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RESEARCH ARTICLE

The evolutionary potential of invasive *Carpobrotus* (Aizoaceae) taxa: are pollen-mediated gene flow potential and hybrid vigor levels connected?

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Abstract Few studies have addressed the importance of native pollinators in shaping the breeding systems and evolutionary potential of invasive plants. We examined the pollination and gamete production of *Carpobrotus affine acinaciformis* (L.) L. Bol. and *C. edulis* (L.) N. E. Br. invading the coasts of southeast France (Provence), and found preliminary evidence that the pollen-mediated gene flow potential (PMGFP) within the four studied populations is positively correlated with their Relative Performance in Hybridization indices (RP_H), suggesting a link between pollinator services, gene flow, and *Carpobrotus* hybridization. Flower density (FD) may be a driver behind pollinator abundance and visitation patterns since it shows a significant, positive relationship with relative pollinator abundance (FR) and a significant negative relationship with per flower visit frequencies (FR/F). In the populations with the highest RP_H indices, and therefore high hybrid vigor, both taxa produce similar quantities of ovules and pollen per flower, whose ratios further indicate (facultative) xenogamy. Pollen diameter distributions include ~25% micro-pollen, and overall viability is less than one half. Viable pollen profiles consist of 3.5% micro, 85.5% normal and 10.9% macro pollen (considered as diplogametes) on average per flower for *C. affine acinaciformis*, and 0.7% micro, 73.0% normal and 26.2% macro pollen for *C. edulis.* Given the co-occurrence of (1) (facultative) xenogamic breeding strategies, (2) a significant, positive relationship between RP_H and PMGFP, and (3) frequent pollen abnormalities, it is probable that hybrid dysgenesis mediated by the local pollinators occurs in these populations. Furthermore, the unusually high frequency of potentially viable, diploid macro-pollen underlines the evolutionary/polyploid potential of these invasive, introgressed populations. Native pollinator interactions may greatly affect the taxonomic status and evolutionary potential of invasive plant complexes.

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Introduction

Pollinator interactions can greatly affect the mating systems and evolutionary potential of plant populations through gene flow via pollination, which in turn can affect such important parameters as genetic diversity, population subdivision and levels of inbreeding depression (Bierzychudek 1981, 1990; Charlesworth 1989, 1992; Charlesworth et al. 1990; Barrett 1996). In the context of invasive plant taxa, considerable pollinator limitation has been found in certain severe invasions (Parker 1997; Suzuki 2000; Parker et al. 2002; Parker and Haubensak 2002; Suehs et al. 2005), indicating that the pollinator communities present at invasion sites may affect the invasion process. Other recent studies have demonstrated that outcrossing and gene flow may be frequent events in invasive plant populations, and to such as extent that inter-specific hybridization may be common among sympatric insect-pollinated congenerics (Gallagher et al. 1997; Milne and Abbott 2000; Pappert et al. 2000; Hurka et al. 2003; Maron et al. 2004; Suehs et al. 2004a, b). Thus, it is becoming increasingly documented that pollen-mediated gene flow is an important process for many invasive taxa.

Pollen-mediated gene flow is also affected by a plant's capacity to produce sufficiently large numbers of pollen to assure fertilization of ovules in spite of losses due to the pollen-consuming activities of pollinators. The resulting ratio of pollen to ovules has proven to be an excellent measure of the reliance of plants on pollinators (Cruden 1977). In addition, pollen quality may have strong effects on plant reproduction, and hybrids are known for low gamete viability and frequent meiotic abnormalities (Rieseberg and Carney 1998; Fontdevila 2004). However this situation, known as hybrid dysgenesis (Fontdevila 2004), is often associated with high evolutionary potential, unreduced gamete production and the rise of polyploid races (Abbott 1992; Bretagnolle and Thompson 1995; Abbott et al. 2003). Given that the evolutionary potential of invasive plant populations via gene flow, hybridization and polyploidization is becoming increasingly stressed in the scientific community (Abbott 1992; Ellstrand and Schierenbeck 2000; Milne and Abbott 2000; Vilà et al. 2000; García-Ramos and Rodríguez 2002; Lee 2002; Hurka et al. 2003; Maron et al. 2004), it seems important to address the role of pollen-mediated gene flow in invasive plant populations, especially those that frequently hybridize or undergo introgression.

Within this context, we call attention to the clonal, invasive, South African *Carpobrotus affine acinaciformis* (L.) L. Bol. and *C. edulis (L.)* N. E. Br (Aizoaceae) populations along the coastline of Provence, southeast France (Suehs et al. 2004a, 2004b). Previous genetic work has determined that *C. affine acinaciformis* is an introgressed derivative of *C. acinaciformis* and *C. edulis*. Correspondingly, the reproductive system of *C. affine acinaciformis* is typified by fertility limitations (Fontdevila 2004) characteristic of hybrids/introgressed taxa: partial self-fertilility, inbreeding depression, and a maximization of seed production via hybridization in controlled pollination experiments with *C. edulis* (Suehs et al. 2004a, 2004b, 2005). In contrast, *C. edulis* is fully self-fertile, has no inbreeding depression, and does not maximize seed production via hybridization with *C. affine acinaciformis*.

Additional factors also indicate that pollen-mediated gene flow could be especially influential *vis-à-*vis the evolutionary future of these populations, and has continuously occurred *in-situ*. First, the seed production of both taxa has been shown to be pollinator limited (Suehs et al. 2005). Second, these populations are characterized by high genetic diversity, and either Hardy–Weinberg equilibrium or an excess of heterozygotes, indicating extensive gene flow (Suehs et al. 2004a, 2004b). Third, because *C. affine acinaciformis* largely maximizes its seed production via hybridization in controlled pollination experiments, and free pollination experiments largely outperform intra-taxon crosses, hybridization is suspected to occur frequently in the field (Suehs et al. 2005). Furthermore, hybridization experiments detected a total absence of reproductive barriers between the two taxa (as is not unusual for recently derived taxa, Klak et al. 2003), and two of these populations have significant hybrid vigor levels (as determined by Relative Performance in Hybridization indices; RP_H), indicating a history of frequent hybridization (Suehs et al. 2005). Interestingly, RP_H indices for these two taxa are not taxon-dependent, but site-dependent despite remarkable environmental similarity between sites (Suehs et al. 2005).

