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## ORIGINAL ARTICLE

# Pollen flow between flowers of the same morph in invasive populations of *Oxalis pes-caprae* L. in the western Mediterranean region

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**Abstract**
*Oxalis pes-caprae* is a tristylous South African geophyte that is invasive in regions with Mediterranean climate. Given the introduction of the short-styled morph only, vegetative reproduction was, until recently, the reproductive mode known for this invaded area. The detection of new floral morphs, fruit and seed production in natural populations of the invaded range, together with the reported weakening in the heteromorphic incompatibility system, raised the question on whether the short-styled flowers are able to exchange pollen among themselves, and if so, which mechanisms account for it. Thus, flower morphometric analysis and field experiments were conducted to assess pollinator visitation rates and pollen flow in three invasive populations. Flower morphological changes suggesting the evolution from tristyly towards semi-homostyly were observed. Moreover, *O. pes-caprae* flowers were visited by different functional groups of pollinators, mostly hymenopteran. Dye flow experiments revealed that the short-styled flowers were able to successfully exchange pollen among themselves in natural conditions. All of this, together with the weakening of the incompatibility system, constitutes further data that might help to explain the recently reported occurrence of sexual reproduction in this invaded range, which by itself bears important implications for the invasion process of *O. pes-caprae*.

**Keywords:** *Heterostyly, invasion, pollen flow, pollinators, sexual reproduction, short-styled morph, western Mediterranean basin*

## Introduction

Heterostyly is a floral polymorphism in which there are two (distyly) or three (tristyly) morphological mating types in a population, differing in the reciprocal positioning of anthers and stigmas. This polymorphism is usually accompanied by a heteromorphic incompatibility system that prevents selfing and fertilizations among individuals of the same floral morph (reviewed in Ganders 1979; Barrett et al. 2000). Consequently, heterostylous species depend on pollinators and compatible morphs in a population for successful sexual reproduction (cf. Baker 1955; Baker 1967; Barrett & Shore 2008).

*Oxalis pes-caprae* L. (Oxalidaceae) is a heterostylous South African geophyte that presents tristylous populations in its native range (Ornduff 1987) and has become a worldwide invasive species in regions with Mediterranean climate (Ornduff 1987; Vilà et al. 2006; Castro et al. 2007; Signorini et al. 2013). In most populations of the invaded range, individuals were forced to asexuality due to the absence of compatible mates after the introduction of the short-styled floral morph (S-morph), only (Michael 1964; Ornduff 1987). Similar to other exotic species (e.g. *Elodea canadiensis*, Bowmer et al. 1995; *Fallopia japonica*, Forman & Kesseli 2003), the ability to reproduce vegetatively by clonal propagation enabled *O. pes-caprae* plants to expand from their initial colony. Despite the drawbacks of this reproductive mode (Holsinger 2000), it represents an initial strong advantage that enables the persistence, growth and spread of exotic species in a new area (Richardson et al. 2000).

Interestingly, our latest studies on this species in the western Mediterranean region revealed the

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occurrence of sexual reproduction, not only in a few newly detected populations composed of different floral morphs, but also in the monomorphic ones composed of S-morph plants (Castro et al. 2007; Castro et al. 2013). The sexual capacity found in the mixed populations was completely expected because of the tristylous plants idiosyncrasy (Ganders 1979; Barrett & Shore 2008; J. Costa, personal observations). However, fruit production by the S-morph plants in the monomorphic populations raised interesting questions on the mechanisms by which these plants alone are able to sexually reproduce.

Similar to what has been found for other exotic species (Crawley 1989; Richardson et al. 2000; Ghazoul 2004; Traveset & Richardson 2006; Lopezaraiza-Mikel et al. 2007; Gibson et al. 2013), previous observations have also shown that *O. pes-caprae* easily integrated the mutualistic networks present in the new range (Ferrero et al. 2013; J. Costa personal observations), theoretically enabling pollen flow, even though the efficiency of pollinators was not evaluated. Also, it has been shown that a weakening in the strength of the heteromorphic incompatibility system in this invaded range (Castro et al. 2013; Costa et al. 2014) broadens the number of compatible mates in the monomorphic populations. Under this scenario, individuals with some levels of compatibility are in advantage in low-density conditions, possibly establishing and spreading more easily than strong self-incompatible ones (Baker 1955; Stebbins 1957; Baker 1967).

