

## RESEARCH PAPER

# Sexual reproduction of the pentaploid, short-styled *Oxalis pes-caprae* allows the production of viable offspring

J. Costa<sup>1</sup>, V. Ferrero<sup>1,2</sup>, J. Loureiro<sup>1</sup>, M. Castro<sup>1</sup>, L. Navarro<sup>2</sup> & S. Castro<sup>1</sup>

<sup>1</sup> Department of Life Sciences, Centre for Functional Ecology (CFE), University of Coimbra, Coimbra, Portugal

<sup>2</sup> Department of Plant Biology, Faculty of Science, University of Vigo, Vigo, Spain

## Keywords

Heterostyly; incompatibility system; invader; pentaploid; tristily; western Mediterranean Basin.

## Correspondence

J. Costa, Department of Life Sciences, Centre for Functional Ecology (CFE), University of Coimbra, PO Box 3046, 3001-401 Coimbra, Portugal.

E-mail: joana.costa@uc.pt

## Editor

D. Byers

Received: 23 May 2012; Accepted: 31 December 2012

doi:10.1111/plb.12010

## ABSTRACT

Reproduction is a key factor for the successful establishment and spread of introduced species. *Oxalis pes-caprae* is a tristylous species with a self- and morph-incompatibility sexual system that, in the invaded range of the western Mediterranean Basin, has been found to reproduce asexually because only the pentaploid, short-styled morph (5x S-morph) was introduced. The objective of this study was to test the ability of the 5x S-morph of *O. pes-caprae* to produce viable offspring in the absence of compatible mates, exploring the hypothesis that new morphs could have emerged by sexual reproduction events of the initially introduced morph. Pollen germination, pollen tube development, fruit and seed production, seed germination and offspring ploidy levels were analysed after controlled hand-pollinations to assess self- and morph-incompatibility and production of viable gametes by the 5x S-morph. The self-incompatibility system is still operating, but a partial breakdown in the morph-incompatibility system combined with the production of viable gametes was observed, allowing sexual reproduction of the 5x S-morph in the invaded range. The ability of the 5x S-morph to reproduce sexually may have major consequences for the dynamics of invasive populations of *O. pes-caprae* and could be one of the factors involved in the occurrence of new floral morphs in this invaded range.

## INTRODUCTION

One key factor for the successful establishment and spread of introduced species after overcoming long-distance dispersal is reproduction and, among other strategies, vegetative propagation has been largely correlated with invasion success (e.g. Godfrey *et al.* 2004; Lloret *et al.* 2005; Pyšek & Richardson 2007). Because clonality affects the spatial distribution of genets and their flowers determine opportunities for cross-fertilisation, clonal species are expected to have increased rates of self-pollination because of the higher probability of pollen dispersal between individuals of the same clone (Handel 1985; Charpentier 2002). In self-incompatible plants, an increase of self-pollination has important reproductive consequences negatively affecting both male (e.g. Harder & Barrett 1996) and female fitness (e.g. Vallejo-Marín & Uyenoyama 2004; Porcher & Lande 2005; Wang *et al.* 2005).

Conflicts between sexual and asexual reproduction can be even more intricate when the invader has a complex breeding system, such as heterostyly. In heterostylous populations, the plants present two or three floral morphs that differ reciprocally in the position of their sexual organs (Barrett 1992). Heterostylous plants are usually self-incompatible and, in addition, present an incompatibility system that only allows crosses among reciprocal anthers and stigmas of compatible morphs (intra-morph incompatibility). In these cases, when just one of the floral morphs is introduced in a new area, the sexual contribution to the fitness of the newly established plant/population is expected to be null (e.g. *Oxalis pes-caprae*, Castro *et al.*

2007; *O. debilis*, Luo *et al.* 2006; *O. corymbosa*, Tsai *et al.* 2010).

Reproduction by vegetative means has several ecological advantages for an invader, enabling, for example, growth and persistence in the new range when conditions are unfavourable for sexual reproduction due to the absence of pollinators (Richardson *et al.* 2000) or to the loss of compatible mating partners (e.g. Barrett 1979; Castro *et al.* 2007). However, asexual reproduction also has strong negative consequences. Populations of obligate clonal plants are expected to have lower levels of genetic variability, being less able to respond adaptively to changing environments (Holsinger 2000). This is clearly a disadvantage for an invader in a new and unpredictable habitat. Under this scenario, selection may favour breakdown of the self-incompatibility, as individuals with some level of compatibility would have an advantage under low-density conditions and would be able to establish new populations after dispersal (Baker's law; Baker 1955, 1967; Stebbins 1957). In heterostylous systems, such a phenomenon has been described in several taxa and is usually associated with a re-arrangement of sexual organ position (*i.e.* secondary homostyly), as a mechanism of reproductive assurance (e.g. distylous taxa: *Amsinckia* spp., Schoen *et al.* 1997; *Primula* spp., Mast *et al.* 2006; *Turnera ulmifolia*, Barrett & Shore 1987; *Psychotria* spp., Sakai & Wright 2008; tristylous taxa: *Eichhornia* spp., Barrett 1985, 2011; *Oxalis corymbosa*, Tsai *et al.* 2010).

