

Assessment of the breeding system in the Algerian narrow coastal endemism *Anacyclus linearilobus* (Anthemideae, Asteraceae)

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Abstract. The breeding system of *Anacyclus linearilobus*, an annual and endemic species restricted to dune ecosystems of the Algerian coast were assessed through the effect of self-fertilization and intra- and inter-population artificial crosses on the probability of producing viable seeds in 20 individuals from two populations in a common garden. The breeding system was determined for each individual by the self-incompatibility index (ISI). These results were compared with those published for other species of the genus, *A. clavatus*, *A. homogamos* and *A. valentinus*. Results of experimental crosses showed that there was high variation in the female reproductive success between individuals of *A. linearilobus*, like that found in its sister species, *A. valentinus*. Unlike the other species in the genus that are self-incompatible, *A. linearilobus* showed a mixed mating system, with a majority of self-incompatible individuals, others partially self-incompatible and some self-compatible. We argue that this strategy may be related to reproductive assurance in this endemic, annual species whose effective population size has been reduced probably due to individual mating incompatibilities.

Keywords. Compositae, mixed mating, reproductive assurance, selfing, western Mediterranean.

Resumen. Se evaluó el sistema de cruzamiento de *Anacyclus linearilobus*, una especie anual y endémica, de reducida distribución en ecosistemas dunares de la costa argelina, mediante la probabilidad de producir semillas en 20 individuos de dos poblaciones, cultivadas en jardín. El sistema de reproducción se determinó para cada individuo por el índice de autoincompatibilidad (ISI). Estos resultados se compararon con los publicados para otras especies del género, *A. clavatus*, *A. homogamos* y *A. valentinus*. Los resultados de los cruces experimentales mostraron que hubo una gran variación en el éxito reproductivo femenino entre individuos de *A. linearilobus*, como la encontrada en su especie hermana, *A. valentinus*. A diferencia de las otras especies del género que son autoincompatibles, *A. linearilobus* mostró un sistema de cruzamiento mixto, con una mayoría de individuos autoincompatibles, otros parcialmente autoincompatibles y algunos autocompatibles. Argumentamos que esta estrategia puede estar relacionada con el aseguramiento reproductivo en esta especie anual endémica cuyo tamaño efectivo de población se ha reducido probablemente debido a incompatibilidad entre individuos emparentados.

Palabras clave. Aseguramiento reproductivo, auto-fertilización, Compositas, Mediterráneo occidental, sistema de cruzamiento mixto.

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INTRODUCTION

Although outcrossing is the most prevalent mating system in angiosperms, a third of flowering plants exhibit mixed mating systems in which reproduction occurs both by self-fertilization and outcrossing (Goodwillie & al. 2005). In such cases, the genetic self-incompatibility system (SI) that is the main barrier to selfing in flowering plants (Igić & al. 2008) is eventually broken. In Astera-

ceae, while most species are self-incompatible (63%), a significant proportion are partially (10%) or totally (27%) self-compatible, pointing to a breakdown of their SI (Ferrer & Good-Avila 2007). Moreover, a complete and irreversible transition from outcrossing to selfing is a prevailing pattern in angiosperm diversification (Stebbins 1974; Igić & al. 2008; Goldberg & al. 2010). However, the maintenance

of selfing requires being advantageous enough to counterbalance the effects of inbreeding depression (Charlesworth & Charlesworth 1987; Byers & Waller 1999). This situation may occur in populations with severe pollen limitation due to low effective population size and/or scarce pollinator presence (Busch & Schoen 2008; Good-Avila & al. 2008), in which selection for reproductive assurance is a prevalent factor (Busch & Delph 2012). This could be especially relevant in annual plants whose reproductive success is limited to one flowering season (Shivanna 2014, 2015).

The annual herb *Anacyclus linearilobus* Boiss. & Reut. is a narrow endemism occurring in coastal dune ecosystems in Algeria. This species was described in the surroundings of Oran by Boissier & Reuter (1852) and only few other populations are known so far (Humphries 1979). The genus *Anacyclus* was recently circumscribed to eight species distributed in western Mediterranean (Vitales & al. 2018; Álvarez 2019). Morphologically, *A. linearilobus* mainly differs from other *Anacyclus* species in the degree of basal leaves division, which in *A. linearilobus* is 1–2 pinnatisect whereas in the remaining species is tri-pinnatisect (Humphries 1979; Vitales & al. 2018). All species in this genus have a relatively wide distribution, except *A. linearilobus* that is restricted to the Algerian coast, and *A. maroccanus* Ball that is an endemism of the Morocco plateau (Humphries 1979; Vitales & al. 2018). Additionally, *A. linearilobus* is the only species in this genus growing in no anthropic habitats.

Anacyclus linearilobus was considered in the past closely related to *A. clavatus* (Desf.) Pers. and *A. homogamos* (Maire) Humphries (Humphries 1979), however recent phylogenetic analyses indicate *A. valentinus* L. as its sister species, in a clade including *A. clavatus* and *A. radiatus* Loisel. (Oberprieler 2004; Vitales & al. 2018). However, *A. linearilobus* and *A. valentinus* are morphologically quite different (i.e., while *A. linearilobus* show radiate capitula, *A. valentinus* present rayless ones). All species in *Anacyclus* are diploid ($2n = 18$; Humphries 1981; Rosato & al. 2017), although significant differences in genome size between them were documented (Agudo & al. 2019; Vitales & al. 2020). It is remarkable that the two extremes in genome size within the genus are represented by these two sister species, with sizes of 8.22 Gbp/2C in *A. valentinus* and 13.14 Gbp/2C in *A. linearilobus* (Vitales & al. 2020). Finally, it is important to consider the possible hybrid origin of *A. valentinus* suggested by Álvarez & al. (2020), since it could also involve its sister species, *A. linearilobus*. In hybrids, allelic incompatibilities (Bateson 1909; Dobzhansky 1936; Muller 1942) and lethal nuclear-cytoplasmic interactions (Levin 2003) may cause low seed sets and high variation in fertility.