However, monomorphic populations of the S-morph of *O. pes-caprae* can also be interpreted as reverse herkogamous systems, i.e. hermaphrodite flowers displaying the female and male sexual organs spatially separated, and characterized by having the anthers above the stigma (Webb & Lloyd 1986). Herkogamy has been hypothesized as a mechanism to prevent sexual interference while promoting outcrossing (Webb & Lloyd 1986; Lloyd & Webb 1986). Still, flowering plants might benefit from the simultaneous presentation of anthers and stigmas at similar heights within a flower, as a mechanism of reproductive assurance through selfing (Lloyd & Schoen 1992). Reproductive biology studies in some heterostylous invasive species have revealed that the weakening or complete breakdown of the hetero-morphic incompatibility system is usually associated with morphological changes within the flower, i.e. the emergence of homostylous or semi-homostylous floral morphs (flowers composed of a long whorl of anthers and a short-level that coincides with the stigma in height; Barrett 1979; Barrett 1989) that would facilitate selfing and increase their weedy behaviour.

Considering all this, the questions addressed in this study were: (1) Is the S-morph able to disperse its pollen to the stigmas of other S-morph flowers? (2) What is the relationship between pollen flow and the observed morphology of the flower? (3) Can the stigmatic pollen deposition patterns be driven by the diversity in floral visitors? To answer these questions, the patterns of pollen flow among individuals were studied in three populations of the S-morph *O. pes-caprae* in the invaded range though morphometric analysis of the flowers, pollinator censuses and transference of fluorescent dye as pollen analogue. We predicted that the S-morph was able to exchange pollen among flowers of the same morph, which was mediated by its floral visitors and was facilitated by changes in the position of anthers and stigma within the flowers.

## Materials and methods

### Plant material and study area

*Oxalis pes-caprae* L. (Oxalidaceae) presents tristylous (long-, mid- and short-styled floral morphs; L-, M- and S-morphs, respectively; Ornduff 1987), actino-morphic yellow flowers arranged in terminal umbellate cymes (Sánchez-Pedraja 2008). In the invaded range of the western Mediterranean basin, it flowers from January to April (Castro et al. 2007) and its flowers are mainly visited by generalist insects, such as *Apis mellifera*, *Anthophora* sp. and *Bombus* sp. (Ferrero et al. 2013).

This study was carried out during the flowering season of 2012 in three natural populations composed of S-morph plants, in Portugal: Coimbra (COI; 40°12′21″N, 8°25′26″W), Alto da Praia Grande (APG; 38°47′52″N, 9°28′35″W) and Cortegaça (CORT; 40°56′25″N, 8°39′19″W).

### Floral characterization

In each population, one flower per plant from 30 distinct individuals was collected and harvested in 70% ethanol for morphological measurements. In the laboratory, the corolla was detached and photographed together with the reproductive organs. The following parameters were measured using the image processing program ImageJ (Abràmoff et al. 2004): (1) corolla length, (2) style length (from the corolla insertion up to the stigma), and (3) stamen height (from the corolla insertion up to the midpoint of the anther for each of the two anther whorls). The separation between anthers (at long- and mid-levels) and stigmas (at short-level) was quantified by dividing the height of the long- and mid-levels by the height of the short-level (ratio of average heights; Lloyd et al. 1990). A second measure of herkogamy was also calculated by taking the difference between the stigma and the mid-level anthers (the closest

male structure to the stigma) heights (herkogamy). Descriptive statistics (mean and standard deviation of the mean) were calculated to characterize populations for corolla, anthers and stigma heights, and herkogamy measurements. Population variability in herkogamy was investigated by calculating the coefficient of variation (CV). Differences in corolla size and herkogamy among populations were assessed by means of one-way ANOVA followed by a Tukey's test at a significance value of 0.05.