In order to investigate the role of varying pollinator activity and pollen quality in the genetic and hybrid vigor level patterns of *Carpobrotus*, we examined flower densities, pollinator visit rates, pollinator quality, and the Pollen-Mediated Gene Flow Potential (PMGFP) within four *Carpobrotus* populations on the southeast coast of France, as well as gamete production, pollen to ovule ratios, and pollen quality in populations known to have especially high hybrid vigor levels. Specifically, we ask the following questions: (1) Do factors related to and/or describing pollinator service demonstrate taxon or site-specific patterns? (2) Is there evidence that the PMGFP of *Carpobrotus* populations, as determined by within-population flowering and pollinator activity, is correlated with known hybrid vigor levels indicative of inter-population gene flow? (3) Do pollen diameter distributions and viabilities indicate meiotic perturbation in populations with high hybrid vigor levels and/or differences between the two *Carpobrotus* taxa? Using this information, we discuss the capacity of native pollinators to rapidly affect invasive *Carpobrotus* populations via hybridization and the resulting hybrid dysgenesis.

Methods

Study species, sites, and hybrid vigor levels

The diploid ($2x$=18) *Carpobrotus* (Aizoaceae, subfamily Ruschioideae) taxa studied are robust, mat-forming South African succulents. They have finger-thick leaves, and solitary, actinomorphic flowers 100–120 mm in diameter (Wisura and Glen 1993). The flowers of *C. edulis* (L.) N. E. Br. are yellow in color, while those of *C. affine acinaciformis* (L.) L. Bol. are typically magenta, but can also be yellow depending on their degree of hybridization/introgression with *C. edulis* (C. Suehs, personal observation). Both taxa possess many (hundreds) petals and stamens (Blake 1969), and 10–16 carpels and pistils (Suehs et al. 2004a, 2004b). To our knowledge, pollen sizes have never been mentioned for this genus, but are reported to reach maximum frequencies at around 30 μm in diameter in the Aizoaceae (Hartmann 1993). An examination of herbarium samples of one individual each of purportedly pure *C. acinaciformis* and *C. edulis* gave unimodal, normal pollen distributions with average diameters of 29.2±1.4 μM and 27.9±1.8 μM (mean±standard deviation; K. Diadema, unpublished data). Pollen viability via staining has been reported to be very high (80–100%) in Australian *Carpobrotus* taxa, and 60–70% in purported Australian *C. edulis* hybrids (Blake 1969). Hybridization is described as rare among Australian taxa, but hybrids between *C. edulis* and *C. virescens* have been reported there (Blake 1969). Several hybrids are mentioned in South Africa (Wisura and Glen 1993): *C. acinaciformis* × *C. edulis*, *C. edulis* × *C. mellei*, *C. edulis* × *C. quadrifidus*. Hybridization between *C. edulis* and *C. chilensis* represents an invasion problem in California (Albert et al. 1997; Gallagher et al. 1997; Vilà and D'antonio 1998a, 1998b). In France, *C. (affine) acinaciformis* and *C. edulis* were introduced in the early 1800's at the Marseille botanical garden (Gouffé De La Cour 1813), and were subsequently mentioned as naturalized on the Hyères coastline and archipelago in the early 1900's (Albert and Jahandiez 1908). Though tetraploid formation is a common occurrence among the Ruschioideae (Hartmann 1993), no polyploids have been reported in the genus *Carpobrotus.* Diads and triads, with diploid pollen formation, have been reported for *C. affine acinaciformis* and *C. edulis* (Diadema, 2002), and suggest a newly arisen polyploid potential within the studied range of invasive *Carpobrotus.*

The four *Carpobrotus* populations studied here were located in the Hyères archipelago of southeast France on the island of Bagaud (off-limits reserve in the National Park of Port Cros) and the adjacent mainland near Brégançon (Fig. 1). Sites Bagaud 1 and Bagaud 2 are separated by approximately 300 m, and consist of *C. affine acinaciformis* and *C. edulis*, respectively. Sites Brégançon 1 and Brégançon 2 are separated by about 200 m, and also consist of *C. affine acinaciformis* and *C. edulis* (Table 2). Past studies have used controlled pollination experiments between *C. affine acinaciformis* and *C. edulis* to quantify the relative performances of issue from manual intra-specific outcrossing experiments and manual inter-specific hybridization experiments in all four study populations based on seed production and progeny quality (Suehs et al. 2005). These results are summarized here using the Relative Performance in Hybridization index (RP_H, Suehs et al. 2005), which is inspired from similar indices in Agren and Schemske (1993) where progeny performance is

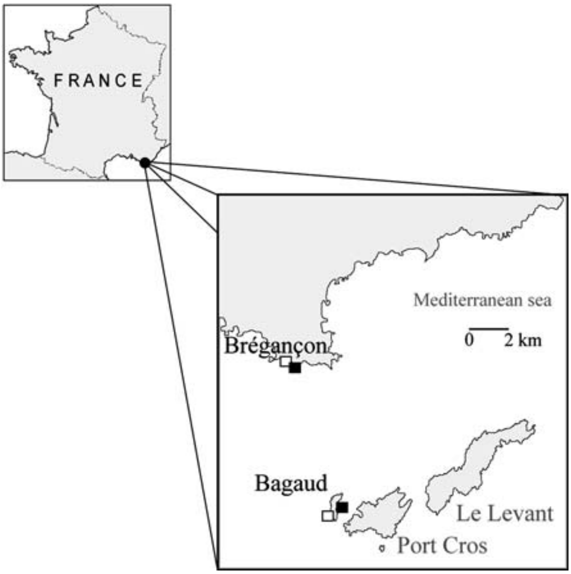

Fig. 1 Map demonstrating the locations of the four *Carpobrotus* populations studied: ☐=*C. affine acinaciformis* (sites Bagaud 1 and Brégançon 1); ■=*C. edulis* (sites Bagaud 2 and Brégançon 2)

Table 1 An interpretation guide to RP_H, the relative performance index in hybridization (Suehs et al. 2005)