*Oxalis pes-caprae* L. is a polyploid (with reported diploids, 2x; tetraploids, 4x; and pentaploids, 5x) tristylous species (Fig. 1) native to South Africa, with a typical heteromorphic

incompatibility system responsible for self- and morph-incompatibility (Ornduff 1987). This geophyte has been introduced into Mediterranean climate regions throughout the world, where it has become a widespread invasive weed (Symon 1960; Baker 1965; Ornduff 1987). In most invaded regions, a shift to predominant asexuality was observed as a result of founder events after the introduction of the pentaploid short-styled morph alone (Baker 1965; Ornduff 1987). However, in the last years, new floral morphs (mid- and long-styled), cytotypes ( $4x$ ) (Castro *et al.* 2007, 2013) and punctual events of sexual reproduction (Vignoli 1937; Ornduff 1987; Castro *et al.* 2007) have been described in the invaded range of the western Mediterranean Basin where the pentaploid short-styled morph ( $5x$  S-morph) is the most frequent floral morph (Castro *et al.* 2007, 2013).

The classical genetic studies in tristylous plants indicate that the floral morph is controlled by two loci, *Ss* and *Mm*, with the long-styled type being homozygous recessive for both of them (*ssmm*), the mid-styled dominant over the long one but recessive for the other (*ssMx*) and the short-styled dominant over both (*Sxxx*) (Lewis & Jones 1992). This system has also been demonstrated in some *Oxalis* species (Weller 1976). In a parallel study, Castro *et al.* (2013) suggest that the occurrence of new morphs in the invaded area could have resulted from a breakdown of the incompatibility system that enabled the occurrence of sexual reproduction events in the short-styled morph and/or from multiple introduction events.

The objective of the present study was to test the ability of the pentaploid short-styled ( $5x$  S-morph) *O. pes-caprae* from the invaded region of the western Mediterranean Basin to produce viable offspring in the absence of compatible mates. It is hypothesised that one of the pathways involved in the emergence of new morphs in this invaded range is by (occasional) sexual reproduction through a breakdown of the incompatibility system in the  $5x$  S-morph. If this is the case, legitimate (between morph) crosses will yield offspring, proving the ability of the  $5x$  S-morph to produce viable gametes, and illegitimate (within morph) crosses will yield viable offspring, proving the ability of the  $5x$  S-morph to reproduce sexually in the absence of compatible mates. Under natural conditions, this will actively contribute to an increase in the genetic

diversity of the invasive populations and, ultimately, may have serious consequences for the invasiveness of this species.

## MATERIAL AND METHODS

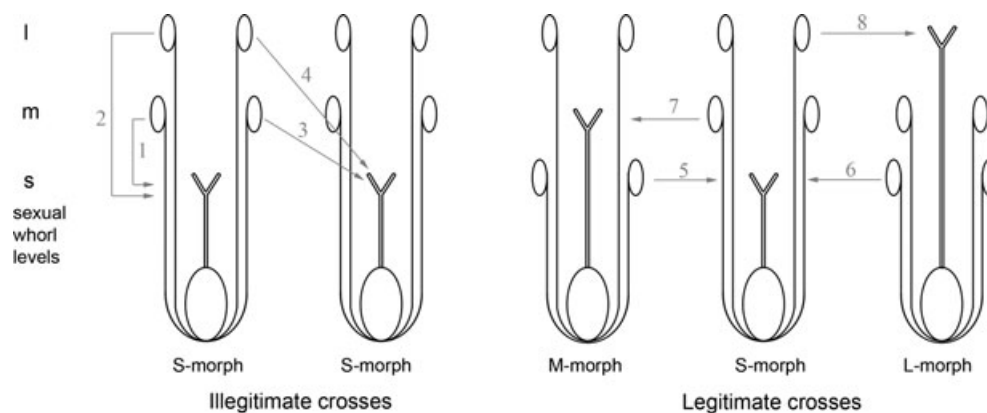
### Plant material and study area

*Oxalis pes-caprae* L. (Oxalidaceae) is a perennial bulbous plant with a profuse production of bulbils that, associated with the contractile properties of its roots, confers a high ability to reproduce asexually (Pütz 1994). *O. pes-caprae* is a tristylous species (short-, mid- and long-styled floral morph, S-morph, M-morph and L-morph, respectively; Fig. 1), with actinomorphic yellow flowers arranged in terminal umbellate cymes (Coutinho 1939; Ornduff 1987; Sánchez-Pedraja 2008). In the invaded region of the Mediterranean Basin, flowering occurs from early January to late March.

This study was carried out during 2009 and 2010 with plants from Almoçageme (Estremadura province, Portugal), a population previously known to be trimorphic (Castro, personal observation). The use of a trimorphic population allowed us to test the ability of the  $5x$  S-morph to produce viable offspring and to assess if it could be involved in the emergence of other floral morphs. The percentage of floral morphs within the population in February 2009 was: 56.0% of the S-morph, 11.9% of the M-morph and 32.1% of the L-morph. Floral morph composition was assessed by evaluating the morph of 100 individuals every 5 m along two longitudinal transects across the population. Plants were collected in the field during December 2009 before flowering. Thirty-five plants per floral morph (S-morph, M-morph and L-morph) were directly transplanted to pots, identified with an ID number and maintained at ambient outdoor conditions in the experimental garden of the Botanical Garden of the University of Coimbra. The selected plants were collected at least 5 m apart to avoid re-sampling of the same genets.

### Ploidy level analysis

Because there are two cytotypes reported to occur in the invaded range of the Mediterranean region ( $4x$  and  $5x$ ; Castro



**Fig. 1.** *Oxalis pes-caprae* floral morphs and crosses performed in hand-pollination experiments: self-pollinations with pollen from the mid- (1) and long-anther (2) levels, intra-morph pollinations with pollen from the mid- (3) and long-anther (4) levels and inter-morph legitimate pollinations with  $5x$  S-morph as pollen recipient (5, 6) and as pollen donor (7, 8). S-morph, M-morph and L-morph for short-, mid- and long-styled floral morphs. The anther levels are represented by l, m and s for long-, mid- and short-whorl, respectively.