### Pollinator visitation rates

Direct field observations were performed during the flowering peak of 2012 to (1) investigate the spectrum of flower visitors foraging *O. pes-caprae* flowers and to (2) assess the visitation rates of each floral visitor to the S-morph flowers. For this, six plots of approximately $2\,m^2$ were arbitrarily selected in each population, and observation sessions of 15 min. per plot were conducted along the day (from 11:30 to 16:15 h, GMT, i.e. the period of corolla opening) until a minimum of 18 h of observations were achieved per population. The identity of each floral visitor and the number of flowers visited were recorded. At the end, one specimen of each insect *taxa* was collected for further identification.

As floral visitors are recognized to take part in the floral phenotypic process (e.g. Johnson 1996; Fenster et al. 2004; Morgan 2006), insects visiting *O. pes-caprae* flowers were classified into three functional groups according to their foraging behaviour: (1) Hymenoptera, insects that entered the flower, in most cases touching the low-level stigma when foraging for nectar and/or pollen; (2) Coleoptera and Diptera, insects that acted mainly as pollen collectors, staying at the long-level anthers for long periods of time and only entering the flower occasionally and (3) Lepidoptera, nectar collectors that coiled the proboscis after nectar feeding.

The total visitation rate for each population was calculated as the total number of flowers visited weighted by the total number of flowers monitored per observation period. The visitation rate per functional group within population was calculated as the total number of flowers visited divided by the total number of flowers monitored per census. For each population, descriptive statistics (mean and standard error of the mean) were calculated for the total visitation rate and for each functional group. Differences in visitation rates among populations and differences in functional groups within and among populations were assessed using Kruskal–Wallis one-way ANOVA on ranks, followed by a multiple comparison test at a significance value of 0.05.

### Pollen flow

Pollen tracking within populations was assessed using fluorescent powder dye as pollen analogue (Waser & Price 1982; Campbell & Waser 1989). Thus, in each plot monitored, five flowers were randomly chosen and fluorescent powder dye was applied to their anthers. After three days, up to 150 inflorescences were collected across the populations and the open flowers were observed with a stereo binocular microscope under UV light. The presence/absence and place of dye deposition were recorded for all the flowers observed.

To estimate natural pollen flow and stigmatic pollen deposition within each population, we calculated the percentage of flowers with fluorescent dye from the total number of flowers observed and the percentage of flowers with fluorescence dye in the stigma from the total number of flowers with dye. Differences in natural dye flow and dye deposition in the stigma among populations were analysed using generalized linear models with a binomial distribution and a logit link function.

All the analyses were performed in R 3.0.1 using "car", "multcomp", "pgirmess" and "stats" packages (R Development Core Team 2013).

