

Genetically based phenotypic differentiation between native and introduced tetraploids of *Oxalis pes-caprae*

Daniela Tavares  · João Loureiro  · Ana Martins  · Mariana Castro  · Sergio Roiloa  · Sílvia Castro 

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Abstract Rapid evolutionary change often plays an important role in determining the success of plant invasions. *Oxalis pes-caprae*, a geophyte native to South Africa, has become a persistent invasive weed in several areas of the world, being particularly widespread in regions with a Mediterranean climate. The objective of this study was to look for the existence of genetically based phenotypic differences regarding competitive ability between *O. pes-caprae* native tetraploids and introduced tetraploids recently discovered in the invaded range of the western Mediterranean basin. For this, shoot emergence time, beginning of flowering, chlorophyll fluorescence parameters, aboveground biomass, final offspring bulb production and survival were measured in a greenhouse experiment with plants from both ranges

growing alone or in competition with *Trifolium repens*. Results demonstrated significant differences between introduced and native tetraploids, with plants from the invaded range emerging earlier, beginning flowering later and producing more aboveground biomass and offspring bulbs than South African plants. Furthermore, introduced plants showed an increase in aboveground biomass when grown with *T. repens*, and affected *T. repens* growth more severely than their native conspecifics, which may be indicative of a greater competitive ability. These findings provide strong evidence for genetic differentiation between introduced and native tetraploids. It is suggested that founder events and rapid post-introduction adaptive evolution may have contributed, independently or in concert, to this divergence.

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Keywords Biological invasions · *Oxalis pes-caprae* · Interspecific competition · Genetic differentiation

D. Tavares (✉) · J. Loureiro · A. Martins · M. Castro · S. Castro
CFE, Centre for Functional Ecology, Department of Life Sciences, Faculty of Sciences and Technology, University of Coimbra, 3000-456 Coimbra, Portugal
e-mail: danielasusanatavares@gmail.com

S. Roiloa
BioCost Group, Department of Animal Biology, Plant Biology and Ecology, Faculty of Science, University of A Coruña, 15071 A Coruña, Spain

Introduction

Over the last few decades, with the increasing awareness of the ecological and economic effects of invasive species, substantial research effort has been dedicated to understanding the mechanisms underlying invasion success (Levine 2000; Keane and Crawley 2002; Hierro et al. 2005; Thuiller et al. 2006; Pyšek

and Richardson 2007). Among the many hypotheses that have been proposed to explain invasion by exotic plants, those focusing on the role of rapid evolutionary change as a determinant of success have become particularly influential (Blossey and Nötzold 1995; Callaway and Ridenour 2004; Bossdorf et al. 2005). Introduction into new ranges frequently comprises marked changes in selection pressures, which may drive adaptive evolution in invading populations (Sakai et al. 2001; Lee 2002; Bossdorf et al. 2005; Prentis et al. 2008). Rapid adaptive evolutionary change has been demonstrated to occur even in founding populations with reduced genetic variation (Dlugosch and Parker 2008a), and may occur for any trait that enhances performance in the invaded range (Lee 2002; Bossdorf et al. 2005).

The evolution of increased competitive ability (EICA) hypothesis (Blossey and Nötzold 1995) has stimulated a lot of research on evolution during plant invasions, arguing that, in the absence of specialist herbivores, selection should favor a shift in resource allocation from defense to growth and fecundity, i.e., traits that confer competitive advantage in the new range. Eventually, this would lead to genetic differentiation between introduced and native populations, with introduced plants presenting reduced resistance to specialist enemies and increased growth (or competitive ability) when compared to plants from native populations.

A direct way of testing for genetically based differences between introduced and native conspecifics is to grow plants from both ranges in a common environment, using propagules from numerous populations sampled widely across each region (Bossdorf et al. 2005; Hierro et al. 2005). If native and introduced plants growing under identical conditions differ significantly, one can say that there is evidence for genetic differentiation. Furthermore, in order to evaluate whether phenotypic differences translate into enhanced competitive ability in plants from introduced populations, it is important to compare native and introduced populations under competitive conditions, i.e., in the presence of intraspecific or interspecific competition (Bossdorf et al. 2005). Increased competitive ability in the invaded range has been demonstrated for several invasive species, such as *Centaurea maculosa* Lam. (Ridenour et al. 2008), *Sapium sebiferum* (L.) Dum.Cours. (Zou et al. 2008), *Artemisia vulgaris* L. (Barney et al. 2009), *Lespedeza*

cuneata G.Don (Beaton et al. 2011), *Solidago canadensis* L. (Yuan et al. 2013) and *Chromolaena odorata* (L.) R.M.King & H.Rob. (Zheng et al. 2015a).

Oxalis pes-caprae L., a geophyte native to South Africa, has become a persistent and troublesome invasive weed in several areas of the world, being particularly widespread in regions with a Mediterranean climate (Ornduff 1987; Castro et al. 2007). This species was first introduced in the Mediterranean basin at the end of the eighteenth century for ornamental purposes (Signorini et al. 2011, 2013). In the native range, *O. pes-caprae* presents similar abundances in natural habitats, such as forest, river, rocky and shrubland habitats, and disturbed habitats, such as roadside and ruderal habitats, while in the invaded range it is more abundant in disturbed habitats, such as roadside, agricultural, urban and ruderal habitats (González-Moreno et al. 2015).