*et al.* 2007, 2013), ploidy level of all plants collected was analysed using flow cytometry (FCM). Samples were prepared following the procedure of Galbraith *et al.* (1993) and the two-step nuclear isolation method with Otto's buffers (Otto 1992; Doležal & Göhde 1995). Briefly, nuclei from fresh leaves of *O. pes-caprae* and *Bellis perennis* (internal reference standard with  $2C = 3.38$  pg; (Schönswetter *et al.* 2007) were released after chopping the leaves in 0.5 ml Otto I solution (100 mM citric acid, 0.5% (v/v) Tween 20); the solution was filtered into a cytometer sample tube using a 50- $\mu$ m nylon filter and 1 ml Otto II solution (400 mM  $\text{Na}_2\text{PO}_4 \cdot 12\text{H}_2\text{O}$ ) added; finally, 50  $\mu\text{g}\cdot\text{ml}^{-1}$  propidium iodide was added to stain the nuclei and 50  $\mu\text{g}\cdot\text{ml}^{-1}$  RNase for digestion of the double stranded RNA (Doležal *et al.* 2007). At least 3000 nuclei per sample were analysed in a Partec CyFlow Space flow cytometer (Partec GmbH, Görlitz, Germany). The flow cytometer was equipped with a green solid state laser (Cobolt Samba 532 nm, 100 mW; Cobolt, Stockholm, Sweden) for PI excitation. Only histograms with a coefficient of variation (CV) below 5% for both sample and standard  $G_1$  peaks were accepted as a quality standard. The DNA index was calculated for all the samples by dividing the *O. pes-caprae*  $G_0/G_1$  peak mean fluorescence by that of *B. perennis* and plants were identified as 4x or 5x for genome size values of  $1.37 \pm 0.056$  ( $n = 39$ ) and  $1.66 \pm 0.030$  ( $n = 248$ ; mean  $\pm$  SD, followed by sample size in parenthesis), respectively (Castro *et al.* 2007).

### Hand-pollination experiments

To assess the ability of the 5x S-morph to produce offspring, both illegitimate and legitimate pollinations were performed (Fig. 1). Illegitimate pollinations were carried out to assess the self- and morph-incompatibility of the 5x S-morph and the following treatments were performed: self-pollinations with pollen from the mid- and long-anther levels (selfing 5x  $S_m$  and selfing 5x  $S_l$ , respectively) and intra-morph pollinations with pollen from the mid- and long-anther levels (5x  $S \times 5x S_m$  and 5x  $S \times 5x S_l$ , respectively; Fig. 1). Legitimate pollinations were carried out to assess the ability of the 5x S-morph to produce viable offspring through its ovules and pollen grains and, thus, the following treatments were performed: inter-morph legitimate pollinations with 5x S-morph as pollen recipient (5x  $S \times 4x M_s$  and 5x  $S \times 4x L_s$ ) and as pollen donor (4x  $M \times 5x S_m$  and 4x  $L \times 5x S_l$ ; Fig. 1). Plants were covered with a nylon mesh before flowering to prevent natural pollinations and maintained bagged until fruiting. Recipient flowers were emasculated to prevent self-pollination. Up to 33 pollinations per treatment were done in distinct individuals. Cross-pollinations were performed by gently rubbing anthers from 3 to 5 distinct individuals against the recipient stigmas.

When the ovaries started to swell, most stigmas and styles were cut and harvested in ethanol 70% to assess pollen germination and pollen tube development in the style. Stigmas and styles were softened with 8 N sodium hydroxide for 3 h, washed in distilled water and placed overnight in 0.05% (w/v) aniline blue prepared in 0.1 N potassium phosphate (Dafni *et al.* 2005). Then they were placed on a microscope slide with a drop of glycerine 50%, squashed beneath a coverslip and observed using a Nikon Eclipse 80i epifluorescence microscope (Nikon Instruments, Kanagawa, Japan) with the UV-2A filter cube. Pollen germination and pollen tube development along the style

were assessed by counting the number of germinated grains from 50 randomly selected grains deposited in the stigmatic papillae and by counting the number of pollen tubes in the lower part of the style, respectively. The mean number of ovules of each floral morph was also assessed in more than 15 flowers from distinct individuals under fluorescence microscopy following the procedure of Dafni *et al.* (2005), as described above.

The fruit and seed production were recorded when mature fruits and seeds were characterised as morphologically viable or aborted. Fruit set was calculated for each pollination treatment as the percentage of treated flowers that developed into fruits.

### Seed germination

The seeds obtained from the hand-pollination experiments were placed to germinate in 6  $\times$  6 cm pots filled with common garden substrate and maintained at ambient outdoor conditions protected from the rain in the experimental garden of the Botanical Garden (University of Coimbra) in September 2010. Pots were monitored weekly over 3 months to count the number of seedlings. Ploidy level of the germinated offspring was assessed following the procedure described in the section 'Ploidy level analysis'.