Index value	Corresponding relative performances	Interpretation
$RP_H=-1$	0 performance in inter-specific hybridization versus an existing performance in intra-specific outcrossing	Complete reproductive barrier between studied taxa
$-1<RP_H<0$	Higher performance in intra-specific outcrossing compared to inter-specific hybridization	Partial reproductive barrier between studied taxa; hybridization depression
$RP_H=0$	Equal performances in intra-specific outcrossing and inter-specific hybridization	Absence of reproductive barrier between studied taxa
$0<RP_H<1$	Higher performance in inter-specific hybridization compared to intra-specific outcrossing	Absence of reproductive barrier between studied taxa with hybrid vigor; hybrid vigor level increases with index
$RP_H=1$	0 performance in manual outcrossing versus an existing performance in hybridization	Impossible natural situation

multiplicative across life history stages. For example, the values reported in this paper are based on the seed count × seed mass (mg) × % seed viability × cotyledon width (mm) × cotyledon length × stem length (mm) of progeny from controlled pollinations (Suehs et al. 2005). $RP_H=(W_h-W_o)/W_{max}$, where W_o is the performance in intra-specific manual outcrossing, W_h is the performance in inter-specific manual hybridization, and W_{max} the greater of W_o and W_h. RP_H indices are indicative of the strength of reproductive barriers between taxa, and hybrid vigor levels. Table 1 provides an RP_H interpretation guide for the reader, and the taxonomic composition, population size (m^2) and RP_H indices of the studied populations are presented in Table 2. The four study populations demonstrate an absence of reproductive barriers between taxa, with hybrid vigor levels varying from none to quite strong (Table 2).

Flower density and pollinator observations

Pollinator observations were carried out for *C. affine acinaciformis* and *C. edulis* in April and May 2001 and 2002 between 9 am and 6 pm, during sunny, warm weather when pollinators were noticed to be active on Bagaud island and at Brégançon (Fig. 1). Pollinator community composition varied among sites, and is summarized in Table 3. All observed pollinators were native to the study area. *C. edulis* patches at Bagaud 2 in 2001 were not observed because the local rat population consumed the anthers that year. The resulting 138 observations consisted of 15 min intervals for which the number of pollinator visits, as well as the length of flower visits (seconds), were recorded for discrete flower patches. Patch size (between 2 and 4 m^2) and the number of flowers open per patch were also noted. Using these data, the following measures were calculated per observation: (1) flower density (FD: flowers/m^2), (2) flower visit frequency (FR: the number of flowers

Table 2 Summary of RP_H index results for the studied populations taken from Suehs et al. (2005)

Taxon	Site	Population size (m^2)	RP_H (mean ± sd)	Hybrid vigor status
C. affine acinaciformis	Bagaud 1	1500	0.83±0.10	present ($P<0.001$)
C. affine acinaciformis	Brégançon 1	400	−0.07±0.13	similar performance (NS)
C. edulis	Bagaud 2	2020	0.21±0.10	present ($P<0.05$)
C. edulis	Brégançon 2	1000	0.08±0.15	similar performance (NS)

Significance values ($P<$threshold or NS for insignificance) refer to tests comparing RP_H means against zero

Table 3 The pollinator community composition of the studied populations

Taxon	Site	Primary pollinator	Secondary pollinator	Other pollinators
C. affine acinaciformis	Bagaud 1	*Bombus terrestris*	*Apis mellifera*	*Eucera* sp., small solitary bees, wasps
C. affine acinaciformis	Brégançon 1	*Athidium* sp.	Small solitary bees	*A. mellifera*
C. edulis	Bagaud 2	Small solitary bees	*A. mellifera*	*B. terrestris*, Diptera
C. edulis	Brégançon 2	*Athidium* sp.	Small solitary bees	*A. mellifera*, *B. terrestris*, Diptera, Lepidoptera

All pollinators are native to the study area

visited per minute), (3) flower visit frequency per flower (FR/F: the number of flowers visited per minute weighted by the number of open flowers in the patch), and (4) the mean flower visit length (VL: seconds). FR is considered a measure of relative pollinator abundance (Kearns and Inouye 1993) and FR/F a measure of the quantitative aspect of pollinator service, while VL represents the quality of pollinator service. Finally, we consider that the PMGFP for a patch of *Carpobrotus* depends on (a) the number of flowers the patch produces and the size of the patch (high flower numbers increase opportunities for gene flow), (b) the pollination frequency of the flowers (high pollinator numbers increase opportunities for gene flow), and (c) how long pollinators stay on one flower (the longer a pollinator visits a flower, the higher the probability that at least one pollen grain will be transferred to the flower). Therefore, a (5) PMGFP index was calculated as FD×FR×VL. Note that the PMGFP index is designed to compare the maximum possible values of pollen-mediated gene flow among populations, but does not actually measure gene flow. We therefore use the term Pollen-Mediated Gene Flow *Potential* (PMGEP) throughout this study to remind the reader of this limitation.

Due to a large amount of heteroscedasticity among groups, Generalized Linear Models (GLM) ANOVAs using normal distributions, identity link functions ($x'=x$), and Type III likelihood tests were chosen to test for taxon and site effects and/or interactions for all observations on each parameter. When significant interactions occurred, differences between groups were confirmed using Type III likelihood ratio tests. A GLM multiple regression using normal distributions, identity link functions ($x'=x$) and Type III likelihood tests was used to examine the relationship between FD and flower visit frequency (FR), flower visit frequency per flower (FR/F), and mean flower visit length (VL). Linear regression was used to examine the relationships between PMGFP and RP_H (in Suehs et al. 2005).

Gamete production

For 14 *C. edulis* individuals and 24 *C. affine acinaciformis* individuals on Bagaud island (sampling was more intense for *C. affine acinaciformis* due to the known genetic and reproductive heterogeneity of this taxon, Suehs et al. 2004a, 2004b, 2005), three test tubes each containing 5 mature stamens were prepared from one flower bud and air-dried. The same flower buds were then preserved in alcohol. These three anther-containing tubes and associated preserved flower correspond to the following measures: (1) the number of pollen grains produced per anther, (2) the measurement of 300 pollen diameters per flower, (3) a pollen viability test via staining per flower, and (4) the number of ovules and the number of stamens per flower.