## Results

### Floral characterization

Floral measurements revealed a close positioning of the stigma and the mid-level anthers across all the studied populations (Figure 1); this result was also confirmed by the calculation of the ratio of average heights (Table I). Flowers from the CORT population presented, on average, the smallest separation between the stigma and the mid-level anthers (1:1.23:1.75); COI population presented intermediate values (1:1.25:1.86), whereas APG presented the biggest separation (1:1.28:1.82) (Figure 1(d)). Also, flowers from APG were the ones showing a higher variation of both mid-level anther and stigma heights when compared with the other two populations (Figure 1(d)). Despite no statistically significant differences were obtained for the mean values of herkogamy among populations ($F_2 = 2.917$, $p = 0.059$), higher values of variation in this measurement were observed in the CORT population than in COI and APG populations (mean ± standard deviation, CV; COI: 1.23 ± 0.30, 24.0%; APG: 1.35 ± 0.32, 24.0%; CORT: 1.15 ± 0.32, 28.2%). Surprisingly, differences among populations were found for corolla size ($F_2 = 14.542$, $p < 0.001$), with the APG population presenting the smallest corolla sizes (Figure 1(d)).
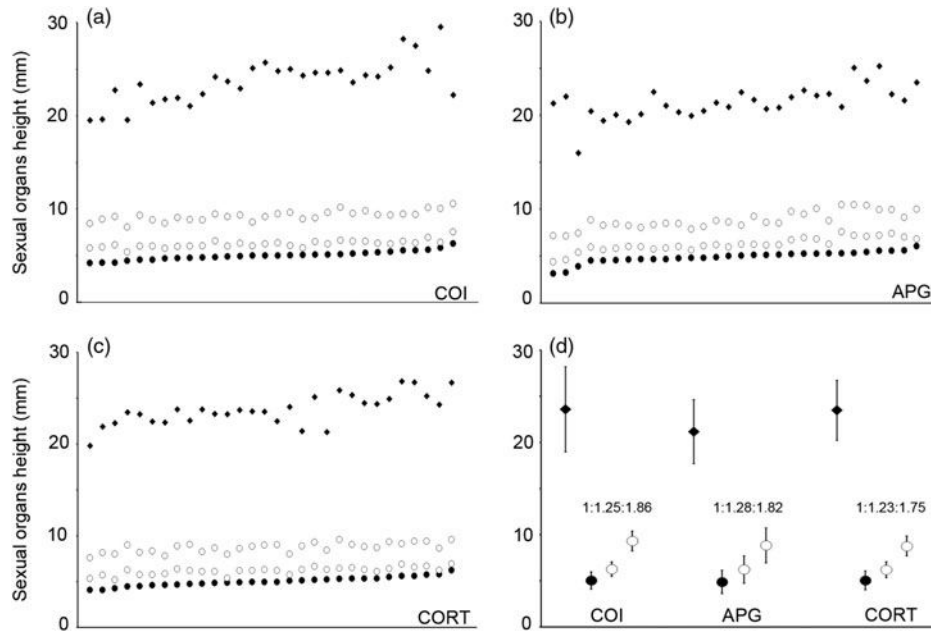
Figure 1. Morphological measurements of the flowers for the studied populations. (a–c) Sexual whorls morphometric measurements for the studied populations; (d) Mean and two times the standard deviation of the mean for morphological measurements of corolla, stigma and stamen levels in the studied populations: COI, APG, CORT. The ratio of average heights values for each population is also provided. Stigma: closed circles, stamen levels: open circles, corolla: closed diamonds.

Table I. Comparison of the position of anthers and stigmas in *Oxalis pes-caprae* and other recognized tristylous Oxalis species.

| Species | Ratio of average heights short: mid:long | Reference |
|---|---|---|
| *Oxalis pes-caprae* | 1:1.26:1.81 | This study (invaded range) |
| *Oxalis pes-caprae* | 1:1.50:2.30 | Turketti (2010) (native range) |
| *Oxalis alpina* | 1:1.58:2.08 | Weller (1976)[a] |
| *Oxalis ciliaris* | 1:1.50:2.10 | Turketti (2010) |
| *Oxalis hirta* | 1:1.50:2.10 | Turketti (2010) |
| *Oxalis squamata* | 1:1.53:2.02 | Marco and Arroyo (1998) |
| *Oxalis suksdorfii* | 1:1.42:2.00 | Ornduff (1964)[b] |
| *Oxalis tuberosa* | 1:1.33:1.86 | Gibbs (1976)[b] |

'a' and 'b', calculations available from Lloyd et al. (1990) and Marco and Arroyo (1998), respectively.

*Pollinator visitation rates*

*Oxalis pes-caprae* flowers were mostly visited by generalist insects, including *Anthophora* sp., *Apis mellifera*, *Bombus terrestris*, *B. pascuorum* and *Psythirus* sp. (Supplemental File I). Visitation rates for each population and functional group within population are given in Table II and Figure 2. Statistically significant differences were observed in total visitation rates among populations ($H_2 = 90.967$, $p < 0.001$), with the APG site presenting a significantly lower total visitation rate ($p < 0.05$; Table II; Figure 2) than COI and CORT populations. Despite not significantly different from the CORT popu-

lation, flowers from COI were the ones registering more insect visits.