### Statistical analysis

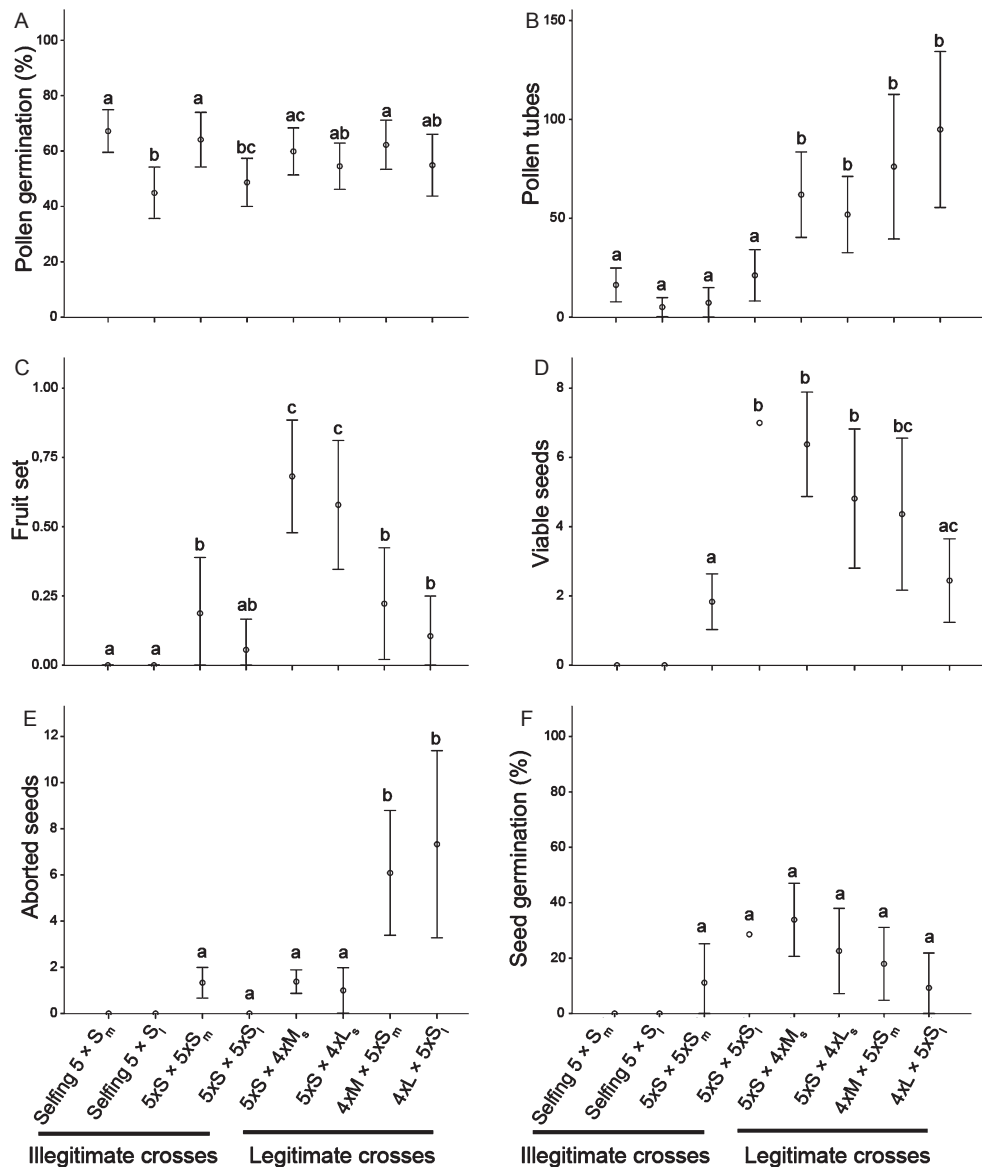
Descriptive statistics (mean and SE of the mean) were calculated for pollen germination, number of pollen tubes developed in the lower part of the style, fruit set, number of morphologically viable and aborted seeds per fruit, and seed germination.

Differences among pollination treatments in pollen germination, number of pollen tubes along the style and aborted seeds were analysed using a generalized linear model (GLM) with a gamma distribution and a power(-1) link function. A similar approach was used for fruit set with a binomial distribution and logit link function. Least square means were used to analyse differences between treatments. The number of viable seeds and seed germination were analysed using a one-way ANOVA, followed by Tukey test for multiple comparisons. The results for viable seeds, aborted seeds and seed germination from the 5x  $S \times 5x S_l$  cross were not included in the analysis because there was only one sample. All the analyses were carried in SAS version 9.2 (SAS Institute Inc., Cary, NC, USA).

## RESULTS

Results from hand-pollination experiments are given in Fig. 2 and Appendix S1. Pollen grains from the 5x S-morph, 4x M-morph and 4x L-morph were able to germinate on the recipient stigmas but statistically significant differences were observed in germination rates ( $\chi^2 = 14.57$ ,  $P = 0.0419$ ): higher germination rates were observed in legitimate crosses (although no significant differences were found for 5x  $S \times 4x L_s$  and 4x  $L \times 5x S_l$  crosses) and in self- and intra-morph pollinations when pollen from the mid-anthers of the 5x S-morph was used (Fig. 2A). Pollen tube development was observed in all illegitimate and legitimate crosses, despite the significant differences observed between pollination treatments ( $\chi^2 = 9.14$ ,  $P < 0.0001$ ), with legitimate pollinations having significantly more pollen tubes than illegitimate ones (Fig. 2B).

The mean number of ovules produced by each floral morph was not significantly different (mean  $\pm$  SE:  $39.6 \pm 1.0$ ;



**Fig. 2.** *Oxalis pes-caprae* sexual reproduction in the invaded range of the Mediterranean basin. A: percentage of pollen germination; B: mean number of pollen tube development in the lower part of the style; C: fruit set; D: mean number of viable seeds; E: mean number of aborted seeds; F: percentage seed germination. Values are given as mean  $\pm$  SE.