Oxalis pes-caprae is a tristylous species, exhibiting three style morphs (long-, mid- and short-styled, hereafter L-, M- and S-morph, respectively) and a heteromorphic incompatibility system responsible for preventing both self-fertilization and intramorph fertilization (Ornduff 1987; Castro et al. 2007; Costa et al. 2017). In the native range, populations are isoplethic (Ornduff 1987; Ferrero et al. 2015), i.e., the populations present similar proportions of the three style morphs, indicating that populations are in equilibrium and that sexual reproduction is expected to be the main reproductive mode (Ferrero et al. 2015). Additionally, individuals of this species present different ploidy levels, with the most common cytotype in the native range being the tetraploid ($2n = 4x = 28$ chromosomes), followed by the diploid ($2n = 2x = 14$ chromosomes) (Krejčíková et al. 2013). The pentaploid cytotype ($2n = 5x = 35$ chromosomes) is considered extremely rare in South Africa (only three individuals were detected in a tetraploid population in Cape Point, Ferrero et al. 2015).

The scenario is completely different in the invaded region of the Mediterranean basin, where the great majority of *O. pes-caprae* populations are composed exclusively of $5x$ S-morph individuals, which, given their odd ploidy level and the absence of suitable mating partners, reproduce almost exclusively asexually through the production of bulbs (Ornduff 1987; Rottenberg and Parker 2004; Castro et al. 2007, 2013). Recently, however, the existence of

several mixed populations containing other forms in variable proportions (namely, 4x L-morph, 4x M-morph and 4x S-morph) and the occurrence of sexual reproduction have been described in the western part of the basin (Castro et al. 2013; Costa et al. 2017). Molecular studies suggest that the presence of the newly discovered tetraploids in this region resulted from multiple introductions from South Africa, while the history of the 5x S-morph remains uncertain (Ferrero et al. 2015).

Comparative studies of plant reproductive traits in native and introduced populations, including the recently discovered introduced tetraploids, have provided evidences of evolutionary changes, with introduced plants presenting higher asexual fitness (Castro et al. 2016), and a weakening in the incompatibility system that may enable sexual reproduction under strong compatible mate limitation (Costa et al. 2017). These changes suggest a potential for rapid evolution, making *O. pes-caprae* an excellent study system to address evolutionary questions and their contribution to invasion success.

The main objective of this study was to look for the existence of genetically based phenotypic differences regarding competitive ability between *O. pes-caprae* native tetraploids and introduced tetraploids recently discovered in the invaded range of the western Mediterranean basin. For this, shoot emergence time, beginning of flowering, chlorophyll fluorescence parameters, aboveground biomass, final offspring bulb production and survival were measured in a greenhouse experiment with plants from both ranges growing alone or with competition. *Trifolium repens* L. was chosen as the competitor because it commonly occurs in agricultural areas and other human-modified habitats that *O. pes-caprae* frequently invades in the Mediterranean region. Chlorophyll fluorescence parameters, aboveground biomass and survival were also measured in *T. repens* to assess the effects of competition with *O. pes-caprae*. An additional objective was to better understand the invasive potential of introduced tetraploid *O. pes-caprae* plants by comparing their competitive ability with that of the invasive and widespread pentaploids. For this, pentaploid plants from the invaded range were also included in the experiment, growing alone or in competition with *T. repens*.

Materials and methods

Study species

Oxalis pes-caprae L. (Oxalidaceae) is a winter-growing bulbous geophyte, reaching up to 40 cm in height (Sánchez Pedraja 2015). Each bulb produces an annual underground stem from which a rosette of leaves arises (Young 1958). A contractile tuberised root develops from the base of the bulb (Young 1958). Inflorescences take the form of umbellate cymes with yellow, actinomorphic flowers (Sánchez Pedraja 2015). The flowers are tristylous, presenting two whorls of five anthers each and one whorl of five stigmas, arranged in three levels according to the style morph of each individual (L-morph, M-morph or S-morph) (Ornduff 1987; Castro et al. 2007). Flowering and peak aboveground growth occur in winter, from May to August in the native range (Dreyer et al. 2006), and from December to April in the invaded range of the Mediterranean basin (Castro et al. 2007). Vegetative reproduction occurs through the formation of offspring bulbs, which develop to final size after the aboveground part of the plant senesces (Vilà et al. 2006a; Verdaguer et al. 2010). A combination of root contraction and underground stem elongation distributes the offspring bulbs along a distance of 20–30 cm (Pütz 1994).

Bulb collection

Bulb collections were performed in the invaded region of the western Mediterranean basin and in the native range in 2010 and 2011, respectively. In the western Mediterranean basin, all the populations sampled were located in highly-invaded areas, distributed along a latitudinal transect from Pontevedra province (Spain) to Essaouira province (Morocco). Since the occurrence of tetraploid plants of the three style morphs is quite restricted in this range, sampling was particularly intensive in the Colares region (Portugal) where trimorphic populations are more common (Castro et al. 2013). This procedure allowed to collect bulbs from 4x L-morph, 4x M-morph, 4x S-morph and 5x S-morph individuals in this invaded region. In the native range, South Africa, collection sites were chosen to span most of the latitudinal and longitudinal distribution of the species. Despite this extensive sampling effort, pentaploid individuals proved once more to be

extremely rare in this range (Ferrero et al. 2015). Consequently, from the native range, only 4x L-morph, 4x M-morph and 4x S-morph individuals were included in this comparative study.

To remove potential maternal effects, bulbs from the invaded and native ranges were grown in the nurseries of the Botanical Garden of the University of Coimbra for three and two generations, respectively.

