by *triangles* or *squares*, respectively. *Symbols* are proportional to ribotype frequency. *Black circles* indicate missing or unsampled ribotypes. (Color figure online)

seeders provided worse fit than simpler models with constant effective population sizes. Best demographic scenarios for 'three-groups' models indicated that, for both Caledon and Cape Peninsula regions, mixed populations originated by secondary contact of distinct seeder and resprouter populations. This secondary contact was older in the Cape Peninsula (1392 generations ago, 95 % CIs: 271–3680) than in the Caledon region (382 generations ago, 95 % CIs: 54–1010), but involved similar admixture rates (11.1 % seeder for the Cape Peninsula and 24.1 % for the Caledon region). A summary of demographic events, as suggested by ABC simulations, is presented in Fig. 4.

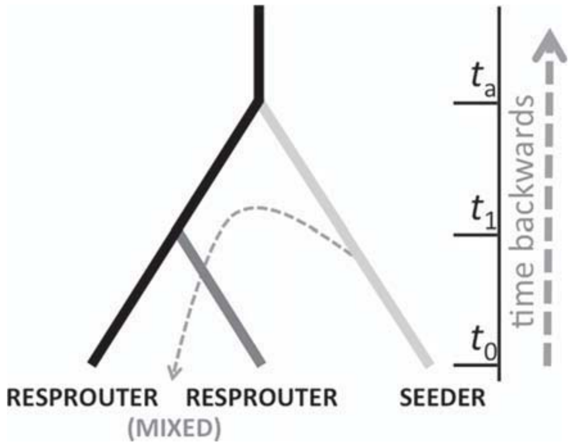

Fig. 4 Summary illustration of the demographic events affecting seeder and resprouter populations of *Erica coccinea*, as inferred from ABC simulations. Times are not scaled

Discussion

Based on nuclear ITSs, *E. coccinea* showed a strong phylogeographic pattern, with distinct features for seeder and resprouter life forms. Ribotypes III and IV were the most common and widespread and, under coalescent theory, likely the oldest ones (Posada and Crandall 2001). Ribotype IV was similarly distributed in seeders and resprouters, while Ribotype III was present and predominant in all resprouter populations. Another resprouter ribotype, Ribotype VI, was central in the median-joining network, despite its restricted distribution to resprouter population R07 (see Figs. 2, 3). Moreover, while seeder populations presented both high frequency of private ribotypes and high average number of mutations from common/central to private ribotypes, private ribotypes were much less frequent and never abundant in resprouter populations. Seeder populations S02, S03, S05 and S06 were remarkable for presenting highly differentiated ribotypes (Fig. 3). Population S06 actually belongs to the distinct subspecies, *E. coccinea* subsp. *uniflora* (Oliver and Oliver 2002), but populations S02, S03 and S05 belong to the type subspecies *E. coccinea* subsp. *coccinea* (Oliver and Oliver 2002) as the rest of the sampled populations, including both seeder and resprouter. So, putative taxonomic differences between seeders and resprouters—which could not be supported by the ITS phylogenetic reconstruction (Figs. S4 and S5 in Supplementary Information)—cannot be argued as a likely explanation for the distinct ribotype patterns. The high frequency of private ribotypes in seeder populations can neither be explained by geographic isolation, since average pairwise geographic distances are actually shorter in seeder than in resprouter populations (Segarra-Moragues and Ojeda 2010), and both seeder and resprouter forms have equal pollination and seed dispersal traits (Malan 2013). A high frequency of private ribotypes has also been suggested as a signature of accelerated ITS evolution (see Jia et al. 2011). Interestingly, our results parallel the higher frequency of private microsatellite alleles in *E. coccinea* seeder populations reported by Segarra-Moragues and Ojeda (2010), which these authors interpreted as the result of faster rates of new alleles arising in seeder populations owing to their comparatively shorter generation times.