First, to determine the number of pollen grains produced per anther, pollen was first extracted from the first tube per individual. 550 μl of pure sulfuric acid were added to each tube, left to soak overnight, and then homogenized in a shaker for 4–5 min before adding

650 µl of 2% triton solution. The samples were subsequently re-homogenized, 1 ml 2% triton solution added, and then centrifuged for 5 min at 2000 rpm. The resulting surnagent was then quickly discarded, and 1 ml of 95% ethanol immediately added. The pollen pellet is resuspended via agitation, recentrifuged for 5 min at 2000 rpm, and the ethanol discarded. These washed pollen pellets are then placed under a hood to dry for 24 h, following which 140 µl of 20% glycerol, 30% saccharose solution are added. Each tube is then placed in an ultrasound bath for 5 min to disperse the pollen grains. One µl is then placed in a Malassez slide, the concentration of pollen grains determined, and the number of pollen grains produced per anther estimated via extrapolation.

Second, pollen grain diameters were determined by homogenizing the contents of the second tube per individual between a slide and cover slip with a drop of demineralized water. The diameters of 300 pollen grains were then measured under a microscope at 150 × magnification.

Third, based on an expected normal pollen diameter peak of ~28–29 µM (see study species and sites), the contents of the third tube per individual was analyzed for pollen viability according to size class (7–22 µM in diameter=micropollen; 23–34 µM in diameter=normal pollen, 35–43 µM in diameter=macro pollen). Specifically, 10 pollen grains per size class per individual were tested for viability by coloration with a solution of 1/6 MTT (3–4,5-diméthylthiazolyl-2–2, 5-diphenyltetrazolium bromide at 10 g/l) + 5/6 sucrose solution at 60%.

Fourth, the number of ovules and stamens per flower were estimated by dissection. First, the flower was divided into fourths, and the number of stamens for one fourth carefully counted. Second, the number of carpels per flower was determined, one carpel isolated using a scalpel, and the number of ovules contained in that one carpel was carefully counted by dissection under 10 × magnification.

Based on these four methods, the following parameters were estimated per flower: (i) the total number of pollen grains produced, (ii) the total number of ovules produced, (iii) pollen to ovule ratios, (iv) micro, normal, and macro pollen frequencies, (v) micro, normal and macro pollen proportion viabilities, (vi) viable micro, normal and macro pollen production.

Gamete production parameters (i, ii, iii) were compared between the two *Carpobrotus* taxa using a one-way MANOVA. Due to heteroscedasticity, mean differences between taxa for pollen quality parameters (iv, v) and viable productions (vi) were tested using GLM ANOVAs with normal distributions, identity link functions ($x'=x$) and Type III likelihood tests. All statistical analyses in this paper were performed using Statistica 6.0 (Statsoft, 2001).

Results

Flower density and pollinator observations

The means, standard deviations, minima and maxima for flower density (FD), flower visit frequency (FR), flower visit frequency per flower (FR/F), the mean flower visit length (VL) and PMGFP indices are presented in Fig. 2. GLMs fit the data well (scaled deviance and scaled Pearson χ^2 close to one; Table 4). Significant taxon effects were detected only for FD and FR/F, and significant site effects were detected only for FD, FR/F, and PMGFP. However, significant taxon by site interactions were found for all parameters (Table 4). Significant group differences ($P<0.05$) detected by two-group GLM comparisons are

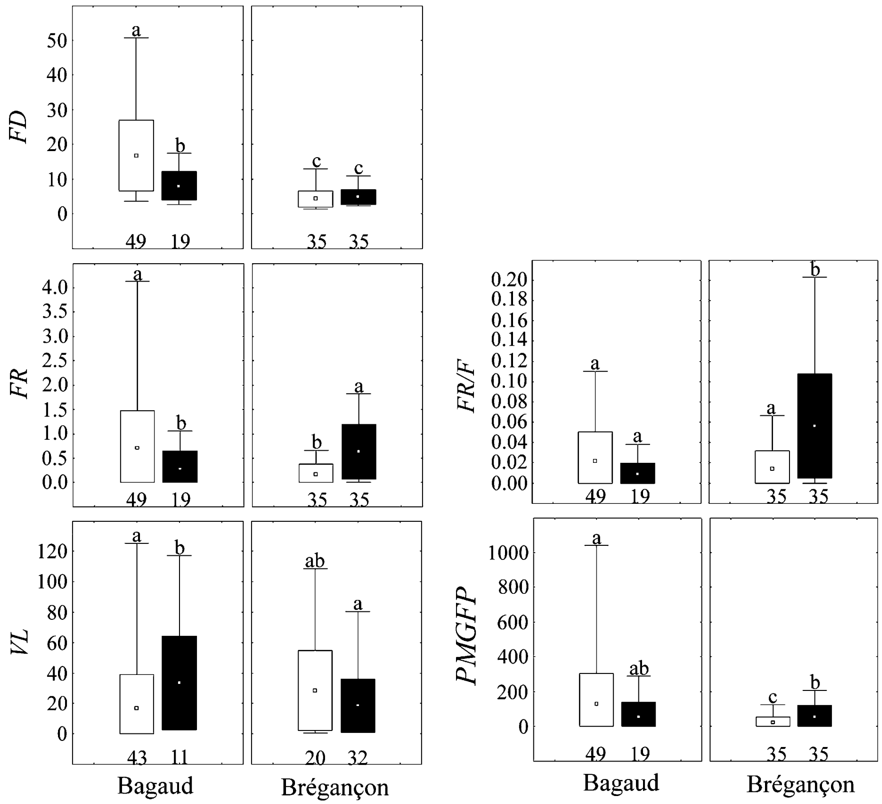

Fig. 2 Mean flower densities (FD; flowers/m^2), pollinator flower visits per minute (FR), pollinator flower visits per minute per flower (FR/F), pollinator visit length (seconds; VL), and Pollen-Mediated gene flow potential (PMGFP) for *C. affine acinaciformis* (white) and *C. edulis* (black) at sites Bagaud and Brégançon. Sample sizes are given below boxes. Points indicate means, boxes standard deviations and bars minima and maxima. Shared lower-case letters above boxes indicate insignificantly different groups (P>0.05) after generalized linear model ANOVA mean comparisons