Ploidy level analysis

The ploidy level of each sampled individual was determined through flow cytometric analyses of fresh leaves. Nuclei were isolated following the procedure of Galbraith et al. (1983) by chopping simultaneously 1 cm² of leaf tissue of *O. pes-caprae* and 1 cm² of leaf tissue of *Solanum lycopersicum* ‘Stupické’ (internal reference standard with $2C = 1.96$ pg; Doležal et al. 1992) in 1 mL of WPB buffer (Loureiro et al. 2007). After filtration with a 50 µm nylon filter, 50 µg mL⁻¹ of propidium iodide and 50 µg mL⁻¹ of RNase were added to the nuclear suspension to stain the DNA and remove double-stranded RNA, respectively. Samples were analyzed in a Partec CyFlow Space flow cytometer (Partec GmbH, Görlitz, Germany) equipped with a 532 nm green solid-state laser, operating at 30 mW. A pooled sample strategy was used, with leaflets from 5 individuals being analyzed simultaneously (Castro et al. 2013); when several peaks were obtained, individual samples were prepared and ploidy levels assigned to each plant. The DNA index was calculated for all samples by dividing the mean fluorescence of *O. pes-caprae* G₁ peak by that of *S. lycopersicum*. According with Castro et al. (2013), plants were identified as tetraploids for DNA index values of 0.70 ± 0.03 (mean \pm SD) and as pentaploids for values of 0.86 ± 0.02 .

Greenhouse experiment

A greenhouse experiment with *O. pes-caprae* tetraploid plants from the native and invaded ranges and pentaploid plants from the invaded range, growing alone or in competition with *T. repens*, was performed in the Botanical Garden of the University of Coimbra (Supplementary Fig. 1).

In August 2013, i.e., at the end of the second and third generation of plants from the native and invaded range, respectively, all the offspring bulbs were

harvested and stored in identified paper envelopes. Two bulbs were then selected from each of 141 mother plants, which included 63 tetraploids from the native range, 55 tetraploids from the invaded range and 23 pentaploids from the invaded range (Supplementary Fig. 1), representing 23 native populations and 12 introduced populations (Supplementary Table 1). This selection was made so that all the selected bulbs had a similar weight (0.38 ± 0.11 g, mean \pm SD). The bulbs were weighed and the initial bulb weight was recorded. Bulbs from the same mother plant, i.e., with the same genotype, were then designated to different harvest times: one bulb was assigned for harvesting at the time of peak *O. pes-caprae* aboveground biomass (set 1), and the other for harvesting at the end of the experiment (set 2), when offspring bulbs were fully developed and the aboveground part of the plants had become senescent (Supplementary Fig. 1). This approach was based on the procedure developed by Sala et al. (2007) and Verdagner et al. (2010) for *O. pes-caprae*, and allowed to assess aboveground biomass and final offspring bulb production for each genotype.

The 141 pairs of bulbs (each pair containing two bulbs with the same genotype and assigned to different harvest times) were distributed among the following competition treatments: control, one individual of *O. pes-caprae* growing in each pot; low competition, a single individual of *O. pes-caprae* growing with two plants of *T. repens*; and high competition, a single individual of *O. pes-caprae* growing with six plants of *T. repens* (Supplementary Fig. 1). The three style morphs were represented similarly in the native and introduced tetraploid genotypes used in the experiment and similar proportions of morphs were distributed among the competition treatments. Additionally, a control treatment that consisted of two *T. repens* plants growing in each pot, without competition with *O. pes-caprae*, was included in the experiment (*T. repens* control, Supplementary Fig. 1). This control treatment of *T. repens* was assigned for harvesting at the time of peak of *O. pes-caprae* aboveground biomass (set 1) and enabled to evaluate the effects of competition with *O. pes-caprae* on *T. repens* development by comparison with the low competition treatment.

In September 2013, *O. pes-caprae* bulbs were planted 2.5 cm deep in 1-L plastic pots (8.6 × 8.6 × 21.5 cm) filled with a mixture of

commercial substrate and sand (1:1). Several *T. repens* seeds (purchased from a horticultural shop) were sown on the soil surface in all the pots designated to the low and high competition treatments and to the *T. repens* control. After germination, seedlings were thinned out to two per pot in the low competition treatment and in the *T. repens* control, and to six per pot in the high competition treatment.

Pots were completely randomized at the beginning of the experiment, except for the ones with *T. repens* growing alone, which were maintained together to prevent the effects of shade generated by *O. pes-caprae* plants, and re-randomized 5 weeks after planting. The greenhouse temperature was set at 20 °C, but fluctuated to some degree depending on the external temperature, with minimum and maximum temperatures reaching 13 °C and 28 °C, respectively. The plants were grown under a natural day/night light cycle, and watered regularly. Shoot emergence occurred for over 98% of the bulbs planted. Shoot emergence time and beginning of flowering were assessed for each *O. pes-caprae* plant and recorded in number of days after the beginning of the experiment (September 26, 2013). For this, pots were checked twice a week for shoot emergence above the soil surface and, later, for fully opened flowers.

In January 2014, at the time of peak of *O. pes-caprae* aboveground biomass, all the plants from set 1 were harvested. The aboveground part of each *O. pes-caprae* and *T. repens* plant was cut at the soil surface, placed in a paper bag identified with the plant code, dried at 68 °C for 48 h, and weighed. Root biomass was not assessed because in the pots assigned to the low and high competition treatments, roots of the two species were too closely interwoven and hard to differentiate, making the separation unfeasible. *T. repens* plants in the low competition treatment and in the *T. repens* control were counted to assess plant survival. Plants in set 2 were left intact and remained in the same conditions as before until the beginning of March; then watering was gradually reduced until it was stopped. The gradual reduction and cessation of watering served to mimic the natural Mediterranean conditions and helped to accelerate bulb maturation. At the end of April 2014, when *O. pes-caprae* offspring bulbs were completely developed, the final harvest was conducted using set 2. Each pot was emptied and bulbs were harvested, counted, dried as described above for the aboveground structures, and

weighed. *O. pes-caprae* plant survival was also recorded.