$F = 0.53$ ,  $P = 0.59$ ). Fruit production and number of viable and aborted seeds per fruit were significantly different between pollination treatments ( $\chi^2_7 = 65.65$ ,  $P < 0.0001$ ;  $F_4 = 3.50$ ,  $P = 0.0126$ ;  $\chi^2_4 = 30.74$ ,  $P < 0.0001$ , respectively; Fig. 2C–E). Selfing crosses did not yield any fruit and significantly higher fruit set was found in legitimate crosses when the 5x S-morph was used as pollen recipient (Fig. 2C). Legitimate crosses tended to produce more viable seeds per fruit than illegitimate crosses, but no significant differences were found between them and the 5x S  $\times$  5x S<sub>l</sub> (Fig. 2D). Concerning the number of aborted seeds, in legitimate crosses two statistically different groups could be distinguished, with higher seed abortion in pollinations where the 5x S-morph was used as pollen donor (Fig. 2E).

Seed germination revealed no statistically significant differences among pollination treatments ( $F_4 = 1.69$ ;  $P = 0.1667$ )

and ranged between 11.1% and 34.7%, it being possible to obtain seedlings from both illegitimate and legitimate crosses (Fig. 2F). Flow cytometric analysis of the germinated offspring revealed that both 4x and 5x were produced in illegitimate and legitimate crosses (Appendix S1). The low number of seedlings obtained from illegitimate crosses made it difficult to disentangle the cytotype patterns in the offspring. In legitimate crosses, 5x offspring was only obtained when the 5x S-morph was used as pollen recipient; nevertheless, 4x was the most frequent cytotype in the offspring; when the 5x S-morph was used as pollen donor, the offspring was composed only of 4x (Appendix S1).

## DISCUSSION

After long-distance dispersal, reproductive strategies are of major importance for the successful colonisation of invasive



species (e.g. Pyšek & Richardson 2007; Barrett 2011). In heterostylous plants, the introduction of only one floral morph leads to the loss of compatible mates, forcing, in many cases, the emergence of novel reproductive adaptations to the new conditions (e.g. Barrett 1979). Under low-density of mating partners and pollen limitation, the transition from incompatibility to compatibility is expected to be advantageous because selection will favour self- and/or morph-compatible individuals (Allee *et al.* 1949; Baker 1966; Charlesworth 1979; Barrett *et al.* 1987). Self-incompatibility breakdown has already been documented in several heterostylous species (Ornduff 1972; Barrett 1989, 1992; Weller 1992), including some invasive ones (Barrett & Shore 2008; Colautti *et al.* 2010). Although fruit and seed production were not completely ruled out in the invaded range of *O. pes-caprae* where the 5x S-morph dominated (Vignoli 1937; Ornduff 1987; Ater 2005; Castro *et al.* 2007), this is the first study quantifying its potential production of viable offspring as a result of a partial breakdown in the morph-incompatibility system.

In the native range, *O. pes-caprae* is known to present a sporophytic, heteromorphic incompatibility system (Ornduff 1987); however at which level the incompatibility occurs is still unknown. Incompatibility responses in heterostylous plants include lack of adhesion, hydration and germination of pollen, inability of pollen tubes to penetrate the stigmatic zone, and cessation of pollen tube growth in the style and ovary (Dulberger 1992; Barrett & Cruzan 1994). The present study shows that in the invaded area of the western Mediterranean region, the self-incompatibility system is still operating, as no fruit and seed production were observed after self-pollinations. However, as pollen tube development along the style was observed, the incompatibility system seems to be operating at several levels of the style and ovary, which suggests a possible late-acting self-incompatibility system in *O. pes-caprae*. This system has been described in several other species such as *Cyrtanthus breviflorus* (Vaughton *et al.* 2010), *Narcissus* spp. (Dulberger 1964; Sage *et al.* 1999; Navarro *et al.* 2012), *Anchusa officinalis* (Schou & Philipp 1983), *Asclepias exaltata* (Lipow & Wyatt 2000) and *Spathodea campanulata* (Bittencourt *et al.* 2003), however further work must be done in order to confirm this in *O. pes-caprae*.

Contrary to self-pollinations, intra-morph crosses resulted in the production of fruits, seeds and seedlings, showing a partial breakdown in the morph-incompatibility system of *O. pes-caprae* in this invaded area. Nevertheless, pollen tube development and fruit and seed production were lower than in legitimate crosses, indicating that the breakdown was not complete and that morph-incompatibility still reduces the reproductive success of within-morph pollinations at several levels of the style and ovary. Low levels of sexual reproduction may have been occurring for some time (Vignoli 1937; Ornduff 1987; Ater 2005), and Castro *et al.* (2007) reported sporadic pollen tube development (although no fruit production was observed) after within-morph pollinations in other populations of *O. pes-caprae* from the same geographic range. Indeed, a recent large-scale reassessment of natural reproductive success across this range reported a remarkable diversity in floral morph and cytotype composition, with variable sexual reproductive outcomes across the surveyed area (Castro *et al.* 2013). The acquisition of morph-compatibility increases the number of mating partners within the

population and has major implications for the population dynamics and, potentially, for genetic structure (Ray & Chikashi 1957; Ganders 1979; O'Brien & Calder 1989). In addition, a partial breakdown in the morph-incompatibility system may be one of the factors involved in the occurrence of additional floral morphs reported recently in this invaded range, although multiple introduction events could also be involved in the process. The partial breakdown in the morph-incompatibility (results herein) and higher sexual reproductive success of M- and L-morphs (Castro *et al.* 2013) may also be involved with the recently observed spread patterns of these morphs.