The viability of interspecific crosses between all *Anacyclus* annual species has already been documented (Humphries 1981), but the breeding system has only been as-

sessed in *A. clavatus*, *A. homogamos*, and *A. valentinus* (Álvarez & al. 2020). In all these cases, the species are considered self-incompatible and inter-fertile although reproductively isolated by postzygotic barriers.

Our goal here was to assess the breeding system in two populations of *Anacyclus linearilobus* and compare it with other annual species in the genus including its sister species, *A. valentinus*. We consider that this study is relevant not only for conservation purposes of *A. linearilobus*, but also for designing future research on the ecological and evolutionary singularity of this species.

MATERIAL AND METHODS

Plant material

Ripened capitula of *Anacyclus linearilobus* were collected in 2016 from two natural populations (Fig. 1; Table 1). The Bousfer population occupied a wide area along Bomo and Corales beaches where it presented a discontinued distribution. Therefore, three subareas were sampled in this case: two in Bomo beach (BMO and MOB), and one in Corales beach (COR). Our access to La Macta population (MAC) was restricted to a small sampling area. During the same collection trip two other localities were visited, although without success: the surroundings of Oran that was cited by Humphries (1979), and “Les Andalouses” near El-Ançor, represented by several herbarium sheets. For seed germination, 10–30 achenes from each individual were sown on wet desiccant paper into Petri dishes during 90 days under a 16h light/8h dark regime and 10–27°C in the Research Greenhouse of the Real Jardín Botánico (CSIC) in Madrid. Once germinated, seedlings were grown individually in a mix of COMPO SANA® Universal Potting Soil (COMPO GbmH, München, Germany) and siliceous sand (3:1) until the first 4 to 6 leaves developed. We selected fifteen grown plants from Bousfer (i.e., five from each subarea), and five from La Macta as pollen receptors, and 8–20 grown plants from each population as pollen donors.

Breeding system and experimental crosses

To estimate the breeding system in *Anacyclus linearilobus* we followed a protocol similar to that previously performed in other *Anacyclus* species (Álvarez & al. 2020). Four different experiments (one per inflorescence –capitulum–) were performed on each individual designated as pollen receptor: (1) no pollen addition to test autogamy; (2) pollen addition of the same individual to test self-compatibility; (3) pollen addition of individuals from the same population than the pollen receptor to test intra-population outcrossing; and (4) pollen addition of individuals from a different population to test inter-population outcrossing. All experiments were carried out in 2017 at the Research