Chlorophyll fluorescence

Chlorophyll fluorescence parameters were measured by the saturation pulse method (Schreiber et al. 1998) with a portable fluorometer (MINI-PAM photosynthesis yield analyzer; Walz, Effeltrich, Germany). A pulse of saturating light ($> 4000 \mu\text{mol photons m}^{-2} \text{s}^{-1}$, 0.8 s pulse length, actinic white light) was applied through an optical fiber at an angle of 60° relative to the sample and a distance of 12 mm from the leaf. Measurements were taken from plants in set 1, on the upper surface of a fully expanded leaf of each plant of *O. pes-caprae* and one plant of *T. repens* per pot, approximately 1 month before harvesting time.

The maximum quantum yield of photosystem II (PSII) was assessed as the ratio $F_v/F_m = (F_m - F_0)/F_m$ (Bolh ar-Nordenkampf et al. 1989), where F_0 and F_m are the minimal and maximal fluorescence yields of a dark-adapted sample, respectively, with all PSII reaction centers fully open, i.e., all primary acceptors oxidized. This parameter was measured after a 30 min period of dark adaptation. F_v/F_m characterizes the photosynthetic process associated with electron transport (light reactions), and is correlated with the amount of carbon gained per unit of light absorbed (Butler and Kitajima 1975; Bolh ar-Nordenkampf and  quist 1993).

Statistical analysis

Native tetraploids versus introduced tetraploids

Differentiation between tetraploid *O. pes-caprae* plants from the native and invaded ranges was assessed with generalized linear mixed models (GLMMs), using range and competition as fixed factors (with interactions allowed) and population as a random factor. When interactions between the factors were significant, the effect of each factor was explored within the levels of the other factor. Initial bulb weight was used as a covariate in the shoot emergence time model, and shoot emergence time as a covariate for all the other response variables. Data on phenological variables (shoot emergence time and beginning of flowering) were taken from set 2, except when shoot emergence time from set 1 was used as a

covariate for variables measured in set 1 (i.e., in maximum quantum yield of photosystem II and aboveground biomass). A Poisson error distribution and a log link function were used to model shoot emergence time, beginning of flowering and number of offspring bulbs; a binomial distribution and logit link function were used to model the probability of survival; a Gaussian distribution and identity link function were used for the maximum quantum yield of photosystem II (raised to 10) and aboveground biomass; for the mean bulb biomass and total bulb biomass, a gamma distribution and inverse link function were employed.

Introduced tetraploids versus introduced pentaploids

Differences among tetraploid and pentaploid *O. pes-caprae* plants from the invaded range of the western Mediterranean basin were evaluated using the same approach, but using cytotype and competition as fixed factors. Error distributions and link functions were set as mentioned above.

Trifolium repens response

The effect of competition (fixed factor) with *O. pes-caprae*, from different ranges and different cytotypes within the invaded range, on the maximum quantum yield of photosystem II, aboveground biomass and probability of survival of *T. repens* was assessed using generalized linear models (GLMs). A Gaussian distribution and identity link function were used to model maximum quantum yield of photosystem II (raised to 10) and aboveground biomass (ln-transformed). The probability of survival was adjusted to a binomial distribution with a logit link function.

All statistical analyses were performed in R version 3.2.2 (R Core Team 2015) using the packages “lme4” for GLMMs (Bates et al. 2015), “car” for Type-II analyses of deviance (Fox and Weisberg 2011), “lsmeans” for least-squares means (Lenth 2015), “multcomp” for multiple comparisons (Hothorn et al. 2008), and “stats” for GLMs (R Core Team 2015).

Results

Native tetraploids versus introduced tetraploids

Results from GLMMs are summarized in Table 1. Shoot emergence time varied significantly among tetraploid plants from different ranges (Table 1), with plants from the invaded range emerging earlier than plants from the native range (Fig. 1a). Initial bulb weight had a significant effect on shoot emergence time (Table 1), with shoots from large bulbs emerging earlier than shoots from small bulbs. Tetraploid plants from the invaded range began flowering significantly later, remaining vegetative for a longer period than tetraploid plants from the native range (Table 1; Fig. 1b). However, there was a significant range \times competition interaction (Table 1), and the analyses by competition treatment showed that differences between ranges were significant for plants in the control and low competition treatments, but not for plants in the high competition treatment.

Mean values of F_v/F_m (0.83 for both ranges) were within the optimal range for this parameter (0.75–0.85, Björkman and Demmig 1987), and did not differ among ranges or competition treatments (Table 1).

Aboveground biomass differed significantly among ranges, being higher in plants from the invaded range (Table 1; Fig. 1c). There was a significant range \times competition interaction (Table 1), which reflected the fact that within the invaded range, plants in the low and high competition treatments produced significantly more aboveground biomass than plants in the control ($P < 0.05$; Fig. 1c). Shoot emergence time had a significant effect on the aboveground biomass (Table 1), with plants that emerged earlier having greater aboveground biomass than plants that emerged later. Concerning final bulb production, significant differences between native tetraploids and introduced tetraploids were obtained for the number of bulbs produced and mean bulb biomass, with the latter producing more bulbs, but with lower mean biomass (Table 1; Fig. 1d, e).