indicated in Fig. 2. The highest flower densities were found for *C. affine acinaciformis* on Bagaud island, as were, high flower visit frequencies (FR). Flower densities were weakest at Brégançon, but *C. edulis* attracted just as many pollinators (FR) on average there as *C. affine acinaciformis* on Bagaud. This results in flower visit frequencies per flower (FR/F) for *C. edulis* at Brégançon being significantly higher than all other groups. Pollinator quality as measured by mean flower visit length (seconds, VL) generally reflects the opposite pattern observed for pollinator abundance, i.e. groups with high pollinator abundance (*C. affine acinaciformis* on Bagaud, *C. edulis* on Brégançon) have the smallest mean visit lengths. Finally, the potential for a patch of *Carpobrotus* to exchange genes via pollen flow (PMGFP) reaches an obvious maximum within the population of *C. affine acinaciformis* on Bagaud, as indicated by the high standard deviation in Fig. 2, though its mean is insignificantly different from that for *C. edulis* on Bagaud, and the latter is insignificantly different from *C. edulis* at Brégançon. *C. affine acinaciformis* at Brégançon has a significantly low PMGFP mean compared to all other groups.

Table 4 Generalized linear model ANOVA goodness of fit (scaled deviance and scaled Pearson χ^2) and test results for taxon (*C. affine acinciformis* or *C. edulis*) and site grouping (Bagaud or Brégançon) effects, as well as their interaction, on flower density (FD), flower visit frequency (FR), flower visit frequency per flower (FR/F), mean flower visit length (VL: seconds), and Pollen-Mediated gene flow potential (PMGFP)

Parameter	Scaled deviance and scaled Pearson χ^2	Taxon effect	Site effect	Taxon x site interaction
FD	1.03	0.001	0.001	0.001
FR	1.03	NS	NS	0.001
FR/F	1.03	0.05	0.001	0.001
VL	1.04	NS	NS	0.01
PMGFP	1.03	NS	0.05	0.01

Probability values are presented at the $P<0.001$, $P<0.01$, $P<0.05$, and $P>0.05$ (NS) levels

A GLM multiple regression on flower density (FD), flower visit frequency (FR), flower visit frequency per flower (FR/F*)* and mean flower visit length (seconds, VL) also fit the data well (scaled deviance and scaled Pearson χ^2 both equal 1.04), and detected a positive relationship between FD and FR (Wald χ^2=39.43, $P<0.001$), a negative significant relationship between FD and FR/F (Wald χ^2=55.07, $P<0.001$), and no significant relationship between FD and VL. A positive significant relationship was found between PMGFP and RP_H ($PMGFP=112.8\times RP_H+34.99$; r=0.95; F=57.26; $P<0.05$; Fig. 3).

Gamete production

Means and standard deviations for pollen and ovule counts per flower, as well as pollen:ovule ratios are presented in Fig. 4. No significant differences between *C. edulis* and *C. affine acinaciformis* for these gamete production parameters were found. Pollen grain diameter ranged from 7.07 to 42.42 μM, with a peak size at around 29 μM corresponding to normal pollen grain formation (see taxa descriptions and Fig. 5). Overall pollen viability was 45.78% for *C. affine acinaciformis* and 40.79% for *C. edulis.* A production in the 7–15 μM range, corresponding perhaps to empty pollen grains, is found only for *C. edulis* (Fig. 5). Other, unviable micro pollen grains are common for both taxa. Macro pollen are also present for both taxa, and are conservatively considered as diplogametes since their diameter range corresponds to approximately 1.25× that of normal pollen (Bretagnolle and Thompson 1995; Ortiz 1997), and because diad and triad pollen formations have been documented in the studied populations (Diadema, 2002). Macro pollen occur in 67% of *C. affine acinaciformis* individuals, and 71% of *C. edulis* individuals. Viability differences between the three pollen classes result in a viable pollen pool for *C. affine acinaciformis* of 3.5% micro-pollen, 85.5% normal pollen and 10.9% macro pollen; similarly, *C. edulis'* viable pollen is composed of 0.7% micro pollen, 73.0% normal pollen and 26.2% macro pollen. When comparing the two taxa via GLM ANOVAs, *C. edulis* produces significantly more viable macro pollen per flower than *C. affine acinaciformis.* No other differences were found between the two taxa (Fig. 6).

Discussion

Pollinator service patterns and *Carpobrotus* hybrid vigor levels

In response to our first question, all parameters associated with pollinator observations (FD, FR, FR/F, VL and PMGFP) demonstrated significant taxon × site interactions

(Table 4), suggesting that pollinator–plant interactions can be highly contextual, resulting in a spatial mosaic of varying levels of potential for pollen-mediated gene flow. In general, taxon differences on Bagaud island are reversed at Brégançon, though the significance of this pattern varies (Fig. 2). This variation in pollinator service is likely to greatly affect *Carpobrotus* populations, as their pollen to ovule ratios (Fig. 4) indicate facultative to strict xenogamy (Cruden 1977) and pollinator limitation has previously been documented in all studied populations (Suehs et al. 2005). Pollinator-assisted plant invasions, including the present study, are becoming increasingly documented (Parker 1997; Brown and Mitchell 2001; Chittka and Schürkens 2001; Brown et al. 2002; Parker, et al., 2002; Parker and Haubensak 2002; Stout et al. 2002). Furthermore, the almost global introduction of key generalist pollinators, such as *Bombus terrestris* and *Apis mellifera*, suggests that generalist pollinators are increasingly available, and at least partly responsible for certain plant invasions (Parker et al. 2002; Parker and Haubensak 2002; Stout et al. 2002). The spatial distribution of local pollinator communities may therefore play an important part in determining success via seed production, and subsequent gene flow of invasive plant populations (Parker 1997; Parker et al. 2002; Parker and Haubensak 2002).