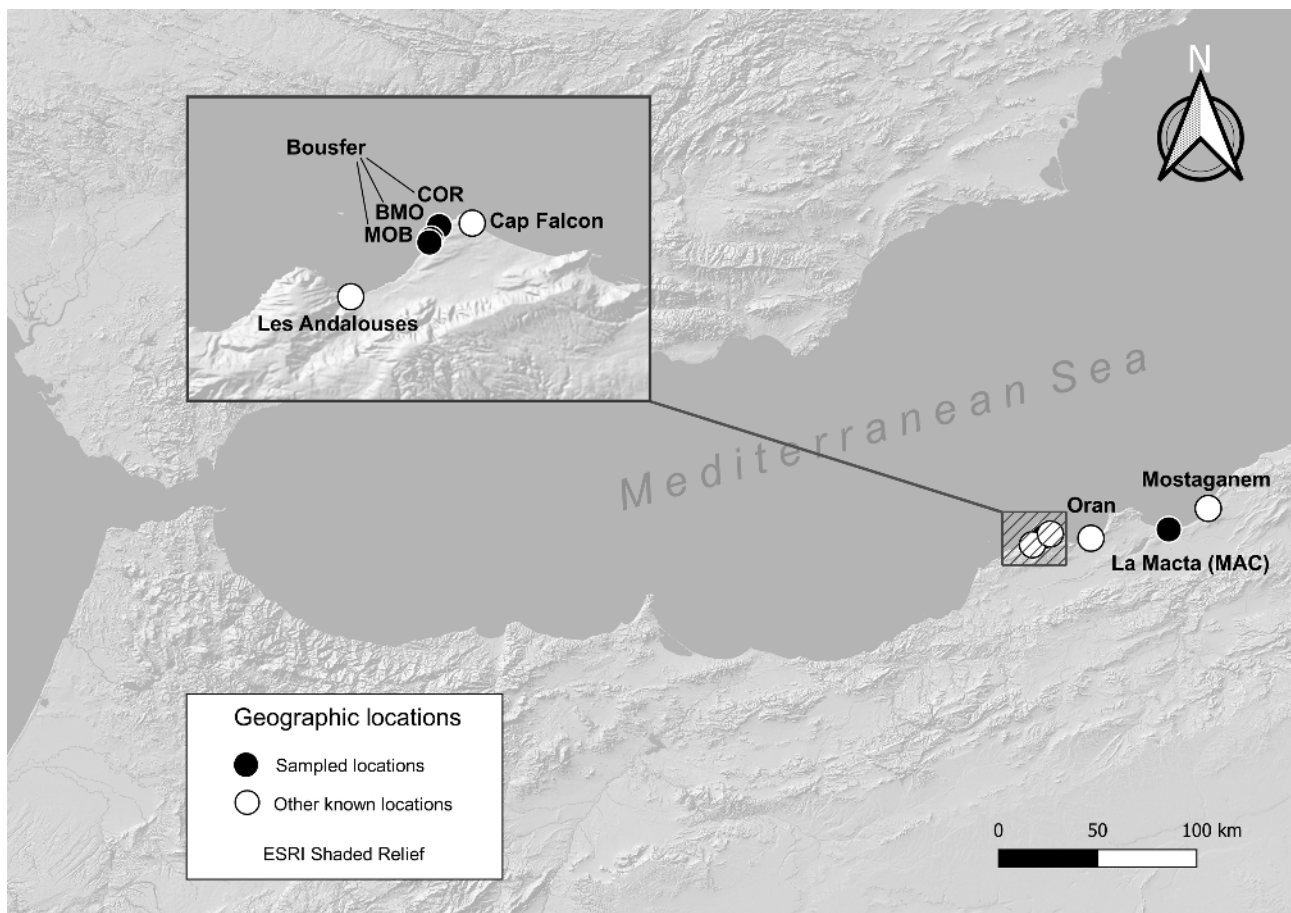


Fig. 1. Sampling sites and other cited localities for *Anacyclus linearilobus*. The zoomed area shows the Bousfer population indicating the three subareas sampled along Bomo and Corales beaches near Cap Falcon in Algeria.

Table 1. Populations of *Anacyclus linearilobus* included in this study, the codes for the areas and subareas sampled, number of individuals collected, and voucher information that includes country, locality, geographic coordinates, altitude above sea level, date, and the collector and number of collection in italics. All vouchers are kept at MA herbarium except for that of COR subarea, which is represented by seeds kept at the Seed Bank of the Real Jardín Botánico-CSIC in Madrid.

Population	Area or subarea code	Number of individuals collected	Origin and voucher information
Bousfer	BMO	3	Algeria: Bousfer, Bomo beach, 35°45'0.32"N, 0°49'50.52"W, 4 m, 23 May 2016, <i>Álvarez 2339</i>
	MOB	1	Algeria: Bousfer, Bomo beach, 35°44'52.08"N, 0°49'56.6"W, 19 m, 23 May 2016, <i>Álvarez 2340</i>
	COR	1	Algeria: Bousfer, Corales beach, 35°45'37"N, 0°49'18"W, 33 m, Jun. 2016, <i>Kaid-Harche s/n</i>
La Macta	MAC	3	Algeria: La Macta, 35°47'25.26"N, 0°9'24.8"W, 2 m, 24 May 2016, <i>Álvarez 2345</i>

Greenhouse of the Real Jardín Botánico (CSIC). All manipulated capitula were bagged before anthesis until fruits were collected. Bags were only open for pollen addition. In experiment 1 no additional manipulation was done once the capitula were bagged. In experiment 2, pollen from di-

fferent capitula of the same individual was added to produce pollen saturation. In experiments 3 and 4, a mix of pollen from different individuals was used in each case to ensure viability, avoiding individual effects, and to favour pollen saturation. In Asteraceae it is difficult to emasculate

without damaging the gynoecium due to the joint development of the sexual organs. Furthermore, in the case of *Anacyclus*, the small size of the hermaphroditic flowers (~ 5 mm) and their high number per capitulum (150–200), make emasculation unfeasible. In this case, we consider outcrossing valid when there is a significant difference between self-fertilization and outcrosses within the same individual. To give time to match phenology and to get the highest number of mature capitula available by pollen donors, the first two capitula formed in each plant selected as pollen receptor were used for experiments 1 and 2. In most Asteraceae, the mature pollen is released and pushed out of the floret as the style elongates, being exposed at the tip of the style (Leins & Erbar 2006). We collected pollen from style tips with tweezers daily and once a day (between 9–11 am) and blended in 1.5 mL Eppendorf Tubes® (Eppendorf, Hamburg, Germany) for immediate application. Pollen addition was performed using a paintbrush for each capitulum, until all flowers in the capitulum were ripened (usually 2–3 weeks, depending on the capitulum size). Ripened capitula were collected at least 4 weeks after the pollen addition was finished in each case. Since in the Asteraceae each flower may produce only one seed, the breeding system was estimated by the seed set (i.e., number of ripened seeds per capitulum/total number of flowers per capitulum) of each individual designated as pollen receptor.

Statistical analyses

The probability of setting a viable seed was analysed by fitting generalised linear mixed models (GLMMs), via restricted maximum likelihood (Patterson & Thompson 1971) using the 'lme4' package for R (Bates & al. 2015). We followed recommendations in Zuur & al. (2010) to ensure that our data met the assumptions of linear modelling, plotting residuals against fitted values, and against each explanatory variable before fitting any model. We modelled the probability of producing viable seeds using a binomial distribution with a logit link function. This model included the type of pollen addition (i.e., experiments enumerated above) as a fixed factor, and the pollen receptor (plant) and the population where it belongs (site) as random factors. We also fitted the same models for each site separately, removing the site as a random factor. All *P*-values of post-hoc comparisons were corrected using Holm's adjustment. The versatility of GLMMs makes them a better choice than linear models for modelling the variables that we considered and discerning between fixed and random factors in the model.

To assess the level and variation of self-incompatibility among these populations, we estimated the index of self-incompatibility for each individual following Lloyd (1992): $ISI = 1 - \text{relative selfed success} / \text{relative outcrossed success}$. For this equation, we used the seed set rate resulting

from the selfing experiment as the relative selfed success and that from the intra-population experiment as the outcrossed success. In the Bousfer population that includes three subareas (BMO, MOB and COR), the intra-population experiment considered was that of the corresponding subarea in each case. According to Raduski & al. (2012), self-incompatible individuals score ≥ 0.8 ; partial self-incompatible plants show values between 0.2 and 0.8, and self-compatible ones ≤ 0.2 . We used analyses of variance (ANOVAs) to test for the significant differences of ISI calculated for each population of each species, using both variables (species and populations) as fixed factors. Seed set rates for *A. clavatus*, *A. homogamos*, and *A. valentinus* were those previously obtained in Álvarez & al. (2020).