Introduced tetraploids versus introduced pentaploids

Results from GLMMs are summarized in Supplementary Table 2. Beginning of flowering was the only variable for which statistically significant differences

Table 1 Results of the analyses of deviance (type II Wald χ^2 tests) from the generalized linear mixed models testing for the effects of range and competition on shoot emergence time, beginning of flowering, maximum quantum yield ofphotosystem II (F_v/F_m), aboveground biomass, number of bulbs, mean bulb biomass, total bulb biomass and probability of survival of tetraploid *Oxalis pes-caprae* plants from the native and invaded ranges

Response variables	<i>n</i>	Range			Competition			Range × competition			Covariate ^a			Population	
		<i>df</i>	χ^2	<i>P</i>	<i>df</i>	χ^2	<i>P</i>	<i>df</i>	χ^2	<i>P</i>	<i>df</i>	χ^2	<i>P</i>	Variance	SD
Shoot emergence time	117	1	8.35	0.004	2	1.84	0.399	2	3.83	0.147	1	20.50	< 0.0001	0.4199	0.6480
Beginning of flowering	83	1	19.00	< 0.0001	2	3.79	0.151	2	6.46	0.040	1	4.49	0.034	0.0134	0.1155
F_v/F_m	115	1	0.01	0.911	2	0.79	0.674	2	5.42	0.067	1	0.16	0.685	0.0001	0.0112
Aboveground biomass	113	1	18.90	< 0.0001	2	9.31	0.010	2	6.71	0.035	1	39.94	< 0.0001	0.1153	0.3396
Number of bulbs	97	1	7.13	0.008	2	1.62	0.445	2	0.50	0.778	1	0.40	0.525	0.0000	0.0000
Mean bulb biomass	97	1	6.66	0.010	2	0.86	0.652	2	0.30	0.862	1	0.01	0.919	0.0000	0.0000
Total bulb biomass	97	1	0.81	0.369	2	2.25	0.325	2	0.15	0.927	1	0.30	0.582	0.0000	0.0000
Probability of survival	104	1	2.15	0.143	2	1.38	0.500	2	0.00	1.000	1	3.35	0.067	0.0000	0.0000

Variance estimation and standard deviation (SD) are shown for the random factor (Population). Significant *P* values are highlighted in bold and sample sizes (*n*) are provided. Thirteen random pots were accidentally lost during harvesting, which resulted in the loss of data on final bulb production and survival for plants in those pots

^aInitial bulb weight was used as a covariate for shoot emergence time, and shoot emergence time as a covariate for all the other response variables

were observed between cytotypes within the invaded range (Supplementary Table 2), reflecting the fact that tetraploids began flowering later than pentaploids (Supplementary Fig. 2a). Also, for this variable there was a significant effect of competition (Supplementary Table 2), with plants in the high competition treatment beginning flowering earlier than plants in the control ($P < 0.05$), and plants in the low competition treatment presenting intermediate values (Supplementary Fig. 2a).

Introduced pentaploids presented the same pattern as introduced tetraploids for aboveground biomass (Supplementary Table 2), namely an increment of biomass with the presence of competition (Supplementary Fig. 2b).

Trifolium repens response

Results from GLMs are summarized in Table 2 and Supplementary Table 3. *T. repens* aboveground

biomass was significantly affected by competition with tetraploid *O. pes-caprae* plants from both ranges (Table 2), with plants in the control presenting the highest values, plants competing with tetraploid *O. pes-caprae* from the native range having low but intermediate values, and plants competing with tetraploid *O. pes-caprae* from the invaded range presenting the lowest values ($P < 0.05$; Fig. 2a). Furthermore, aboveground biomass differed significantly between plants competing with introduced tetraploids and introduced pentaploids (Supplementary Table 3), with plants competing with pentaploids presenting lower values ($P < 0.05$; Supplementary Fig. 3a).

GLM results revealed a significant effect of competition with tetraploid *O. pes-caprae* plants on *T. repens* survival (Table 2). However, the multiple comparison test revealed only marginal differences between plants in the control and plants competing with tetraploids from the invaded range, with the latter