As concerns the site differences detected in this study, this variation may be linked to the pollinator community composition at the study sites (Table 3), where well known generalist pollinators such as *Bombus terrestris, Apis mellifera*, and small solitary bees are quite common on Bagaud island, whereas *Athidium* sp. represent the primary pollinator at Brégançon. Flower density (FD) may also be a driver behind pollinator abundance and visitation patterns (Totland and Matthews 1998) since it shows a significant, positive relationship with flower visit frequency (FR) and a significant negative relationship with flower visit frequency per flower (FR/F). Indeed, *Carpobrotus* populations with higher FDs attract more pollinators, but not enough to maintain high values of FR/F. A relationship between resource allocation to pollinator attraction (e.g. flower density or display), and the necessity of outcrossing for seed production is known to occur (Charlesworth 1989). In such situations, plants that produce larger floral displays in terms of numbers of flowers

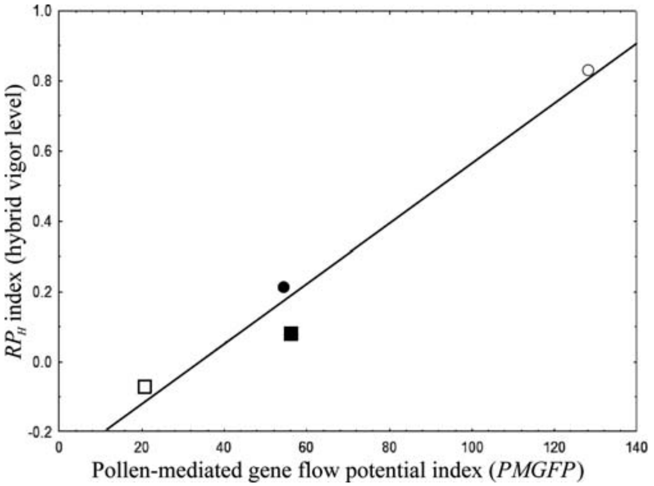

Fig. 3 Scatterplot demonstrating the relationship between PMGFP and hybrid vigor [based on RP_H values from Suehs et al (2005)] for populations of *C. affine acinaciformis* (white) and *C. edulis* (black) on Bagaud island (circles) and Brégançon (squares)

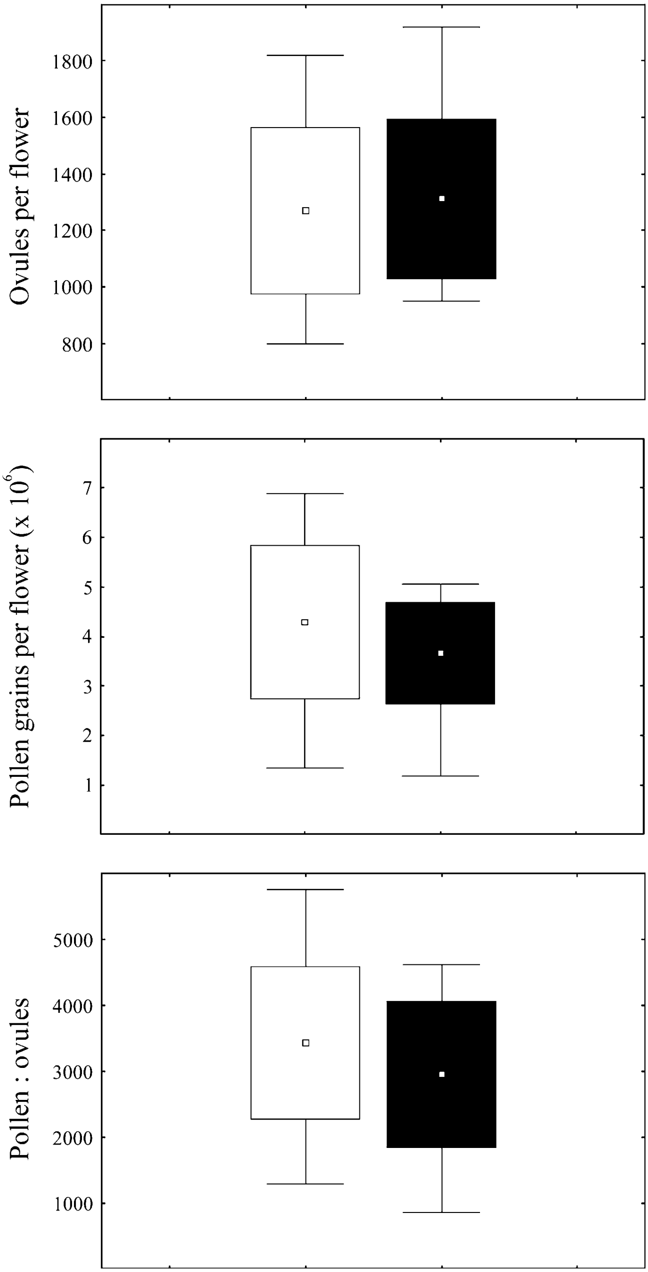

Fig. 4 Mean gamete production parameters for *C. affine acinaciformis* (white, sample size=24) and *C. edulis* (black, sample size=14) on Bagaud island. Points indicate means, boxes standard deviations and bars minima and maxima

attract more pollinators per plant, thus increasing the overall PMGFP to the plant, but not necessarily more pollinators per flower. According to past results, *C. affine acinaciformis* in only partially self-fertile, and suffers from inbreeding depression, while *C. edulis* is fully self-fertile with no inbreeding depression (Suehs et al. 2005), indicating that the former is dependent on cross-pollination for seed production, and the latter not. The taxon effects found in the present paper, i.e. higher FD and lower FR/F for *C. affine acinaciformis* compared to *C. edulis*, seem to well accompany these past results.