The three functional groups recognized to visit *O. pes-caprae* flowers assumed unequal weights in the total visitation rates within populations. Irrespective of the population, hymenopteran were the most frequently observed floral visitors ($p < 0.05$; Table II), accounting for 78.2% and 96.8% of the total visits in APG and COI populations, respectively. In COI and CORT populations, Coleoptera and Diptera were the second most important functional group, whereas in the APG population, Lepidoptera assumed that role, with Coleoptera and Diptera presenting very low values of visitation rate (only 4.4% of the total visits). Indeed, while in COI and CORT populations, Lepidoptera accounted for less than 1.0% of the floral visits, in the APG population, insects from this functional group were responsible for 14.0% of the visits. This difference was not significant, most probably because of the large standard deviation values observed in visitation rates.

*Pollen flow*

Statistically significant differences among populations were detected in dye flow ($\chi_2^2 = 17.191$; $p < 0.001$; Figure 3(a)), with the COI population presenting the highest number of flowers with fluorescence dye ($p < 0.05$; Table II). Despite not significantly different from the values observed in the CORT population, the APG population was the one

Table II. Visitation rates for each population studied (total and per functional group) and percentage of stigmas with fluorescence dye.

| | | | Visitation rates | | | | | | | |
| | | | Functional groups | | | | | | | |
| Population | $n_1$ | Total | 1 | 2 | 3 | Statistical test (within populations) | $n_2$ | Flowers with dye | $n_3$ | Dye in the stigma |
|---|---|---|---|---|---|---|---|---|---|---|
| Coimbra | 83 | $0.298 \pm 0.037^a$ | $0.288 \pm 0.035^{a*}$ | $0.007 \pm 0.002^{□}$ | $0.002 \pm 0.001^{□□}$ | $H_2 = 130.586$, $p < 0.001$ | 250 | $13.00^a$ | 32 | $18.75^a$ |
| Alto da Praia Grande | 78 | $0.042 \pm 0.017^b$ | $0.033 \pm 0.016^{b*}$ | $0.002 \pm 0.001^{a*}$ | $0.006 \pm 0.005^{a*}$ | $H_2 = 7.730$, $p = 0.021$ | 191 | $3.00^b$ | 5 | $60.00^{ab}$ |
| Cortegaça | 84 | $0.213 \pm 0.026^a$ | $0.186 \pm 0.024^{a*}$ | $0.020 \pm 0.004^{a□}$ | $0.002 \pm 0.001^{a**}$ | $H_2 = 107.692$, $p < 0.001$ | 496 | $7.00^b$ | 35 | $91.43^b$ |
| Statistical test (among populations) | | $H_2 = 90.967$, $p < 0.001$ | $H_2 = 84.010$, $p < 0.001$ | $H_2 = 20.637$, $p < 0.001$ | $H_2 = 1.683$, $p = 0.431$ | | | $\chi_2^2 = 17.191$, $p < 0.001$ | | $\chi_2^2 = 40.329$, $p < 0.001$ |

Notes: Functional groups: 1, 2 and 3 for Hymenoptera, pollen collectors (Coleoptera and Diptera) and Lepidoptera, respectively. The total number of flowers observed ($n_2$) and the number of flowers with dye are also provided ($n_3$). Values are given as mean and standard error of the mean, except for "flowers with dye" and "dye in the stigma" that are given as the percentage of the number of flowers from the total observed that received dye and with dye in the stigma, respectively. Different letters and symbols represent statistically significant differences among and within populations, respectively at $p < 0.05$.

with the lowest values of dye flow (Table II, Figure 3 (a)).

Similarly, statistically significant differences were found among populations in stigmatic pollen transfer ($\chi_2^2 = 40.329$; $p < 0.001$; Figure 3(b)). When considering only the flowers with dye, the COI population presented a significantly lower percentage of flowers with dye in the stigma (18.75%), whereas the opposite was observed in the CORT population (91.43%; $p < 0.05$; Table II; Figure 3(b)).