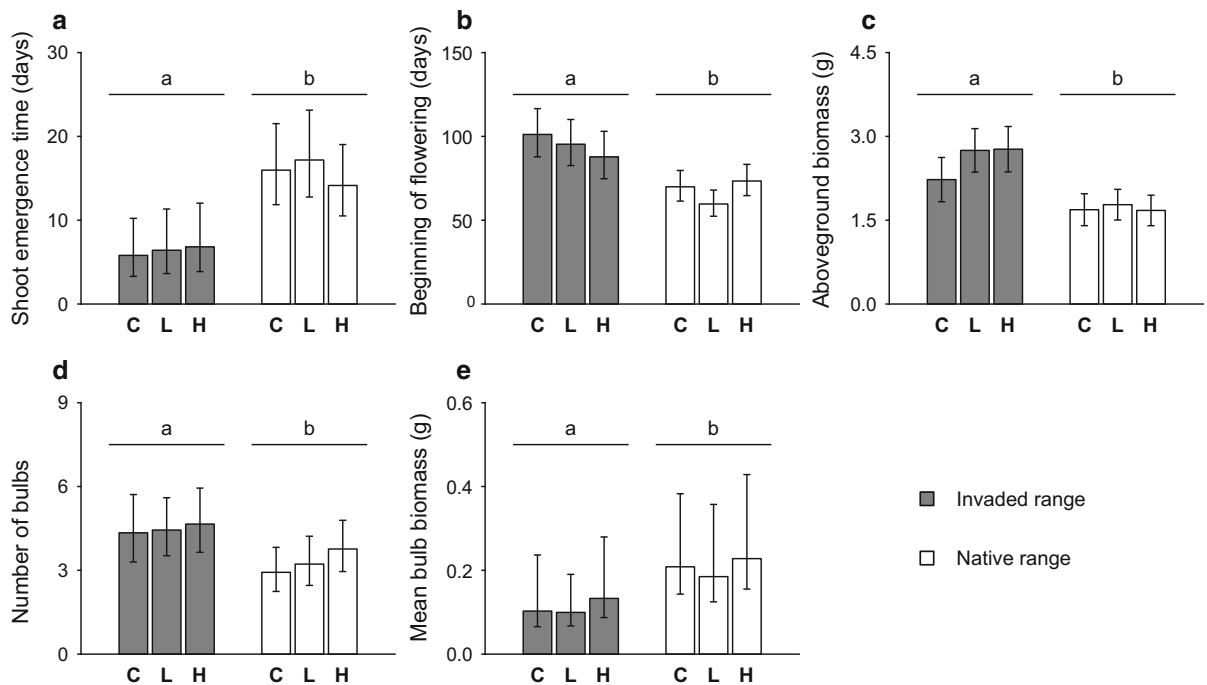


Fig. 1 Comparison between tetraploid *Oxalis pes-caprae* plants from the invaded (grey) and native (white) ranges growing alone (C), under low competition (L), and under high competition (H): time of emergence (a), beginning of flowering (b), aboveground

biomass (c), number of bulbs (d) and mean bulb biomass (e). Values are given as model-adjusted back-transformed least-squares means and 95% confidence intervals. Different letters denote significant differences among ranges at $P < 0.05$

Table 2 Results of the analyses of deviance (type II χ^2 tests) from the generalized linear models testing for the effect of competition with tetraploid *Oxalis pes-caprae* plants from the native and invaded ranges on maximum quantum yield of photosystem II (F_v/F_m), aboveground biomass and probability of survival of *Trifolium repens*

Response variables	Competition			n
	df	χ^2	P	
F_v/F_m	2	4.26	0.119	57
Aboveground biomass	2	115.69	< 0.0001	59
Probability of survival	2	6.36	0.042	122

Significant P values are highlighted in bold and sample sizes (n) are provided

having lower survival values ($P = 0.099$; Fig. 2b). The same happened for the comparison between *T. repens* competing with introduced tetraploids and introduced pentaploids (Supplementary Table 3), with the multiple comparison test revealing only marginal differences between plants in the control and plants

competing with pentaploids, with the latter presenting lower survival ($P = 0.061$; Supplementary Fig. 3b).

Discussion

The results of the present study demonstrate significant genetic based differences in life-history traits between native and introduced tetraploid plants of *O. pes-caprae*. Shoots of tetraploid plants from the invaded range emerged earlier, the plants began flowering later and produced more aboveground biomass and a greater number of offspring bulbs when compared to native tetraploids. Furthermore, introduced tetraploids showed an increase in the aboveground biomass in the competition treatments, and affected *T. repens* growth more severely than their native conspecifics, suggesting an increased competitive ability of introduced tetraploid *O. pes-caprae*. These patterns are in accordance with studies of other invasive plants that have demonstrated that, when grown in a common environment, plants from

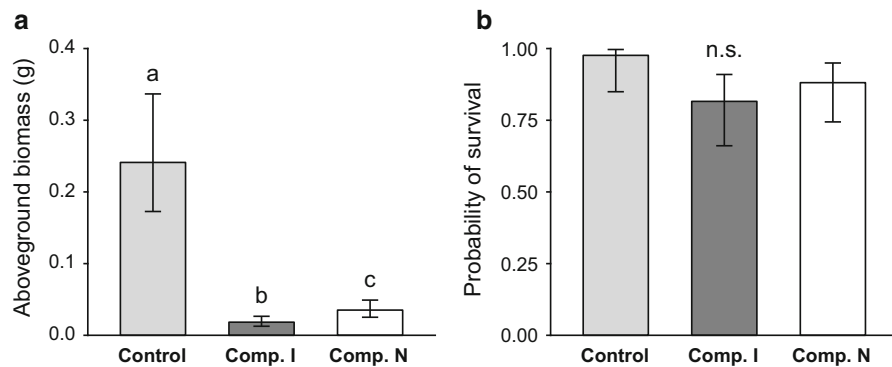


Fig. 2 Comparison between *Trifolium repens* plants grown alone (Control; light grey) and in competition with tetraploid *Oxalis pes-caprae* plants from the invaded (Comp. I; grey) and native (Comp. N; white) ranges: aboveground biomass (a) and

probability of survival (b). Values are given as model-adjusted back-transformed least-squares means and 95% confidence intervals. Different letters denote significant differences among treatments at $P < 0.05$; n.s. no significant differences

introduced populations performed better than those from native populations (e.g., Blair and Wolfe 2004; Brown and Eckert 2005; Ridenour et al. 2008; Zou et al. 2008; Barney et al. 2009; Hahn et al. 2012; Turner et al. 2014).

Phenological traits, in particular the emergence and flowering times, may evolve rapidly in a new environment in response to both biotic and abiotic factors (Lee 2002; Elzinga et al. 2007; Donohue et al. 2010). Genetically determined differences in phenological traits between native and introduced populations of invasive plant species have been demonstrated by several authors (e.g., Blair and Wolfe 2004; Erfmeier and Bruelheide 2005; Dlugosch and Parker 2008a; Barney et al. 2009; Turner et al. 2014). In this study, tetraploid *O. pes-caprae* from the invaded range emerged earlier and began flowering later than native tetraploids. Early emergence is assumed to provide a competitive advantage (for example, when competition for light is intense) and to benefit growth and fecundity by allowing plants to attain a larger size before reproduction and/or increase their reproductive period (Verdú and Traveset 2005; Donohue et al. 2010). Later flowering is often associated with a larger size at maturity, which may translate into higher overall reproductive output (Weber and Schmid 1998; Colautti et al. 2010). Indeed, in this study, the differences in phenology between ranges were accompanied by differences in aboveground biomass. Considering the possible association between phenological traits and growth, shoot emergence time was used as a covariate in the statistical analyses to

control for its effects. The fact that range had a significant effect on aboveground biomass after controlling for the effect of shoot emergence time, suggests that the difference in aboveground biomass between ranges is not solely explained by the differences in time of emergence. Therefore, the earlier emergence and later onset of flowering of tetraploid *O. pes-caprae* plants from introduced populations may confer an advantage under certain conditions, but do not seem to completely explain the increased aboveground biomass in these populations. This change towards increased growth in the invaded range is consistent with the patterns found in other invasive species (e.g., Siemann and Rogers 2001; Blair and Wolfe 2004; Dlugosch and Parker 2008a; Turner et al. 2014).