In response to our second objective, we found preliminary evidence that the PMGFP of the four studied populations is positively correlated with RP_H values, and in this particular case hybrid vigor levels (Fig. 3; Table 1), suggesting a link between pollinator services, pollen-mediated gene flow, and *Carpobrotus* hybridization. The spatial pattern of pollinator activity resulting in different levels of potential for pollen-mediated gene flow therefore appears linked to a corresponding spatial pattern of RP_H levels, indicative of a hybridization history (Suehs et al. 2005). Though this result should be used with caution because the PMGRP index does not measure actual gene flow between populations, and because correlation does not prove causation or coupling, the proposed synergy between hybridization, hybrid vigor levels, pollinator service, flower density, and PMGFP corroborates the site effects (Fig. 2) demonstrated in this paper with past demonstrations of high genetic diversity via introgression on Bagaud island (Suehs et al. 2004a, 2004b). Furthermore, this potential relationship has developed over the timespan of only ~200 years since these taxa were introduced to the study region in the early 1800's (Gouffé De La Cour 1813; Albert and Jahandiez 1908). Even if our results are based on a low number of populations, and could benefit from several more years of work to incorporate

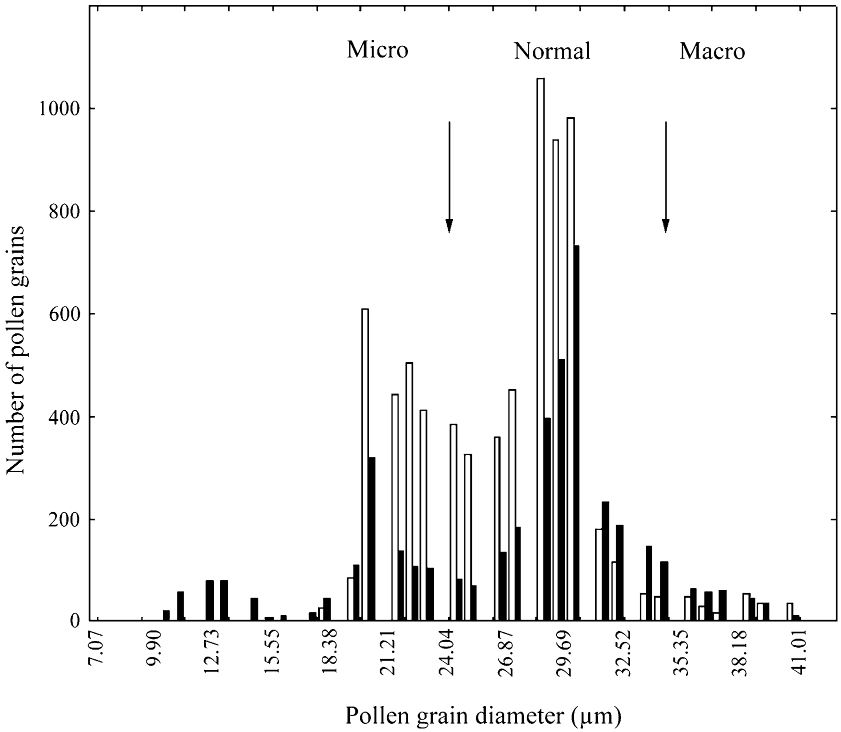

Fig. 5 Histogram demonstrating the distribution of pollen grain diameters for *C. affine acinaciformis* (white) and *C. edulis* (black) on Bagaud island. Arrows indicate divisions between micro, normal, and macro pollen classes

more data, it is hard to imagine that the relationship demonstrated between PMGFP and RP_H index values (Fig. 3) is simply due to chance, and underscores the importance of pollinator interactions in shaping the breeding behavior and genetic diversity of invasive plant populations.

Gamete production and hybrid dysgenesis

In response to our third question, pollen production in the populations with the highest RP_H indices/hybrid vigor levels appears to be meiotically perturbed. Pollen viability is less than indications for pure taxa (see methods section and Fig. 6), and pollen abnormalities in the form of micro- and macro-pollen are frequent (Figs. 5 and 6). These island populations are therefore doubly dependent on pollinators, as over twice the amount of pollen deposition will be necessary to transfer an equal amount of viable pollen, and stresses the pollinator limitation present in all populations (Suehs et al. 2005). Such abnormalities often correspond to meiotic or genic perturbations, a frequent problem among hybrids (Rieseberg and Carney 1998) known as hybrid dysgenesis (Fontdevila 2004), and is probably occurring in these populations. This result is further supported by observations of the following in the study populations' pollen: (i) univalent and sometimes multivalent series during metaphase I, (ii) late chromosomes following asynchronized division in metaphase II, (iii) chromosomal fragments in metaphase I and II, as well as (iv) diad, triad and polyad formations resulting in diploid and aneuploid gametes (Diadema, 2002).

One of the major consequences of hybrid dysgenesis is lowered fertility for early generation hybrids (Rieseberg and Carney 1998; Fontdevila 2004). At this point, we draw

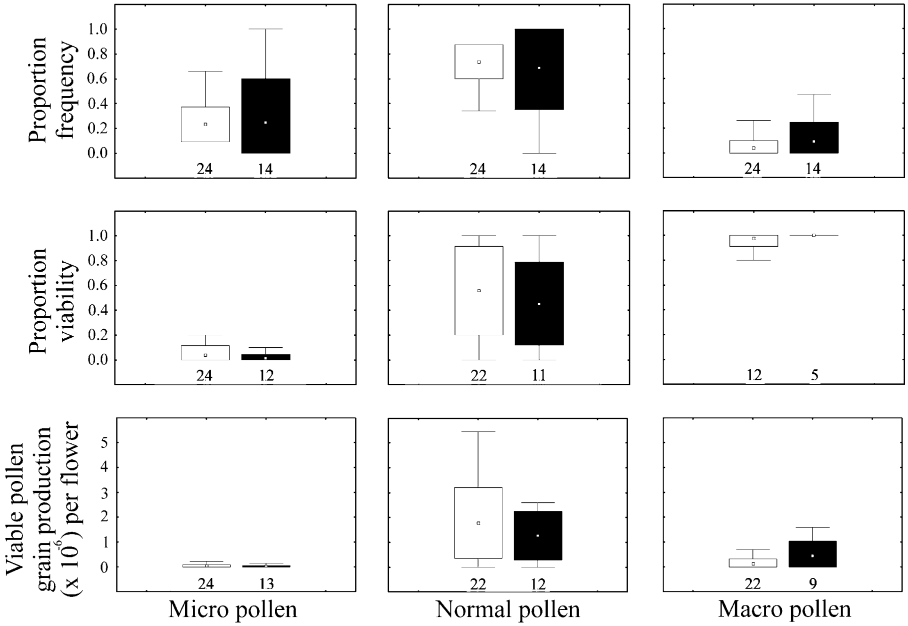

Fig. 6 Mean pollen class (micro, normal or macro) frequencies, proportion viabilities, and viable pollen grain production per flower for *C. affine acinaciformis* (white) and *C. edulis* (black) on Bagaud island. Sample sizes are given below boxes. Points indicate means, boxes standard deviations and bars minima and maxima