## Discussion

This study showed that the S-morph of *Oxalis pes-caprae* is able to disperse its pollen to the stigmas of other S-morph flowers. Most probably, this was the combined result of the successful pollination interactions already established in the invaded range with native fauna (results herein and Ferrero et al. 2013), and of the changes observed in the morphology of the flowers, namely the reduction in the separation between female and male organs. These observations, together with previous findings [i.e. heteromorphic incompatibility system weakening (Costa et al. 2014) and fruit and seed production (Castro et al. 2013)], are expected to comprise significant impacts in the demographic and genetic traits of *O. pes-caprae* populations and, consequently, on its invasion potential. Moreover, they point out to forthcoming evolutionary pathways of this floral
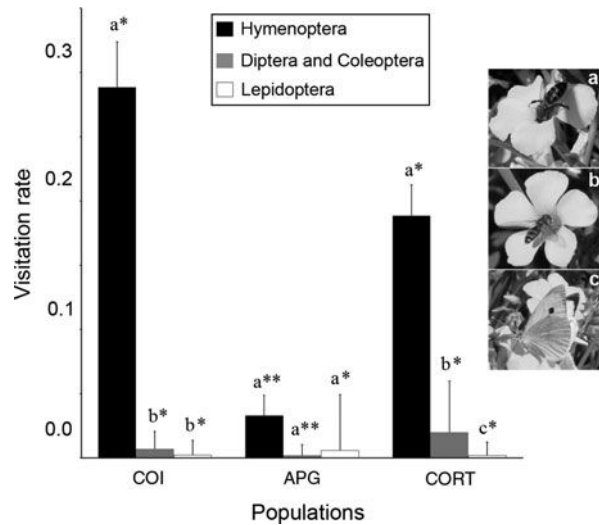


Figure 2. Visitation rates per functional group for the studied populations. Functional groups: black, Hymenoptera; light grey, Coleoptera and Diptera; white, Lepidoptera. Populations: COI, Coimbra; APG, Alto da Praia Grande, CORT, Cortegaça. Different letters and symbols represent statistically significant differences in visitation rates (at $p < 0.05$) within populations among functional groups, and among functional groups and populations, respectively. (a–c) Representantives of each functional group described: (a) *Apis mellifera*; (b) *Episyrphus balteatus*; (c) *Pieris brassicae*.
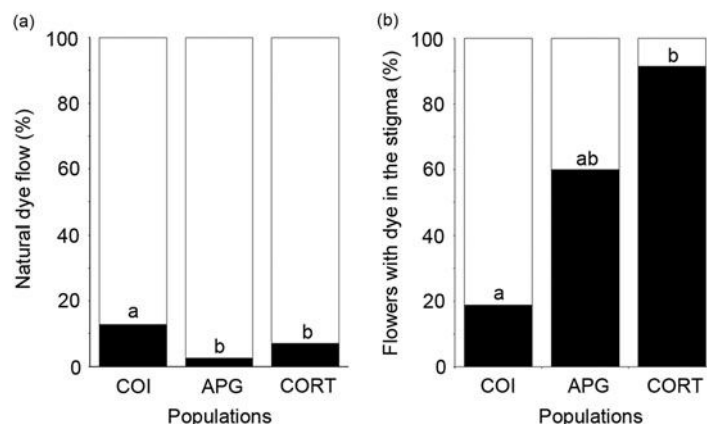
Figure 3. Pollen flow, given as fluorescence dye flow, within the studied populations: (a) Natural dye flow given as percentage of flowers with fluorescece dye (black bars); (b) Flowers with dye in the stigma given as percentage from the total number of flowers with dye (black bars). Populations: COI, Coimbra; APG, Alto da Praia Grande; CORT, Cortegaça. Different letters represent statistically significant differences at $p < 0.05$.

polymorphism in the invaded area of the western Mediterranean basin.