RESULTS

Seed germination rate, flowering, and pollen production

In average, the rate of germination in individuals from Bousfer population (70.8%, $n = 30$) was within the range found in other *Anacyclus* species (54–91% in *A. clavatus*, 66–94% in *A. homogamos*, and 56–85% in *A. valentinus*; Torices & al. 2013), and it was slightly lower in La Macta population (43%, $n = 30$). However, a detailed analysis by pollen receptor indicated a high variation in seed germination rate at the individual level, ranging from 26.7% to 86.7% in Bousfer, and from 10% to 90% in La Macta.

Generally, cultivated plants produced the first capitulum around 30–40 days after transplantation. Individuals COR 1 and BMO 1 from Bousfer, and MAC 8 from La Macta showed difficulties in pollen releasing, and therefore their pollen availability was lower.

Breeding system

In line with results obtained for other *Anacyclus* species (Álvarez & al. 2020), most *A. linearilobus* individuals showed seed set values < 0.1 for self-compatibility treatments (Table 2). However, in the two studied populations, discordant results with this pattern were observed in two individuals from Bousfer, and in four out of five individuals from La Macta.

Seed set for outcrossing, including both intra- and inter-population treatments, was ≥ 0.4 , except for six individuals from Bousfer (Table 2). Variation in seed set under outcrossing pollination treatments was already observed in *A. valentinus* (Álvarez & al. 2020), ranging between 0.351–0.981. On the contrary, seed set in the outcrossing treatments were higher and with lower variation in *A. clavatus* (0.831–0.939) and in *A. homogamos* (0.789–0.978). Additionally, in *A. linearilobus*, the variation was observed also at individual level in several cases. For example, the individual COR 1 showed seed sets < 0.4 and

Table 2. Individuals of *Anacyclus linearilobus* selected as pollen receptors, its population of origin, ID (identification code), the seed set for each type of experiment (auto: autogamy, self: self-compatibility, intra- and inter-population outcrosses indicating the origin –subarea– of the pollen), and the index of self-incompatibility (ISI). In square brackets are the number of flowers used for each experiment.

Popula- tion	ID	auto	self	intra-population outcrosses			inter-population outcrosses			ISI
				BMO	COR	MOB	MAC	-	-	
Bousfer	BMO 7	0.000 [172]	0.014 [138]	0.603 [136]	-	0.692 [120]	0.647 [119]	-	-	0.976
	BMO 1	0.076 [225]	0.056 [195]	0.481 [206]	-	-	-	-	-	0.883
	BMO 6	0.190 [163]	0.054 [205]	0.828 [169]	0.413 [80]	0.000 [117]	0.727 [110]	-	-	0.935
	BMO 2	0.000 [223]	0.017 [120]	0.871 [209]	0.708 [137]	0.628 [94]	0.608 [102]	-	-	0.981
	BMO 3	0.000 [126]	0.016 [125]	0.906 [139]	0.923 [156]	0.739 [165]	0.796 [103]	-	-	0.982
	COR 1	0.021 [146]	0.012 [161]	0.907 [97]	0.034 [146]	0.006 [158]	0.344 [128]	-	-	0.637
	COR 2	0.079 [202]	0.229 [144]	0.193 [109]	0.688 [157]	0.444 [81]	0.650 [143]	-	-	0.667
	COR 6	0.519 [154]	0.669 [130]	-	-	-	0.629 [97]	-	-	-
	COR 7	0.000 [215]	0.014 [147]	0.518 [110]	0.801 [136]	0.376 [85]	0.836 [110]	-	-	0.983
	COR 9	0.006 [168]	0.023 [130]	-	0.456 [79]	-	0.648 [108]	-	-	0.949
	MOB 3	0.000 [182]	0.000 [175]	0.447 [199]	0.565 [184]	0.579 [178]	0.490 [202]	-	-	1.000
	MOB 4	0.000 [255]	0.012 [247]	0.521 [177]	-	0.416 [209]	0.523 [107]	-	-	0.971
	MOB 5	0.000 [222]	0.000 [158]	-	-	0.248 [113]	-	-	-	1.000
	MOB 6	0.000 [141]	0.007 [138]	-	-	-	-	-	-	-
	MOB 8	0.000 [307]	0.004 [226]	0.186 [253]	0.826 [121]	0.895 [275]	0.813 [166]	-	-	0.995
Popula- tion	ID	auto	self	intra-population outcrosses			inter-population outcrosses			ISI
				MAC	-	-	BMO	COR	MOB	
La Macta	MAC 7	-	0.231 [212]	0.908 [196]	-	-	0.880 [183]	-	0.560 [100]	0.745
	MAC 1	0.320 [250]	0.502 [207]	0.528 [233]	-	-	0.602 [226]	-	-	0.048
	MAC 8	0.602 [211]	0.682 [173]	0.497 [143]	-	-	-	-	-	0.374
	MAC 17	0.013 [235]	0.016 [256]	0.953 [255]	-	-	0.649 [191]	0.824 [140]	0.890 [145]	0.984
	MAC 18	0.060 [216]	0.439 [230]	0.775 [218]	-	-	0.832 [125]	0.600 [100]	-	0.434

Table 3. Hand pollination effects on seed set of *Anacyclus linearilobus*. Main effects were assessed using type-II tests. Site was included as random factor in the model including all sites, and individual plant was included as random factor in all models [n: number of fertilized flowers; N: number of individual plants used].

Sites	n	N	d.f.	χ^2	P
All sites	15645	20	3	3138.1	< 0.001
Bousfer	11370	15	3	1973.0	< 0.001
La Macta	4275	5	3	793.8	< 0.001

~ 0.9 after different intra-population treatments (Table 2).

