1 **The function of the floral corona in the pollination of a Mediterranean style dimorphic**
2 **daffodil**

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1 **Summary**

- 2 • In style dimorphic species, pollinator attraction is crucial to promote cross-pollination
- 3 between floral morphs. *Narcissus papyraceus* presents dimorphic and monomorphic
- 4 populations, visited primarily by long-tongued and short-tongued pollinators
- 5 respectively. The maintenance of the polymorphism is associated to long-tongued
- 6 insects (moths), as they deliver pollen to short-styled flowers (S). Here we aimed at
- 7 identifying the importance of the floral corona as visually and fragrantly attractive
- 8 trait, and its consequences for the fertility of the morphs.
- 9 • We described UV reflectance patterns of the floral parts, and used neutral red to
- 10 identify parts with higher osmophore concentration. We also characterised volatile
- 11 organic compounds in intact flowers and in flowers with trimmed coronas. Finally, we
- 12 conducted a field experiment to evaluate the consequences of corona removal for seed
- 13 production, in stands with solitary plants and in groups to control by pollen limitation.
- 14 • *Narcissus papyraceus* flowers are white, with reflectance of tepals and corona above
- 15 400 nm wavelength. The largest concentration of osmophores appeared in the corona,
- 16 but all floral parts produced similar volatiles. Across dimorphic and monomorphic
- 17 regions, S flowers without corona suffered a reduction in seed production of ca. 50%,
- 18 while fertility in L flowers virtually remained unchanged. Plants in solitary stands
- 19 suffered a strong reduction in fertility, which was more pronounced in the
- 20 monomorphic region.
- 21 • Our results support the hypothesis that the corona in *Narcissus* is important for floral
- 22 scent production and attraction of long-tongued pollinators, which are critical for the
- 23 pollination of S flowers and the maintenance of the polymorphism.
- 24

1 **Key words:** diurnal and nocturnal pollination, flower reflectance, flower corona, flower
2 fragrance, moths, *Narcissus*, style polymorphism, syrphids, winter flowering.

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4 Running head: Floral advertisement, female fecundity and style dimorphism

5

1 **Introduction**

2 The great diversity of floral forms in Angiosperms has largely been interpreted in the
3 context of pollination and the evolution of adaptations to attract animal pollinators (Faegri &
4 van der Pijl, 1979; Fenster *et al.* 2004). Much research on plant-pollinator interactions has
5 focused on floral traits associated to visual signals to understand floral evolution (Schemske
6 1980; Chittka & Menzel 1992; Harder & Barrett 1995; Borges *et al.* 2003; Armbruster *et al.*
7 2005; Harder & Johnson 2005; Parachnowitsch & Kessler 2010; Fenster *et al.* 2015). Floral
8 scents have also been recognized as important traits to attract animal pollinators (Raguso
9 2008; Schiestl 2015), although quantitative estimates of their adaptive value are less abundant
10 (but see Schiestl *et al.* 2011; Parachnowitsch *et al.* 2012). This is surprising because, as
11 phenotypic trait, floral fragrances are extremely variable across lineages, species and ecotypes
12 and populations (Galen *et al.* 1987; Galen & Newport 1988, Dobson *et al.* 1997; Odell *et al.*
13 1999; Ashman *et al.* 2005; Burdon *et al.* 2015; Prieto-Benitez *et al.* 2016b). Furthermore,
14 floral scent has played an important role to depict pollination syndromes, particularly to
15 describe pollination associated to nocturnal moths, mammals, carrion- and dung-seeking
16 insects and male euglossine bees (Fenster *et al.* 2004, Dobson 2006, and references therein).
17 Furthermore, several studies have shown that some pollinators are more attracted to particular
18 floral volatile compounds (Knudsen & Tollsten 1993; Andersson 2003; Milet-Pinheiro *et al.*
19 2012; Schietl & Dötterl 2012; Riffel *et al.* 2013; de Vega *et al.* 2014; Larue *et al.* 2016),
20 whereas an important role has also been identify for defence against insect herbivory (see
21 Schiestl 2010 for a review).