Growth and reproductive performance are important components of plant fitness, and clonal reproduction, in particular, has been regarded as one of the determinants of *O. pes-caprae* invasion success (Vilà et al. 2006a; Vilà and Gimeno 2006; Verdagner et al. 2010; Castro et al. 2016). In a comparative study of reproductive traits using plants from the same populations used here, but under optimal resource conditions, Castro et al. (2016) showed that, when compared to native tetraploids, introduced tetraploids invested significantly more in bulb production, producing a greater number of bulbs, which translated into higher total bulb biomass. Interestingly, in the present study, which was carried out under more limiting conditions (reduced soil volume—1 L pots), the total biomass invested in the production of offspring bulbs did not

differ between ranges; however, while introduced tetraploids produced a higher number of smaller bulbs, native tetraploids produced less, but larger bulbs. Parent bulb size seems to be important for *O. pes-caprae* shoot emergence and initial development, as indicated by the significant effect of initial bulb weight on shoot emergence time in this experiment. However, further plant growth apparently becomes independent of resources stored in the parent bulbs once these become depleted and plants start to photosynthesize (Chawdhry and Sagar 1973). Consistent with this, parent bulb size has been suggested to have a small overall effect on maximum plant biomass and reproductive output of the pentaploid *O. pes-caprae* (Sala et al. 2007; Verdaguer et al. 2010). Still, under stressful conditions, such as in nutrient-deficient soils and shaded habitats, increased bulb size may improve plant emergence, growth and reproduction (Sala et al. 2007; Verdaguer et al. 2010). Furthermore, the production of bulbs in this species is plastic and highly dependent on the conditions in which plants are grown, with differences in offspring bulb size as a response to growth conditions (e.g., nutrient availability and bulb depth) having been reported for the pentaploids (Sala et al. 2007; Verdaguer et al. 2010). Despite all this, in the fertile soils that this species tends to occupy in the invaded range (commonly in agricultural areas), the production of many small bulbs, which are equally capable of growing and reproducing prolifically, may be advantageous, contributing to enhance the spread of *O. pes-caprae* without additional costs. Thus, smaller bulb sizes of introduced tetraploids could relate to an occurrence in disturbed less shaded habitats in the invaded range, where competition is probably not as important as in the native range. Therefore, the production of a greater number of bulbs by introduced tetraploids in this study seems to indicate that these have evolved higher clonal dispersal potential, as previously suggested by Castro et al. (2016).

Comparing native and introduced populations under competitive conditions is fundamental to evaluate whether phenotypic differences actually translate into enhanced competitive ability in the invaded range (Bossdorf et al. 2005). In the present study, although *T. repens* proved to be a weak competitor, tetraploid *O. pes-caprae* plants from different ranges differed significantly in their competitive responses and effects. Introduced tetraploids grown with *T. repens*

tended to begin flowering earlier than introduced tetraploids in the control. Furthermore, introduced tetraploids showed an increase in aboveground biomass when grown with *T. repens*, and affected *T. repens* growth more severely than tetraploids from the native range, which may be indicative of a greater competitive ability. The greater aboveground biomass of introduced tetraploids grown with *T. repens* indicates facilitation by *T. repens*. One possible explanation for this may be the occurrence of belowground nitrogen transfer from *T. repens*, which is a N₂-fixing legume, to *O. pes-caprae*. Indeed, *T. repens* has been shown to be an efficient N donor (Pirhofer-Walzl et al. 2012) and to promote N facilitation (Hernandez and Picon-Cochard 2016). Differences in the ability to take up belowground N between native and introduced tetraploids, with a better acquisition of N by introduced plants, might explain why *T. repens* presence had a positive effect on introduced plants, but no effect on native ones.

Studies comparing competitive ability between introduced and native populations of other invasive plant species have produced mixed results. While in some cases plants from introduced populations were shown to have higher competitive ability than their native conspecifics (e.g., Ridenour et al. 2008; Zou et al. 2008; Barney et al. 2009; Beaton et al. 2011; Huang et al. 2012; Yuan et al. 2013; Zheng et al. 2015a), in other cases introduced populations presented similar or lower competitive ability when compared with native populations (e.g., Leger and Rice 2003; Vilà et al. 2003; Blair and Wolfe 2004; Bossdorf et al. 2004; McKenney et al. 2007; Leifso et al. 2012; Gruntman et al. 2014). Furthermore, the differences in competitive ability between ranges may vary when measured in different biotic and abiotic conditions (Liao et al. 2013; Qin et al. 2013; Lin et al. 2015) and depending on the identity of the competitors [e.g., intraspecific versus interspecific competition, Joshi et al. (2014), Zheng et al. (2015b); competition with resident species from the native range versus competition with resident species from the invaded range, Zheng et al. (2015b); competition with a single competitor versus competition with a multi-species community, Oduor et al. (2015)]. Despite the competitive superiority demonstrated here by *O. pes-caprae* plants from both ranges when growing with *T. repens*, the competitive responses and effects of this species may differ when growing with other

competitors. Sala et al. (2007) found that competition with *Lolium rigidum* Gaudin had a strong negative effect on invasive pentaploid *O. pes-caprae* plants. However, a comparison with native populations was not included in that experiment.