As in other *Anacyclus* species studied, in *A. linearilobus*, the probability of setting a viable seed differed significantly between the outcrossing experiments on the one hand, and the autogamy and selfing ones on the other (Fig. 2). In the same way, the type of pollen addition was a significant explanatory variable at population level (Table 3). In both sites, the analysis of all paired treatments revealed

significant differences between them, being the inter- and intra-population outcrosses those producing the highest seed sets (Fig. 2a, b). There were two main differences between sites: inter-population outcrossing led to the highest seed set in Bousfer but not in La Macta, in which they did not significantly differ from the intra-population ones. In addition, in La Macta the probabilities of setting a seed by autogamy and selfing treatments were remarkably higher than in Bousfer (Fig. 2a, b).

The self-incompatibility index (ISI) only differed significantly at population level (ANOVA, $n = 32$, $F_{6,22} = 3.32$, $P = 0.018$), and not at the species level (ANOVA, $n = 32$, $F_{3,22} = 2.10$, $P = 0.142$). In most of the cases, ISI was > 0.8, indicating self-incompatibility (Table 2). The exceptions found included individuals COR 1 and COR 2 that with values of 0.637 and 0.667, respectively were considered partially self-incompatible, as well as individuals MAC 8, MAC 18 and MAC 7 showing values of 0.374, 0.434, and 0.745, respectively. In addition, individual MAC 1 turned out to be self-compatible with

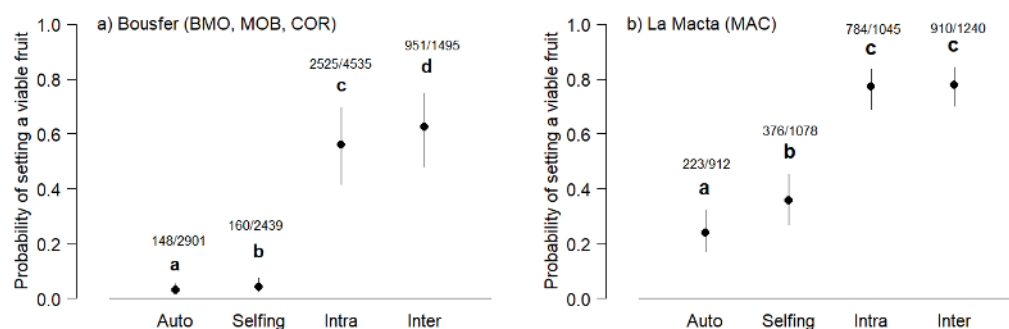
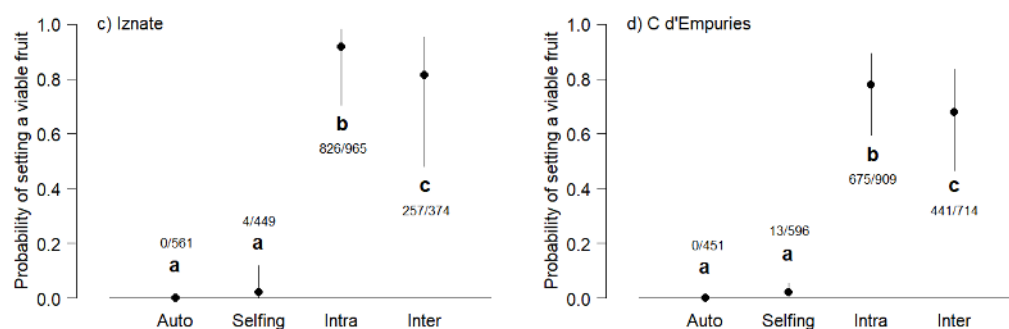
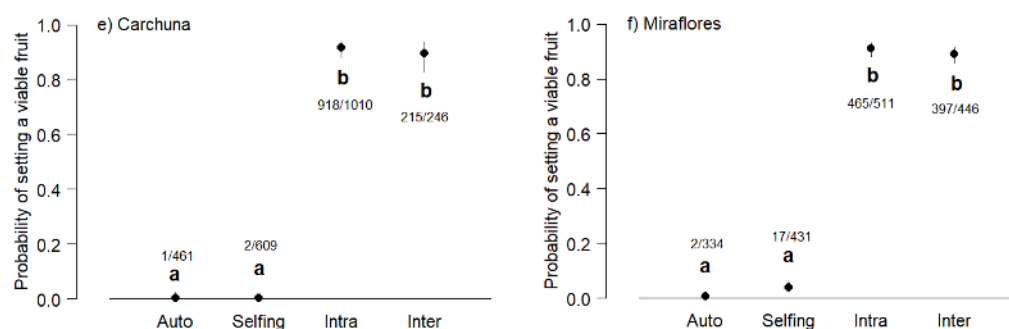
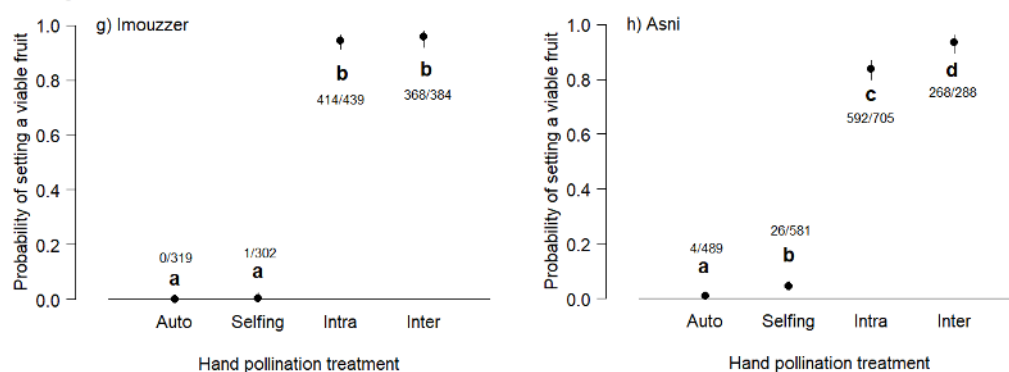
A. linearilobus*A. valentinus**A. clavatus**A. homogamos*