Legitimate pollinations were performed to assess the ability of the 5x S-morph to produce viable offspring through its ovules and pollen grains. Plants with odd ploidy levels, such as triploids and pentaploids, are reported to have meiotic abnormalities and to produce a high number of aneuploids, as well as 1x, 2x, 3x, 4x and/or 5x gametes in lower numbers (Ramsey & Schemske 1998; Riso-Pascotto *et al.* 2003), consequently they are expected to be mostly sterile (Ramsey & Schemske 1998). Meiotic abnormalities producing microspores with a variable number of chromosomes have been described in *O. pes-caprae* (Vignoli 1937). Although no differences were observed in pollen tube development along the style, the 5x S-morph individuals were more successful as pollen recipient than as pollen donor. Still, our results showed that 5x S-morph individuals were able to produce some viable pollen grains and ovules that, after legitimate pollinations, yielded viable offspring. The prevalence of 4x in the offspring also seems to indicate that 2x gametes were favourably recruited for seed production. Bi-nucleate microspores and 2n microspores resulting from nucleus restitution were already reported in the pentaploid *Brachiaria brizantha* (Riso-Pascotto *et al.* 2003). Moreover, exploring the ploidy of the offspring produced by triploids of *Aloineae*, Brandham (1982) showed that plants with odd ploidy levels (3x) still have some fertility, contributing either 1x or 2x gametes when crossed with 2x or 4x plants, respectively. The bias in the frequency of progeny ploidy levels resulted from seed abortion when the ratio of maternal to paternal genomes in the endosperm tissue deviated from 2:1 (Brandham 1982; Grossniklaus *et al.* 2001). A similar mechanism could be actually guiding the prevalence of 4x seedlings in the 4x × 5x and 5x × 4x crosses with *O. pes-caprae* plants.

In conclusion, a partial breakdown in the morph-incompatibility system, combined with the ability of the 5x S-morph to produce some viable gametes, opened the possibility of sexual reproduction and may be one of the mechanisms involved in the emergence of new floral morphs and cytotypes of *O. pes-caprae* in this invaded region. These results are in accordance with our hypothesis; however, in order to fully understand the patterns of the incompatibility breakdown and their contribution to the reproductive success and morph proportions of *O. pes-caprae* in this invaded region, large-scale pollination experiments are currently being performed throughout the invasive range of the western Mediterranean region.

## ACKNOWLEDGEMENTS

This research was supported by Portuguese Foundation for Science and Technology (FCT) with the project PTDC/BIA-BIC/110824/2009, by CRUP *Acções Integradas Luso-Espanholas*

2010 with the project E10/10, by the Spanish DGICYT (CGL2009-10466), FEDER funds from the European Union, and the *Xunta de Galicia* (INCITE09-3103009PR). FCT also financed the work of Joana Costa (CB/C05/2009/209; PTDC/BIA-BIC/110824/2009) and Sílvia Castro (FCT/BPD/41200/2007). *Fundación Ramón Areces* financed the work of Victoria Ferrero through a post-doctoral grant.