attention to *C. affine acinaciformis*, which is known to be highly introgressed (Suehs et al. 2004a, 2004b) and to have an overall lower seed production than *C. edulis* (Suehs et al. 2004a, 2004b, 2005). This contrasts with the very similar ovule production of these two taxa (Fig. 4), and results in much lower seed to ovule ratios for *C. affine acinaciformis* (0.16 and 0.57 for manual outcrossing and manual hybridization, respectively) compared to *C. edulis* (0.95 and 0.78, respectively). Perhaps not all *C. affine acinaciformis'* ovules were fertilized due to insufficient pollen deposition, the inviability of deposited pollen, or differences in the phenology of pollen maturation. However, this is unlikely because controlled hybridization experiments using all pollen types on *C. edulis* gave high seed to ovule ratios, indicating a good level of pollen saturation. Given that over 10–20% viable pollen production potentially consists of diplogametes, and the production of other abnormalities can be assumed due to the high inviability of normal pollen and the production of micro pollen, aneuploid and triploid blocks (seed inviability due to inappropriate ratios of embryo:endosperm genome numbers: Ramsey and Schemske 1998) may very well be contributing to lowered seed set in *C. affine acinaciformis*. Meiotic perturbation in the macrogametes of *C. affine acinaciformis* is also a plausible explanation (Bretagnolle and Thompson 1995), and remains to be studied. Curiously, low seed production is not a problem for *C. edulis*, and all reported cases of hybridization in the genus *Carpobrotus* have involved *C. edulis* (see methods section). This species is also agamospermic (Vilà et al. 1998; Suehs et al. 2004a, 2004b, 2005), a character strongly associated with polyploidy (Bierzychudek 1985; Bretagnolle et al. 1998). We therefore suspect that this species may have a mechanism that facilitates allosyndesis (interspecific chromosome pairing) and that it may also be able to tolerate polyploidization, and not experience aneuploid or triploid blocks. Though no polyploids have yet been reported for the genus *Carpobrotus*, their presence would certainly help to explain the high frequency of aneuploid (micropollen) and potential diplogametes found for both taxa (Bretagnolle et al. 1998; Ramsey and Schemske 2002), but remains to be confirmed via screening in the studied populations. Given that tetraploidy is a common occurrence in the Ruschioideae (the subfamilily of the Aizoaceae to which *Carpobrotus* belongs, Hartmann 1993), and the occurrence of individual plants capable of producing macrogametes and the frequency of macrogametes is unusually high (compared to Bretagnolle and Thompson 1995), we conclude that the polyploid potential of the studied populations is also unusually high.

Implications in the invasion process

We have evidence that pollinator interactions mediate different levels of PMGFP in the studied populations, which are in turn correlated with $\mathrm{RP_H}$ index values and thus hybrid vigor levels. The pollen diameter distributions and pollen viabilities of the populations with the highest $\mathrm{RP_H}$ indices/hybrid vigor levels are characteristic of hybridization-associated meiotic perturbations (hybrid dysgenesis), and indicate high polyploid potential. Different levels of pollinator activity therefore appear to mediate the frequency of hybridization, resulting in different $\mathrm{RP_H}$ levels, with highly introgressed populations experiencing hybrid dysgenesis. This suite of events has many implications in the invasion process. First, native pollinator mutualists are shown to actively and quickly affect invasive plant mating systems, pollen-mediated gene flow, and species integrity (within introgressed populations). Second, hybridization and polyploidization occurrences within populations are likely to be correlated with their PMGFPs, and these two processes are known to produce especially aggressive invaders with high evolutionary potential which may result in rapid adaptation and further aggressiveness in invasive taxa (Roy 1990; Thompson

1990; Song et al. 1995; Ramsey and Schemske 1998; Vilà et al. 2000; Wendel 2000; Sakai et al. 2001; Hänfling and Kollman 2002; Ramsey and Schemske 2002; Ainouche et al. 2003; Fontdevila 2004). Hybridization is also of special concern because hybrid *Carpobrotus* progeny are known to have especially large seedling sizes (Suehs et al. 2005), and this vigor may favor hybrid establishment at this critical life history stage.

Given the high PMGFP means and hybrid vigors on Bagaud island, especially for *C. affine acinaciformis* (Fig. 2), hybridization/introgression is likely to be an ongoing process in these populations. Continued introgressive hybridization can have several outcomes as concerns the evolution of species complexes (Arnold 1992, 1997). Genetic swamping and assimilation can occur, resulting in the incorporation of one (a presumably rare) genome into that of another (Arnold 1997; Vilà et al. 2000). This is a possibility for *C. affine acinaciformis* on Bagaud, due to its low self-compatibility coupled with strong hybrid vigor (Suehs et al. 2005). A second alternative is the derivation of novel genotypes with the potential to occupy new habitats (Arnold 1992; Rieseberg and Wendel 1993; Arnold 1997), or the potential for novel evolutionary trajectories, such as homoploid speciation (Arnold 1992; Rieseberg and Wendel 1993; Arnold 1997; Rieseberg 1997). However the latter process is likely to take place only under stringent isolation mechanisms (Rieseberg 1997). Continued hybridization (and any subsequent polyploidization) can also result in fertility selection and improvement in just a few generations (Rieseberg and Carney 1998; Ramsey and Schemske 2002; Fontdevila 2004). Should *C. affine acinaciformis*, currently less common than *C. edulis* but more vegetatively aggressive, recover high seed production, this taxon could become more problematic than *C. edulis.* From a large-invasion management perspective, examination of the pollinator services to invasive populations can help prioritize eradication/control programs. The avoidance of sympatry via eradication between invasive congeners is also highly recommended.

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