Fig. 2. Least-square means (95% CI) of the probability of setting a viable seed: **a, b**, Bousfer and La Macta populations of *Anacyclus linearilobus*, respectively; **c, d**, Iznate and Castelló d'Empuries populations of *A. valentinus*, respectively; **e, f**, Carchuna and Miraflores populations of *A. clavatus*, respectively; **g, h**, Imouzzar and Asni populations of *A. homogamos*, respectively. Different letters above each group indicate means statistically different between groups within each population. Sample size for each group is indicated for each treatment (number of seeds / number of total flowers). Data used for graphics c-h were published in Álvarez & al. (2020).

ISI = 0.048. In contrast, all individuals of *A. clavatus*, *A. homogamos*, and *A. valentinus* analysed from a previous study (Álvarez & al. 2020) were self-incompatible showing ISI values = 0.868–1 ($N = 16$, 5–6 individuals from two populations from each species).

DISCUSSION

Our results indicate that *Anacyclus linearilobus* has a mixed breeding system, in which most individuals are self-incompatible and coexist with other self-compatible and partially self-compatible ones. In contrast, its sister species, *A. valentinus*, as well as two other annual species in the genus, *A. clavatus* and *A. homogamos*, do not show a mixed reproductive system and are all considered self-incompatible (Álvarez & al. 2020). Therefore, it can be assumed that a breakage of the SI occurred along *A. linearilobus* evolution, allowing a shift to self-compatibility. The fact that the mating mixed system is present in the two populations studied and that transition to selfing is unidirectional (Igić & al. 2008) suggests that this reproductive strategy might be fixed at species level. However, occasionally, mixed mating systems may stabilize. For example, in the annual *Hypochaeris salzmänniana* DC. (Asteraceae), the proportion of selfing depends on pollinators availability (Arista & al. 2017), maintaining a mixed mating system over time. The paucity of pollinators might also be related with a partial shift to selfing in two isolated populations of the Madeiran endemism *Tolpis succulenta* (Aiton) Lowe (Asteraceae) (Crawford & al. 2019; Kerbs & al. 2020). The shift to self-compatibility may be associated with the floral biology, life cycle, and ecology of the species (Barrett 2014; Arista & al. 2017; Barrett & Harder 2017). Interestingly, unlike its sister species and others closely related, *A. linearilobus* is the only one growing in sand dunes, occupying a different habitat than its congeners. Besides, *A. linearilobus* individuals grow under shrubs canopy and therefore their occurrence is contingent to the shrub's presence. While other *Anacyclus* species that occupy anthropic habitats may form dense continuous populations, in *A. linearilobus* the density of individuals might be lower with a more scattered distribution. Moreover, the presence and abundance of pollinators in these ecosystems might be heterogeneous and dependent as well on "vegetation islands" within the sand dune matrix. Under this situation pollen availability might be a limiting factor for outcrosses, while the effective population size decreases, driving to a need for a reproductive assurance strategy (Busch & Schoen 2008; Good-Avila & al. 2008), which is essential for species with short life cycles like annuals (Shivanna 2014). A similar scenario occurs in oceanic islands, in which selfing and mixed mating systems may be advantageous. In fact, in a study comparing species of Asteraceae, Brassicaceae and Solanaceae from islands and mainland, 66% of island

species resulted self-compatible, compared to 41% of mainland ones (Grossenbacher & al. 2017).

The female reproductive success in *Anacyclus linearilobus* was variable both at individual and at population level (Fig. 2a, b). Similar type of variation has been observed at population level in *A. valentinus* (Fig. 2c, d), but not in other *Anacyclus* species studied (Fig. 2e–h). This variation in *A. valentinus* has been attributed to its possible hybrid origin (Álvarez & al. 2020). As *A. linearilobus* is the sister species of *A. valentinus*, we might consider a similar hybrid origin, and therefore, the variation observed in female reproductive success could also have similar causes. Other evidence supporting this hypothesis is the high variation in germination rates in *A. linearilobus* as well as in *A. valentinus* (Álvarez & al. 2020), and the difficulty in pollen release observed in some individuals of *A. linearilobus*, which is in agreement with the lower fitness expected in hybrids. Genic and allelic incompatibilities between pollen receptors and donors reduce mating availability, giving rise to a reduction in effective population size, which might be increased by inbreeding depression (Angeloni & al. 2011). In this case, it would be expected that seed set values for intra-population crosses were lower than for the inter-population ones. This was observed in the Bousfer population (Fig. 2a), but not in La Macta (Fig. 2b). As La Macta population has partially lost self-incompatibility, it is not expected that intra-population incompatibility would act as much as in the Bousfer population, resulting in a less variable and higher female reproductive success in La Macta (Fig. 2a, b). Other factors, such as species distribution, and population size and structure, have also relevant effects on reproductive performance. This seems evident in *A. valentinus*, which shows the highest variation in female reproductive success overall, but in which the seed set values for the intra-population crosses are significantly higher than for the inter-population ones (Fig. 2c, d). In this case, the large size of its populations occurring along roadsides, and its wide distribution in western Mediterranean coasts might have counterbalanced the effects of incompatibilities between individuals from the same population.