The finding that native and introduced tetraploids growing in a common environment differed significantly in several life-history characters provides strong evidence for evolution of invasiveness, with the observation of a change towards a more aggressive phenotype in introduced tetraploids. This divergence can be the result of factors associated with founding events or rapid post-introduction evolution, or even a combination of these two non-exclusive processes. All introduced species have to experience founding events, which may comprise large reductions in genetic diversity (Klüber and Eckert 2005; Dlugosch and Parker 2008b; Zhang et al. 2010). Ferrero et al. (2015) found that, although *O. pes-caprae* populations from the western Mediterranean basin were composed of numerous genotypes, the genetic diversity of these populations was significantly lower than that of tetraploid populations from South Africa. Under such circumstances, it is impossible to rule out the possibility that introduced tetraploids may have originated from a small subset of plants of vigorous genotypes, not necessarily representative of the native genotypes.

Regardless of the role that founding events might have played, the shifts in phenology and the changes toward increased competitive ability, growth, and production of asexual propagules in introduced tetraploids of *O. pes-caprae* may be, at least partly, explained by post-introduction evolutionary changes. Introduced populations often experience markedly different selection pressures in the new environment and may undergo rapid evolutionary adaptation even in cases where it would seem unlikely due to genetic bottlenecks (Dlugosch and Parker 2008a; Rollins et al. 2013; Schrieber and Lachmuth 2017). In the specific case of *O. pes-caprae* invasion in the western Mediterranean basin, recent studies, including the introduced tetraploids used in this study, have provided striking evidences of evolutionary changes in several reproductive traits. Plants from the invaded range were shown to have higher asexual fitness than those from the native region (Castro et al. 2016). Additionally, a weakening in the incompatibility system has been reported for this invaded range, with introduced plants having a higher reproductive success

following illegitimate pollinations (self- and intra-morph pollinations) than South African plants, which are strongly morph- and self-incompatible (Costa et al. 2017). Also, Vilà and Gimeno (2006) found a genetically based higher propagation potential (i.e., greater production of bulbs) in Mediterranean insular populations compared to neighboring mainland populations of the 5x S-morph, suggesting adaptive evolution as one of the possible explanations. Altogether, these findings indicate that *O. pes-caprae* may have a great potential for rapid evolutionary change.

Evolutionary changes regarding competitive ability in introduced tetraploids of *O. pes-caprae* could be associated with a reallocation of resources from defense to growth in the absence of coevolved herbivores, as predicted by the evolution of increased competitive ability (EICA) hypothesis (Blossey and Nötzold 1995). The fact that plants from the invaded range produced more aboveground biomass than those from the native range provides partial support for the EICA hypothesis; however, the accompanying changes in defense were not assessed in this study. In order to better assess the EICA hypothesis, further investigations should consider different levels of herbivory by specialist and generalist herbivores in native and introduced populations to evaluate both plant resistance and tolerance. Another mechanism that could lead to evolutionary increases in competitive ability following introduction is selection for increased production of allelochemicals in the invaded range (Callaway and Ridenour 2004; Uesugi and Kessler 2013, 2016; Yuan et al. 2013; Gruntman et al. 2016; Zheng et al. 2015a). Therefore, it would be interesting to test whether evolution of increased allelopathy also occurred in introduced populations of *O. pes-caprae*.

Although the pre-adaptation of certain cytotypes has been suggested to partly explain the success of some invasive species (Treier et al. 2009; Thébault et al. 2011; te Beest et al. 2012; Sun et al. 2016), the rarity of the pentaploid *O. pes-caprae* cytotype in South Africa and the unclear origin of invasive pentaploids make it difficult to disentangle the role of ploidy level from the role of evolutionary changes in explaining the success of this invasive form. In this study, tetraploid and pentaploid plants from the invaded range did not differ in most of the traits measured. However, in the comparative study of reproductive traits by Castro et al. (2016) introduced

tetraploids presented greater dispersal potential, producing more seeds and more, yet smaller, bulbs than pentaploids. The authors suggested that different introduction timings, with the initial introduction and spread of pentaploids being followed by the introduction of sexual tetraploids more recently, may be in the origin of the current distribution patterns of *O. pes-caprae* in the western Mediterranean. Considering the similarity between introduced pentaploid and tetraploid individuals observed here, as well as the superior reproductive fitness of tetraploids reported by Castro et al. (2016) and the weedy character presented by tetraploids in the native range (Ornduff 1987), introduced tetraploids seem to have the potential to become widespread in the future. Still, it would be interesting to assess if the different *O. pes-caprae* cytotypes differ in other traits related to invasive potential, such as tolerance to disturbances.

Concluding remarks

This study suggests that founder events and rapid post-introduction adaptive evolution may have contributed, independently or in concert, to the genetic differentiation between native and introduced tetraploids of *O. pes-caprae*, leading to the appearance of a phenotype with higher invasive potential in the invaded range of the western Mediterranean basin. Additionally, the similarity between tetraploids and pentaploids found within this invaded region implies that both cytotypes have the potential to behave as invaders with negative impacts on the native flora. This is particularly important considering that the ruderal communities that this species invades represent a valuable element of the Mediterranean flora providing important ecosystem services (Lambdon 2006; Vilà et al. 2006b).

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Compliance with ethical standards

Conflict of interest The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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