22 The hypothesis of pollinators as important ecological drivers for plant reproduction
23 was firstly put forward by Sprengel (1793, 1996). This work inspired Darwin to develop an
24 evolutionary perspective (reviewed in Barrett 2010), when he recognized cross-pollination
25 and avoidance of selfing as important mechanisms to increase female fertility (Darwin 1876;

1 1877), and animal pollinators as relatively efficient agents to collect pollen from anthers and
 2 delivering it to stigmas (Darwin 1862; 1877). However, the vast majority of angiosperms are
 3 hermaphrodites, and very often pollen lands on the stigma of the same flower or another
 4 flower from the same plant, resulting in self- and geitonogamous pollination, respectively.
 5 Detrimental effects of selfing have favoured the evolution of traits to avoid self-pollination
 6 and promote cross-pollination, such as spatial (hercogamy) and temporal (dichogamy)
 7 separation of sex organs (Lloyd & Webb 1986; Webb & Lloyd 1986), and genetic
 8 incompatibility systems (Nettancourt 1997). These mechanisms increase plant dependency on
 9 animal pollinators, and selection on accuracy of the organs involved in pollen transfer
 10 (Armbruster *et al.* 2009). One of the most studied floral adaptations to promote effective
 11 pollen pick up from anthers and delivery to stigmas are stylar polymorphisms, which include
 12 heterostyly, style dimorphism and related cases (Barrett 2002). Heterostylous species include
 13 two (sometimes three) discrete floral morphs within populations. These morphs have anthers
 14 and stigmas placed at reciprocal heights, in such a way that stigmas receive pollen mostly
 15 from flowers of the opposite morph; that is, they undergo disassortative mating (reviewed in
 16 Barrett *et al.* 2000). Lloyd & Webb (1992) proposed that stylar polymorphisms are likely to
 17 evolve if appropriate pollinators place pollen from the different morphs on different parts of
 18 their bodies (Barrett 2002; Armbruster *et al.* 2006), an argument first launched by Darwin
 19 (1877). Thus, pollinator behaviour becomes prominent in building up and maintaining stylar
 20 polymorphisms, although unequivocal evidence for that is scarce, being experimental (Stone
 21 & Thomson 1994; Lau & Bosque 2003; Pérez-Barrales & Arroyo 2010, Simón-Porcar *et al.*
 22 2014) and comparative in populations and species with different pollinator arrays (Arroyo &
 23 Dafni 1995; Pérez-Barrales *et al.* 2006; Santos-Gally *et al.* 2013b). The evidence also shows
 24 that both the abundance and distribution of floral morphs (Thompson *et al.* 2003; Cesaro *et al.*
 25 2004; Stehlik *et al.* 2006; Brys *et al.* 2007; Meeus *et al.* 2012), and the fit between flowers

and pollinators (Massinga *et al.* 2005, Armbruster *et al.*, 2006, Perez-Barrales *et al.* 2007, 2014) are critical for the maintenance of the polymorphism. In contrast, less is known about the influence of floral display on female fertility of the morphs (but see Brys *et al.* 2004; Garcia-Robledo & Mora 2007; Brys & Jacquemyn 2010), or how floral fragrances influence their pollination (but see Ashman 2009).

Narcissus papyraceus is a Mediterranean style dimorphic bulb geophyte with white and sweet scented flowers. Flowers typically present a wide corona and a long and narrow floral tube at the bottom of which nectar accumulates. This combination of traits fits with an apparent nocturnal moth pollination syndrome (Faegri & van der Pijl 1979), and it has been suggested that the floral corona present in most of the species functions as scent producing organ (Vogel & Müller-Doblies 1975; Effmert *et al.* 2006). Flowers are either long- (L) or short-styled (S), the stigma of the latter being placed deep into the floral tube below the anthers; thus, only long-tongued pollinators deliver pollen on the stigmas of S-flowers, whereas L-flowers can be reached by long and short tongued pollinators (Simón-Porcar *et al.* 2014). Populations of *N. papyraceus* are either dimorphic (i.e. two morphs are present in the populations), or long-styled monomorphic, and they differ in the composition of the pollinator fauna: dimorphic populations are mainly visited by nectar seeking long-tongued insects, including nocturnal moths, and monomorphic populations are mostly visited by pollen collecting short-tongued hoverflies (Pérez-Barrales *et al.* 2007; Pérez-Barrales & Arroyo 2010, Santos-Gally *et al.* 2013b). The species is self-incompatible but plants of the same morph are fully cross-compatible (Arroyo *et al.* 2002; Simón-Porcar *et al.* 2015a), therefore plants require visits by insects to set seeds. Arroyo *et al.* (2002) described a gradual loss of S-plants in populations and proposed that such pattern was driven by the shift in pollinator type because short-tongued insects cannot reach short stigmas. Moths seem to be attracted by *N. papyraceus* fragrance, particularly by its high content in the monoterpene trans- β -ocimene

1 and the aromatic benzyl acetate (Dobson *et al.* 1997), whereas the white flat tepals and a
 2 central yellow spot (i.e. anthers) represent a model that has been shown to be attractive for
 3 pollen-seeker hoverflies (Dinkel & Lunau 2001).