The assessment of breeding systems of rare or endemic species may be critical for the development of successful conservation strategies (Pérez & al. 2018). Since mixed mating systems may reduce species vulnerability (Yates & Ladd 2004), the finding that *Anacyclus linearilobus* shows this strategy as reproductive assurance is hopeful for the viability of its populations. However, the fact that this endemic species may experience high pollen limitation in the communities where it occurs (Alonso & al. 2010), its scattered distribution contingent to shrub canopy in the habitat it occupies, and its limitation in successful mating, make this endemic species a good candidate to be considered in conservation programmes. In

addition, populations of *A. linearilobus* might be heavily impacted by development of industrial and tourism activities as well as the increase in the urban population in northern Algerian coastline (Snoussi & Aoul 2000). It might be possible that both the population of “Les Andalouses” near El-Ançor and that of the surroundings of Oran may have become extinct due to urbanistic development in these areas. *Anacyclus linearilobus* has a restricted distribution on the Algerian coast, with an extent of occurrence estimated as 4,227 km² and an area of occupancy estimated as 8–36 km² (of which only around 7 km² are suitable habitat). Therefore, we have suggested to the International Union for Conservation of Nature (IUCN) its assessment for the category of EN (Endangered).

In conclusion, in contrast with other species in *Anacyclus* that are considered self-incompatible, the narrow endemism *A. linearilobus* showed a mixed mating system in which most individuals were self-incompatible whereas others were partially self-incompatible, and others were self-compatible. Effective population sizes in *A. linearilobus* are diminished by intrinsic factors such the genic incompatibilities between different individuals giving rise to limitation in mating, but also maybe by environmental factors such as scattered habitat availability. Reproductive assurance under these circumstances may be favoured by the mixed mating system. Our results are also congruent with the hypothesis of a possible hybrid origin for *A. linearilobus* that showed a variable and relatively low female reproductive success both at population and individual levels. However, further research including genomic and cytogenetic data is required to be conclusive on the origin of this singular evolutionary entity. In the same line, the search for other populations of *A. linearilobus* along coastal sand dunes in Algeria and their demographic tracking would help in better assessing the state of conservation of this species.

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REFERENCES

- Agudo A.B., Torices R., Loureiro J., Castro S., Castro M. & Álvarez I. 2019. Genome size variation in a hybridizing diploid species complex in *Anacyclus* (Asteraceae: Anthemideae). *International Journal of Plant Sciences* 180: 374–385.
- Alonso C., Vamasi J.C., Knight T.M., Steets J.A. & Ashman T.L. 2010. Is reproduction of endemic plant species particularly pollen limited in biodiversity hotspots? *Oikos* 119: 1192–1200.
- Álvarez I. 2019. *Anacyclus* L. In Benedí C., Buira A., Rico E., Crespo M.B., Quintanar A., Aedo C. (eds.), *Flora iberica* vol. 16(3), Compositae (partim). Real Jardín Botánico-CSIC, Madrid.
- Álvarez I., Agudo A.B., Herrero A. & Torices R. 2020. The Mendelian inheritance of gynomonoeecy: insights from *Anacyclus* hybridizing species. *American Journal of Botany* 107: 116–125.
- Angeloni F., Ouborg N.J. & Leimu R. 2011. Meta-analysis on the association of population size and life history with inbreeding depression in plants. *Biological Conservation* 144: 35–43.
- Arista M., Berjano R., Viruel J., Ortiz M.A., Talavera M., Ortiz P.L. 2017. Uncertain pollination environment promotes the evolution of a stable mixed reproductive system in the self-incompatible *Hypochaeris salzmänniana* (Asteraceae). *Annals of Botany* 120: 447–456.
- Barrett S.C.H. 2014. Evolution of mating systems: outcrossing versus selfing. In Losos J.B. (ed.), *The Princeton Guide to Evolution*. Princeton University Press, Princeton.
- Barrett S.C.H. & Harder L.D. 2017. The ecology of mating and its evolutionary consequences in seed plants. *Annual Review of Ecology, Evolution and Systematics* 48: 135–157.
- Bates D., Mächler M., Bolker B.M. & Walker S. 2015. Fitting Linear Mixed-Effects Models using lme4. *Journal of Statistical Software* 67: 1–48.
- Bateson W. 1909. Heredity and variation in modern lights. In Seward A.C. (ed.), *Darwin and modern science*. Cambridge University Press, Cambridge.
- Boissier P.E. & Reuter G.F. 1852. *Pugillus Plantarum Novarum Africae Borealis Hispaniaeque Australis*. Ferd. Ramboz et socii, Geneva.
- Busch J.W. & Delph L.F. 2012. The relative importance of reproductive assurance and automatic selection as hypotheses for the evolution of self-fertilization. *Annals of Botany* 109: 553–562.
- Busch J.W. & Schoen D.J. 2008. The evolution of self-incompatibility when mates are limiting. *Trends in Plant Science* 13: 128–136.
- Byers D.L. & Waller D.M. 1999. Do plant populations purge their genetic load? Effects of population size and mating history on inbreeding depression. *Annual Review of Ecology and Systematics* 30: 479–513.
- Charlesworth D. & Charlesworth B. 1987. Inbreeding depression and its evolutionary consequences. *Annual Review of Ecology, Evolution and Systematics* 18: 237–268.
- Crawford D.J., Moura M., Borges Silva L., Mort M.E., Kerbs B., Schaefer H. & Kelly J.K. 2019. The transition to selfing in Azorean *Tolpis* (Asteraceae). *Plant Systematics and Evolution* 305: 305–317.
- Dobzhansky T. 1936. Studies on hybrid sterility. II. Localization of sterility factors in *Drosophila pseudoobscura* hybrids. *Genetics* 21: 113–135.
- Ferrer M. & Good-Avila S. 2007. Macrophylogenetic analyses of the gain and loss of self-incompatibility in the Asteraceae. *New Phytologist* 173: 401–414.