To start, morphometric analysis of S-morph flowers of *O. pes-caprae* revealed a lower ratio of average heights than it is commonly recognized not only for tristylous species (1.0:1.5:2.0; Lloyd et al. 1990) but also for populations of this species in the native range (1.0:1.5:2.3; Turketti 2010). This points out for a closer positioning between the stigma and the mid-level anthers in flowers of the S-morph in invasive populations than in native ones. This close positioning might promote illegitimate pollen transfer and explain the high rates of dye transference between flowers of the same morph. Such differences in organ position might also be indicative of ongoing changes in the reproductive system towards semi-homostyly (see Lewis 1954; Barrett 1989). Indeed, it has been previously reported that the evolutionary breakdown of tristyly involves a shift from outcrossing to selfing that is usually accompanied by morphological changes in the height of the reproductive organs (Ganders 1979; Barrett 1985; Barrett 1988; Lewis & Jones 1992), through recombination phenomena or the accumulation of modifier genes in a medium–long term (Ganders 1979). Semi-homostyly has already been described for several heterostylous species (reviewed in Turketti 2010) and seems to be advantageous under low-density conditions or limited pollination services (see Baker 1955; Baker 1967). Despite the genetic basis of semi-homostyly in tristylous species being rather complex overall, *O. pes-caprae* seems to be following a pathway similar to that observed in other heterostylous species: first, a weakening in the incompatibility system has already been described in this invaded range (Castro et al. 2013; Costa et al. 2014) and second, the ratio of average heights is lower than the optimally expected for tristylous

species, with a few flowers already presenting the short-level of anthers coinciding with the stigma in height (results herein). Facing these observations, the actual role of semi-homostyly in this system requires further consideration in the future.

Despite the differences between fluorescence dyes and pollen grains' properties (Thomson et al. 1986), fluorescence dyes proved to be a useful tool to assess pollen flow under natural conditions (e.g. Waser & Price 1982; Adler & Irwin 2006; Van Rossum et al. 2011). Overall and as expected, the fluorescence dye flow mediated by floral visitors followed the same pattern of total visitation rates. Visitation rates differed among populations and were most probably the combination of biotic (e.g. co-flowering rewarding species, Horvitz & Schemske 1988; Stone et al. 2003) and abiotic factors (e.g. solar radiation, Herrera 1995; temperature, Murcia 1990; reviewed in Kearns & Inouye 1993), both recognized to influence pollinators' activity. Indeed, in the APG population, *O. pes-caprae* was flowering simultaneously with *Acacia longifolia*, known to produce high amounts of floral rewards (Stone et al. 2003), and thus, it could be competing with *O. pes-caprae* for pollinators, significantly reducing the visitation rates to our study species (J. Costa, personal observations). Moreover, CORT and APG populations were located near the coast where strong abiotic factors, such as strong winds and high temperature variation, might have affected pollinators' activity and assemblage.

Intramorph pollen transfer has been reported in studies of pollen flow in heterostylous systems (e.g. Mulcahy & Carporello 1970; Ornduff 1970; Olesen 1979; Ornduff 1979; Price & Barrett 1984). Despite reciprocal herkogamy being considered a mechanism that promotes outcrossing, sexual interference through intramorph pollen transfer has been com-

monly recognized as a side-effect that cannot be totally avoided (Ganders 1979; Barrett & Harder 2006). Thus, some level of intramorph pollen transfer was expected in *O. pes-caprae* natural populations composed of the S-morph. Two distinct factors may account for this: (1) floral morphology that constraints pollinators' entry and exit patterns (reviewed in Stone & Thomson 1994) and (2) differences in pollinators' array and foraging behaviour (e.g. Wolfe & Barrett 1987; Goulson & Wright 1997; Ferrero et al. 2011). In APG and CORT populations, fluorescence dye transfer to the stigmas was surprisingly high with 60% and 91% of the flowers with dye presenting stigmatic dye deposition, respectively. This observation points out for a considerably high precision of pollinators in intramorph stigmatic pollen transfer, reaching percentages that are theoretically higher than expected for tristylous species. Contrarily, the COI population registered only 19% of stigmatic dye deposition, which is considerably lower than that observed in the remaining populations. These differences could be related to differences in pollinator's foraging behaviour.