4 In the present study, the influence of the floral corona on the female fertility of *N.*
 5 *papyraceus* flowers was investigated under the contrasting pollination environments, with
 6 predominantly long-tongued and short-tongued pollinators. Firstly, the potential role of the
 7 corona to attract pollinators visually or by means of scent production was examined. The
 8 reflectance spectra of the floral corona and tepals were characterized in L and S flowers.
 9 Then, neutral red stain was used to describe the distribution of tissues with osmophoric
 10 function in flowers, and volatile organic components (VOCs) were measured in intact flowers
 11 and in flowers where the corona had been removed. Secondly, to understand the importance
 12 of the corona on pollination, seed production was studied under experimental conditions in a
 13 factorial design, where the floral corona was removed from plants distributed individually or
 14 in groups. Specifically, we tested the hypothesis that, if the corona is an important organ to
 15 attract long-tongued pollinators (e.g. nocturnal moths), its removal will have a stronger
 16 impact on seed production of S-flowers than L-flowers, as the latter can be pollinated by
 17 different pollinator arrays. In addition, the importance of pollinator visits and pollen transfer
 18 dynamics on seed production prompted by the two pollinator arrays (hoverflies usually visit
 19 all flowers in a plant and a patch while moths make flights among distant flowers, Herrera
 20 1987) was examined by manipulating the size (solitary vs 3-plant groups) and distance (>150
 21 m) among experimental plots, to test for a possible distance effect (see Barthelmess *et al.*
 22 2006).

1 **Material and Methods**

2 *Study species and populations*

3 *Narcissus papyraceus* is distributed along the Mediterranean basin, and it is more abundant in
4 the Strait of Gibraltar region (SW Iberian Peninsula and NW Morocco). Populations are large
5 and dimorphic in the core region of the Strait of Gibraltar, while smaller and L-styled
6 monomorphic in the northern range margin (Arroyo *et al.* 2002). This geographic pattern of
7 variation in the polymorphism follows a clinal gradient, from isoplethy (equal morph-ratio) to
8 anisoplethy (L>S) and to L-monomorphism (Fig. 1). Flowering season usually starts in mid-
9 November in isoplethic populations from coastal areas near the Strait of Gibraltar and it ends
10 in late March in L-monomorphic populations from inland and highland areas. An individual
11 flower blooms during 7-15 days depending on pollination success, and an entire population
12 blooms approximately between six to eight weeks (Arroyo *et al.* 2002). Flowers are displayed
13 in umbels of 5-15 flowers, but only the first 7-8 flowers produce fruits and thus only these
14 were subjected to experiments described below. Of the 26 compounds identified in the floral
15 scent, the most abundant is trans- β -ocimene (70%), followed by benzyl acetate and indole
16 (Dobson *et al.* 1997). Populations are visited by short-tongued diurnal pollinators (67% and
17 75% of the visits in dimorphic and monomorphic populations), most of them being hoverflies
18 (61% and 74% respectively) which collect pollen. Dimorphic and monomorphic populations
19 differ on the visitation rates from short-tongued insects (0.1 visits min⁻¹ in and 0.8 visits min⁻¹
20 respectively). In contrast, long-tongued nocturnal insects (mostly moths) are relatively more
21 frequent in dimorphic populations, and more efficient pollinators than diurnal insects (Pérez-
22 Barrales *et al.* 2007; Pérez-Barrales & Arroyo 2010; Santos-Gally *et al.* 2013b).