- Goldberg E.E., Kohn J.R., Lande R., Robertson K.A., Smith S.A. & Igić B. 2010. Species selection maintains self-incompatibility. *Science* 330: 493–495.
- Good-Avila S.V., Mena-Alí J.I. & Stephenson A.G. 2008. Evolutionary consequences of variations in self-fertility in self-incompatible species. In Franklin-Tong E. (ed.), *Self-incompatibility in flowering plants—evolution, diversity, and mechanisms*. Springer, Berlin.
- Goodwillie C., Kalisz S. & Eckert C.G. 2005. The evolutionary enigma of mixed mating systems in plants: occurrence, theoretical explanations, and empirical evidence. *Annual Review of Ecology, Evolution and Systematics* 36: 47–79.
- Grossenbacher D.L., Brandvain Y., Auld J.R., Burd M., Cheptou P.-O., Conner J.K., Grant A.G., Hovick S.M., Pannell J.R., Pauw A., Petanidou T., Randle A.M., Rubio de Casas R., Vamosi J., Winn A., Igić B., Busch J.W., Kalisz S. & Goldberg E.E. 2017. Self-compatibility is over-represented on islands. *New Phytologist* 215: 469–478.
- Humphries C.J. 1979. A revision of the genus *Anacyclus* L. (Compositae: Anthemideae). *Bulletin of the British Museum (Natural History) Botany* 7: 83–142.
- Humphries C.J. 1981. Cytogenetic and cladistic studies in *Anacyclus* (Compositae: Anthemideae). *Nordic Journal of Botany* 1: 83–96.
- Igić B., Lande R. & Kohn J.R. 2008. Loss of self-incompatibility and its evolutionary consequences. *International Journal of Plant Sciences* 169: 93–104.
- Kerbs B., Crawford D.J., White G., Moura M., Borges Silva L., Schaefer H., Brown K., Mort M.E. & Kelly J.K. 2020. How rapidly do self-compatible populations evolve selfing? Mating system estimation within recently evolved self-compatible populations of Azorean *Tolpis succulenta* (Asteraceae). *Ecology and Evolution* 10: 13990–13999.
- Leins P. & Erbar C. 2006. Secondary pollen presentation syndromes of the Asterales—a phylogenetic perspective. *Botanische Jahrbücher für Systematik, Pflanzengeschichte und Pflanzengeographie* 127: 83–103.
- Levin D.A. 2003. The cytoplasmic factor in plant speciation. *Systematic Botany* 28: 5–11.
- Lloyd D.G. 1992. Self- and cross-fertilization in plants II. The selection of self-fertilization. *International Journal of Plant Sciences* 153: 370–380.
- Muller H.J. 1942. Isolating mechanisms, evolution, and temperature. *Biology Symposium* 6: 71–125.
- Oberprieler C. 2004. On the taxonomic status and the phylogenetic relationships of some unispecific Mediterranean genera of Compositae-Anthemideae I. *Brochia, Endopappus* and *Heliocauta*. *Willdenowia* 34: 39–57.
- Patterson H.D. & Thompson R. 1971. Recovery of inter-block information when block sizes are unequal. *Biometrika* 58: 545–554.
- Pérez M.E., Meléndez-Ackerman E.J. & Monsegur-Rivera O.A. 2018. Breeding system and pollination of *Gesneria pauciflora* (Gesneriaceae), a threatened Caribbean species. *Flora* 242: 8–15.
- Raduski A.R., Haney E.B., Igić B. 2012. The expression of self-incompatibility in angiosperms is bimodal. *Evolution* 66: 1275–1283.
- Rosato M., Álvarez I., Nieto Feliner G. & Rosselló J.A. 2017. High and uneven levels of 45S rDNA site-number variation across wild populations of a diploid plant genus (*Anacyclus*, Asteraceae). *PLoS One* 12: e0187131.
- Shivanna K.R. 2014. Reproductive assurance through autogamy in some annual weed species. *Proceedings of the National Academy of Sciences, India Section B: Biological Sciences* 84: 681–687.
- Shivanna K.R. 2015. Reproductive assurance through autogamous self-pollination across diverse sexual and breeding systems. *Current Science* 109: 1255–1263.
- Snoussi M. & Aoul E.H.T. 2000. Integrated coastal zone management programme northwest African region case. *Ocean & Coastal Management* 43: 1033–1045.
- Stebbins G.L. 1974. *Flowering plants. Evolution above the species level*. Belknap Press, Cambridge.
- Torices R., Agudo A. & Álvarez I. 2013. Not only size matters: achene morphology affects time of seedling emergence in three heterocarpic species of *Anacyclus* (Anthemideae, Asteraceae). *Anales del Jardín Botánico de Madrid* 70: 48–55.
- Vitales D., Nieto Feliner G., Vallès J., Garnatje T., Firat M. & Álvarez I. 2018. A new circumscription of the Mediterranean *Anacyclus* (Anthemideae, Asteraceae) based on plastid and nuclear DNA markers. *Phytotaxa* 349: 1–17.
- Vitales D., Álvarez I., Garcia S., Hidalgo O., Nieto Feliner G., Pellicer J., Vallès J. & Garnatje T. 2020. Genome size variation at constant chromosome number is not correlated with repetitive DNA dynamism in *Anacyclus* (Asteraceae). *Annals of Botany* 125: 611–623.
- Yates C.J. & Ladd P.G. 2004. Breeding system, pollination and demography in the rare granite endemic shrub *Verticordia staminosa* ssp. *staminosa* in south-west Western Australia. *Austral Ecology* 29: 189–200.
- Zuur A.F., Leno E.N. & Elphick C.S. 2010. A protocol for data exploration to avoid common statistical problems. *Methods in Ecology and Evolution* 1: 3–14.