Indeed, *O. pes-caprae* flowers display open corollas with readily accessible rewards and were visited by several generalist insects from three different functional groups. These results are in accordance with those obtained by Ferrero et al. (2013) in another invasive population of this species. Members of the order Hymenoptera were the most important floral visitors of *O. pes-caprae* flowers across all the populations; still differences in insect *taxa* and differences in their efficiency in pollen transfer were observed. These differences might explain the patterns of stigmatic dye deposition observed among populations. In the COI population, i.e. the site with the lowest intramorph dye transfer regardless of the high visitation rates, *Apis mellifera* and *Anthophora* sp. were the main floral visitors. While *Anthophora* sp. was more ascertain, rapidly and systematically visiting the flowers while foraging for nectar (e.g. Kadmon 1992; Ferrero et al. 2011; Nocentini et al. 2012; J. Costa, personal observations), *A. mellifera* predominantly stayed for longer periods at the long- and, eventually, mid-level anthers while foraging for pollen, rarely entering completely the corolla tube (results herein; Wolfe & Barrett 1987). From these observations and considering previous studies (Barrett & Wolfe 1986; Harder & Barrett 1993), we suggest that *A. mellifera* was responsible for a faster rate of pollen loss in the long-level anthers than in the mid-level ones, decreasing the amount of pollen available and the levels of pollen transfer when *Anthophora* sp. visited the flowers. In addition, grooming and pollen packaging activities of the honeybees may have contributed to the redistribution of pollen along the whole insect body,

once again decreasing the amount of pollen available for pollination and, most importantly, the efficiency in stigmatic pollen deposition (Thomson 1986; Rademaker et al. 1997; Holmquist et al. 2012).

By contrast, high efficiency in stigmatic dye deposition was found in APG and CORT populations. These results were particularly surprising for the APG population given that it presented the highest value of herkogamy and the lowest visitation rates from all populations. In this site, Lepidoptera were important floral visitors. Despite butterflies being frequently described as low efficiency pollinators (e.g. Herrera 1987; Fishbein & Venable 1996), some studies revealed high seed production after a single visit of a butterfly (e.g. Jennersten & Nilsson 1993; Kandori 2002; Bloch et al. 2006; Sahli & Conner 2007), which points out their high efficiency as pollinators. Moreover, Ferrero et al. (2011) observed that they were good pollinators for *Lithodora fruticosa* and *Glandora* spp., due to pollen deposition only on the proboscis without pollen losses in body regions that do not contact the stigmas. *Bombus terrestris* was also a main floral visitor in the APG population; this insect constantly foraged for pollen and nectar and approached the flowers frontally (J. Costa, personal observations). Considering the relative big size of *O. pes-caprae* flowers (and its anthers and stigma separation) and the large size of *B. terrestris*, pollen pick-up and deposition all over the insect's body was expected and may have contributed to a higher probability of stigmatic dye deposition. In the CORT population, *Psithyrus* sp. accounted for the majority of flower visits. Pollinators from this genus presented a bumbling behaviour that resulted in pollen distribution all over the insect's body (J Costa, personal observations). This behaviour could have a similar effect as that described above for *B. terrestris*, and together with the close positioning of the stigma and mid-level anthers observed in this population might contribute to the high rates of stigmatic dye deposition. Thus, despite members of all the functional groups considered being present in all the studied populations, the behaviour of the predominant pollinators could have been the main explanatory factor for the differences observed in stigmatic dye transference observed among populations. However, future studies investigating pollinators' efficiency in pollen transfer are needed to completely rule out their role in the patterns of pollen flow observed.

Our results did not allow to distinguish whether the fruits already found in populations of the invaded range are a result of self- or intramorph pollinations, but confirm the ability of *O. pes-caprae* individuals to reproduce sexually in populations bearing flowers with the same morphology. These results might have important consequences for both the demographic

and genetic aspects of populations of this species in the invaded range. Future studies, such as a large scale assessment of flower morphology in this region, as well as, manipulative experiments focused on assessing pollinator efficiency in pollen pick-up and deposition, considering the variation in stigma–anther separation, are being developed and will certainly help to clarify the mechanisms underlying our conclusions.

## Supplemental data

## Funding

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