## REFERENCES

- Allee W.C., Emerson A.E., Park O., Park T., Schmidt K.P. (1949) *Principles of animal ecology*. W. B. Saunders Company, Philadelphia, PA, USA.
- Ater M. (2005) Biologie de la reproduction d'*Oxalis pes-caprae* au Maroc. In: Menéndez J., Bastida F., Fernández-Quintanilla C., González J.L., Recasens J., (Eds), *Malherbologia Ibérica: soluciones comunes a problemas comunes*. Universidad de Huelva Publicaciones, Huelva, Spain.
- Baker H.G. (1955) Self-compatibility and establishment after "long-distance" dispersal. *Evolution*, **9**, 347–348.
- Baker H.G. (1965) Characteristics and modes of origin of weeds. In: Baker H.C., Stebbins G.L. (Eds), *The genetics of colonizing species*. Academic Press, New York, NY, USA, pp 147–168.
- Baker H.G. (1966) The evolution, functioning and breakdown of heteromorphic incompatibility systems. I. The Plumbaginaceae. *Evolution*, **20**, 349–368.
- Baker H.G. (1967) Support for Baker's Law – as a rule. *Evolution*, **21**, 853–856.
- Barrett S.C.H. (1979) The evolutionary breakdown of tristylly in *Eichhornia crassipes* (Mart.) Solms (water hyacinth). *Evolution*, **33**, 499–510.
- Barrett S.C.H. (1985) Floral trimorphism and monomorphism in continental and island populations of *Eichhornia paniculata* (Spreng.) Solms. (Pontederiaceae). *Biological Journal of the Linnean Society*, **25**, 21–40.
- Barrett S.C.H. (1989) The evolutionary breakdown of heterostyly. In: Bock J.H., Linhart Y.B. (Eds), *The evolutionary ecology of plants*. Westview Press, Colorado, CO, USA, pp 151–169.
- Barrett S.C.H. (1992) Heterostylous genetic polymorphisms: model systems for evolutionary analysis. In: Barrett S.C.H. (Ed), *Evolution and function of heterostyly*. Springer, Berlin, Germany.
- Barrett S.C.H. (2011) Why reproductive systems matter for the invasion biology of plants. In: Richardson D.M. (Ed), *Fifty years of invasion ecology: the legacy of Charles Elton*. Blackwell, Oxford, UK, pp 195–210.
- Barrett S.C.H., Cruzan M.B. (1994) Incompatibility in heterostylous plants. In: Williams E.G., Clarke A.E., Knox R.B. (Eds), *Genetic control of self-incompatibility and reproductive development in flowering plants*. Kluwer Academic, Boston, MA, USA.
- Barrett S.C.H., Shore J.S. (1987) Variation and evolution of breeding systems in the *Turnera ulmifolia* L. complex (Turneraceae). *Evolution*, **41**, 340–354.
- Barrett S.C.H., Shore J.S. (2008) New insights on heterostyly: comparative biology, ecology and genetics. In: Franklin-Tong V.E. (Ed), *Self-incompatibility in flowering plants – evolution, diversity, and mechanisms*. Springer, Berlin, Germany, pp 3–32.
- Barrett S.C.H., Brown A.D.H., Shore J.S. (1987) Disassortative mating in tristylous *Eichhornia paniculata* (Pontederiaceae). *Heredity*, **58**, 49–55.
- Bittencourt N.S.J., Gibbs P.E., Semir J. (2003) Histological study of post-pollination events in *Spathodea campanulata* Beauv. (Bignoniaceae), a species with late-acting self-incompatibility. *Annals of Botany*, **91**, 827–834.
- Brandham P.E. (1982) Inter-embryo competition in the progeny of autotriploid *Aloineae* (Liliaceae). *Genetica*, **59**, 29–42.
- Castro S., Loureiro J., Santos C., Ater M., Ayensa G., Navarro L. (2007) Distribution of flower morphs, ploidy level and sexual reproduction of the invasive weed *Oxalis pes-caprae* in the western area of the Mediterranean region. *Annals of Botany*, **99**, 507–517.
- Castro S., Ferrero V., Costa J., Sousa A.J., Navarro L., Loureiro J. (2013) Reproductive strategy of the invasive *Oxalis pes-caprae*: distribution patterns of flower morphs, ploidy levels and sexual reproduction. *Biological Invasions*, doi: 10.1007/s10530-013-0414-2.
- Charlesworth D. (1979) The evolution and breakdown of tristylly. *Evolution*, **33**, 486–498.
- Charpentier A. (2002) Consequences of clonal growth for plant mating. *Evolutionary Ecology*, **15**, 521–530.
- Colautti R.I., White N.A., Barrett S.C.H. (2010) Variation of self-incompatibility within invasive populations of purple loosestrife (*Lythrum salicaria* L.) from Eastern North America. *International Journal of Plant Science*, **171**, 158–166.
- Coutinho A.X.P. (1939). *Flora de Portugal*. Bertrand Ltd., Lisboa, Portugal.
- Dafni A., Pacini E., Nepi M. (2005) Pollen and stigma biology. In: Dafni A., Kevan P., Husband B. (Eds), *Practical pollination biology*. Enviroquest, Ontario, ON, Canada, pp 83–142.
- Doležel J., Göhde W. (1995) Sex determination in dioecious plants *Melandrium album* and *M. rubrum* using high-resolution flow cytometry. *Cytometry*, **19**, 103–106.
- Doležel J., Greilhuber J., Suda J. (2007) Estimation of nuclear DNA content in plants using flow cytometry. *Nature Protocols*, **2**, 2233–2244.
- Dulberger R. (1964) Floral dimorphism and self-incompatibility in *Narcissus tazetta* L. *Evolution*, **18**, 361–363.
- Dulberger R. (1992) Floral polymorphisms and their functional significance in the heterostylous syndrome. In: Barrett S.C.H. (Ed), *Evolution and function of heterostyly*. Springer, Berlin, Germany, pp 41–84.
- Galbraith D.W., Harkins K.R., Maddox J.M., Ayres N.M., Sharma D.P., Firoozabady E. (1993) Rapid flow cytometric analysis of the cell-cycle in intact plant-tissues. *Science*, **220**, 1049–1051.
- Ganders F.R. (1979) The biology of heterostyly. *New Zealand Journal of Botany*, **17**, 607–635.
- Godfrey R., Lepshi B., Mallinson D. (2004) Ecological filtering of exotic plants in an Australian sub-alpine environment. *Journal of Vegetation Science*, **15**, 227–236.
- Grossniklaus U., Spillane C., Page D.R., Köhler C. (2001) Genomic imprinting and seed development: endosperm formation with and without sex. *Current Opinion in Plant Biology*, **4**, 21–27.
- Handel S.N. (1985) The intrusion of clonal growth patterns on plant breeding systems. *American Naturalist*, **125**, 367–383.
- Harder L.D., Barrett S.C.H. (1996) Pollen dispersal and mating patterns in animal-pollinated plants. In: Lloyd D.G., Barrett S.C.H. (Eds), *Floral biology: studies on floral evolution in animal-pollinated plants*. Chapman & Hall, New York, NY, USA, pp 140–190.
- Holsinger K.E. (2000) Reproductive systems and evolution in vascular plants. *Proceedings of the National Academy of Sciences USA*, **97**, 7037–7042.
- Lewis D., Jones D.A. (1992) The genetics of heterostyly. In: Barrett S.C.H. (Ed), *Evolution and function of heterostyly*. Springer, Berlin, Germany, pp 129–150.
- Lipow S.R., Wyatt R. (2000) Single gene control of postzygotic self-incompatibility in poke milkweed, *Asclepias exaltata* L. *Genetics*, **154**, 893–907.
- Lloret F., Médail F., Brundu G., Camarda I., Moragues E., Rita J., Lambdon P., Hulme P.E. (2005) Species attributes and invasion success by alien plants on Mediterranean islands. *Journal of Ecology*, **93**, 512–520.
- Luo S., Zhang D., Renner S.S. (2006) *Oxalis debilis* in China: distribution of flower morphs, sterile pollen and polyploidy. *Annals of Botany*, **98**, 459–464.
- Mast A.R., Kelso S., Conti E. (2006) Are any primroses (*Primula*) primitively monomorphic? *New Phytologist*, **171**, 605–616.
- Navarro L., Ayensa G., Ferrero V., Sánchez J.M. (2012) The avoidance of self-interference in the endemic daffodil *Narcissus cyclamineus* (Amaryllidaceae). *Plant Ecology*, **213**, 1813–1822.
- O'Brien S.P., Calder D.M. (1989) The breeding biology of *Epacris impressa*. Is this species heterostylous?. *Australian Journal of Botany*, **37**, 43–54.
- Ornduff R. (1972) The breakdown of trimorphic incompatibility in *Oxalis* section Corniculatae. *Evolution*, **26**, 52–65.
- Ornduff R. (1987) Reproductive systems and chromosome races of *Oxalis pes-caprae* L. and their bearing on the genesis of a noxious weed. *Annals of the Missouri Botanical Garden*, **74**, 79–84.
- Otto F. (1992) Preparation and staining of cells for high-resolution DNA analysis. In: Radbruch A. (Ed), *Flow cytometry and cell sorting*. Springer, Berlin, Germany, pp 101–104.
- Porcher E., Lande R. (2005) The evolution of self-fertilization and inbreeding depression under pollen discounting and pollen limitation. *Journal of Evolutionary Biology*, **18**, 497–508.
- Pütz N. (1994) Vegetative spreading of *Oxalis pes-caprae* (Oxalidaceae). *Plant Systematics and Evolution*, **191**, 57–67.
- Pyšek P., Richardson D.M. (2007) Traits associated with invasiveness in alien plants: Where do we stand? In: Nentwig W. (Ed), *Biological invasions, ecological studies*. Springer, Berlin, Germany, pp 99–126.
- Ramsey J., Schemske D.W. (1998) Pathways, mechanisms, and rates of polyploid formation in flowering

## SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

**Appendix S1.** Results from controlled hand-pollination experiments

- plants. *Annual Review of Ecology and Systematics*, **29**, 467–501.
- Ray P.M., Chisaki D.H.F. (1957) Studies on *Amsinckia*. *American Journal of Botany*, **44**, 529–544.
- Richardson D.M., Allsopp N., D'Antonio C.M., Milton S.J., Rejmánek M. (2000) Plant invasions – the role of mutualisms. *Biological Reviews of the Cambridge Philosophical Society*, **75**, 65–93.
- Risso-Pascotto C., Pagliarini M.S., Do Valle C.B., Mendes-Bonato A.B. (2003) Chromosome number and microsporogenesis in a pentaploid accession of *Bra-chiaria brizantha* (Gramineae). *Plant Breeding*, **122**, 136–140.
- Sage T.L., Strumas F., Cole W.W., Barret S.C.H. (1999) Differential ovule development following self- and cross-pollination: the basis of self-sterility in *Narcis-sus triandrus* (Amaryllidaceae). *American Journal of Botany*, **86**, 855–870.
- Sakai S., Wright S.J. (2008) Reproductive ecology of 21 coexisting *Psychotria* species (Rubiaceae): when is heterostyly lost? *Biological Journal of the Linnean Society*, **93**, 125–134.
- Sánchez-Pedraja O. (2008) *Oxalis* L. In: Garmendia F.M., Navarro C. (Eds), *Flora Iberica*. Real Jardín Botánico, C. S. I.C., Madrid, Spain.
- Schoen D.T., Johnston M.O., L'Heureux A.-M., Mas-rsolais J.V. (1997) Evolutionary history of the mat-ing system in *Amsinckia* (Boraginaceae). *Evolution*, **51**, 1090–1099.
- Schönswetter P., Suda J., Popp M., Weiss-Sheeweiss H., Brochmann C. (2007) Circumpolar phylogeogra-phy of *Juncus biglumis* (Juncaceae) inferred from AFLP Fingerprints, cpDNA sequences, nuclear DNA content and chromosome number. *Molecular Phylogenetics and Evolution*, **42**, 92–103.
- Schou O., Philipp M. (1983) An unusual heteromor-phic incompatibility system. II. Pollen tube growth and seed sets following compatible and incompatible crossing within *Anchusa officinalis* L. (Boraginaceae). In: Mulcahy D.L., Ottaviano E. (Eds), *Pollen: biology and implications for plant breeding*. Elsevier, New York, NY, USA, pp 219–227.
- Stebbins G.L. (1957) Self fertilization and population variability in the higher plants. *American Naturalist*, **91**, 337–354.
- Symon D.E. (1960) The species of *Oxalis* established in South Australia. *Transactions of the Royal Society of South Australia*, **84**, 71–77.
- Tsai M.-Y., Chen S.-H., Kao W.-Y. (2010) Floral morphs, pollen viability, and ploidy level of *Oxalis corymbosa* DC. in Taiwan. *Botanical Studies*, **51**, 81–88.
- Vallejo-Marín M., Uyenoyama M.K. (2004) On the evolutionary costs of self-incompability: incomplete reproductive compensation due to pollen limitation. *Evolution*, **58**, 1924–1935.
- Vaughton G., Ramsey M., Johnson S.D. (2010) Polli-nation and late-acting self-incompatibility in *Cyrtanthus breviflorus* (Amaryllidaceae): implica-tions for seed production. *Annals of Botany*, **106**, 547–555.
- Vignoli L. (1937) Fenomeni riproduttivi di *Oxalis cer-nua* Thunb. *Lavori del Real Istituto Botanico di Palermo*, **8**, 5–30.
- Wang Y., Wang Q.-F., Guo Y.-H., Barrett S.C.H. (2005) Reproductive consequences of interactions between clonal growth and sexual reproduction in *Nymphoides peltata*: a distylous aquatic plant. *New Phytologist*, **165**, 329–336.
- Weller S.G. (1976) The genetic control of tristylly in *Oxalis* section *Ionoxalis*. *Heredity*, **37**, 387–393.
- Weller S.G. (1992) Evolutionary modifications of tristylous breeding systems. In: Barrett S.C.H. (Ed), *Evolution and function of heterostyly*. Springer, Ber-lin, Germany, pp 247–270.