While phylogenetic analysis of ITS did not resolve the ancestral life form in *E. coccinea*, the ABC analysis of the microsatellite data presented in this study suggests that resprouter populations are ancestral in this species. Thus, the derived seeder state can be interpreted as the result of an ontogenetic loss of the resprouting ability (Verdaguer and Ojeda 2005), likely as a consequence of a loss-of-function mutation. The 'mutant' seeder phenotype would have replaced an originally resprouter population under particular environmental conditions associated with a stable, mild Mediterranean climate (Ojeda et al. 2005), characteristic of the southwestern CFR at least since the early Pleistocene (Verboom et al. 2014). The ABC analysis dated this branching event some 3000 generations before present, i.e. about 60,000 years ago assuming 20-year fire return intervals (Ojeda et al. 2005). Interestingly, this dates back to the penultimate Pleistocene glaciation, when the Agulhas Bank was exposed above the sea level, providing new opportunities for plant diversification (Cowling et al. 2009; Verboom et al. 2014). During this period, owing the lower temperatures and more regular frontal rains (Midgley et al. 2005; Marean et al. 2014), Mediterranean climate in the southwestern CFR was probably milder, a feature which would have favoured seeders (Ojeda et al. 2005).

Moreover, according to the ABC simulations, the subdivision of resprouter *E. coccinea* populations into western and eastern groups (Segarra-Moragues and Ojeda 2010) occurred more recently than the appearance of the seeder form and, at about the same time, they came into secondary contact with seeders. This contact happened some 1300 generations ago in the Cape Peninsula and more recently in the Caledon region, likely as a consequence of environmental changes that favoured the expansion and genetic differentiation of seeder populations across coastal mountains of the southwestern CFR. Coincidentally, 1300 generations (i.e. 26,000 years) ago dates back to the Last Glacial Maximum when, once again, the Agulhas Bank emerged (Verboom et al. 2014) and the Mediterranean climate was again milder (Midgley et al. 2005; Marean et al. 2014) and, hence, more suitable for seeders in the southwestern CFR (Ojeda et al. 2005). This seeder expansion could not reach the NW nor the SE of the CFR because of the demographic constraints of seeder populations under either strongly seasonal or non-seasonal regimes (Ojeda et al. 2005), thus accounting for the lack of seeder and mixed populations further inland within the CFR (Fig. 1).

It must be stressed that most shared ribotypes between seeders and resprouters were found within or close to mixed populations, suggesting that a relatively recent secondary contact and subsequent gene flow may account for this ribotype sharing. Similar patterns of introgressive gene flow after secondary contact of previously separated lineages have been found in other plant species (e.g. in temperate or tropical trees, Latta and Mitton 1999; Born et al. 2008). Although the flowering phenologies of seeder and resprouter *E. coccinea* populations hardly overlap (see "Introduction"), there may still be—or could have been in the recent past—some opportunities for hybridization and gene flow across regeneration forms. Nevertheless, this must be further investigated (e.g. using genomic approaches), since common ancestry rather than secondary contact may still underlie some of the shared ribotypes. Ancestral shared polymorphism has also been repeteadly reported in different plant taxa (e.g. Bouillé and Bousquet 2005; Blanco-Pastor et al. 2012).

Apart from the undeniable importance of geological and climatic stability for understanding the high biodiversity of the CFR flora (Schnitzler et al. 2011; Cowling et al. 2015), fire has also been suggested as one of the key causal factors (Cowling 1987; Ellis et al. 2014). However, the mechanism by which fire-driven diversification has occurred has been explained only in terms of the evolution of the two fire regeneration forms, seeder and resprouter, in sister species (Schnitzler et al. 2011; Ellis et al. 2014). This study provides additional microevolutionary insights to those by Segarra-Moragues and Ojeda (2010), not only to help understand the evolutionary history of the genus *Erica* in the CFR, but also to understand how fire may have spurred diversification in seeder lineages of pyrophytic fynbos taxa (McDonald et al. 1995).

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