23

24 *Floral colour, floral organ for scent production and identification of floral volatile organic*
25 *compounds (VOCs) in N. papyraceus*

1 The colour of *N. papyraceus* flowers was characterized using an Ocean Optics USB-2000
 2 spectrometer and a Top Sensor System Deuterium-Halogen DH-2000 lamp as a standardized
 3 light source (DT-MINI-GS-2). Reflectance was measured as the proportion of a standard
 4 white reference tile (WS-1 SS; Ocean Optics, Duiven, The Netherlands). A coaxial fibre cable
 5 (QR-400-7-UV-VIS-BX; Ocean Optics) was used for all measurements and the distance
 6 between the sample and the measuring probe was held constant. The angle of illumination and
 7 reflection was fixed at 45°. Spectra data were processed with the software Spectrasuite
 8 (version 10.4.11; Ocean Optics) and calculated in 5 nm wide spectral intervals over the range
 9 of 300-700 nm, which is the visual range for most pollinators. Reflectance was measured in
 10 the external and internal tepals, and the outer and inner part of the corona in 24 flowers. In
 11 addition, reflectance was measured in five anthers of a flower and in the stem of 10 plants as a
 12 control measure.

13 In order to locate the floral parts with osmophores, 10 flowers from both dimorphic
 14 (five flowers from each morph) and monomorphic populations were selected and submerged
 15 for two hours in an aqueous solution of 0.001% (w/v) neutral red (Dafni 1992; Kearns &
 16 Inouye 1993). To quantify floral VOCs, 10 plants (five for each morph) were collected from a
 17 dimorphic population, and transported to the greenhouse at the University of Seville until
 18 flowering to extract floral VOCs. In order to control for individual effects, two flowers per
 19 inflorescence and per morph were selected, and one of them remained intact (control),
 20 whereas in the other the crown was removed (treatment). This was replicated across the ten
 21 collected individuals. Floral VOCs were collected from the potted plants using dynamic
 22 headspace sorption. A single flower was enclosed within a microwave-safe oven bag (ITS ®
 23 Nagelpoelweg, Netherlands). Volatile compounds were adsorbed during 15 minutes on a trap
 24 of 5 mg of Porapak Q (Mesh size 80/100; Alltech Associates Inc., Deerfield, IL, USA)
 25 connected to a battery-operated vacuum pump (SKC Inc., Eighty Four, PA, USA), which

1 drew the air through the bag and trapped VOCs at a constant rate of ca. 150 mL min⁻¹. Filters
 2 were sealed and stored at -20°C until extraction in the laboratory. All floral odour samples
 3 were analysed with a gas chromatograph (GC; Agilent 6890 N) and a mass selective detector
 4 (MSD). The separation of VOCs was conducted with an HP5 column (5%- Phenyl-
 5 methylpolysiloxane, 30m x 0.32mmØ x 0.25 lm film thickness; Agilent Technologies), with
 6 helium as a carrier gas. One microliter of the odour sample was injected splitless at a
 7 temperature of 50 °C (3 min) followed by opening of the split valve and programming to 250
 8 °C at a rate of 5 °C min⁻¹. Chromatogram outputs were recorded by the Chemstation program
 9 (Agilent Technologies). VOCs were identified by comparing their mass spectra and retention
 10 times with those integrated in the NIST library and those of authentic standards. To calculate
 11 absolute amounts from peak areas, a response factor was used, which was calculated as the
 12 mean response of 100ng of three reference compounds (cresol, limonene, 3-hexen-1-ol) in the
 13 GC-MS. This approach did not always allow us to identify which isomer was present, and two
 14 compounds could not be identified (RT 3.47 and RT 3.81). Because we only compared the
 15 amount of VOCs between morphs with and without corona, we did not calculate the absolute
 16 amounts of compounds. Instead, peak areas of total ion chromatograms were used and $\ln(1 +$
 17 $x)$ transformed for comparative analyses.

18

19 *Corona removal, pollen limitation and seed production of L and S plants in experimental*
 20 *populations*

21 The experiment was conducted in the dimorphic isoplethic region (Sierras de Algeciras cork
 22 oak open woodlands, Cádiz prov., 36° 15' 15''N, 5° 21' 31''W, #2 in Fig. 1) and the
 23 monomorphic region (Aznalcázar pine woodlands, Seville prov., 37° 15' 47''N, 6° 13' 12''W,
 24 #1 in Fig. 1). Between December 2004 and January 2005, long-styled and short-styled plants
 25 in a similar floral bud developmental stage were collected from a large dimorphic isoplethic

1 population (#3 in Fig. 1). Plants were transferred to pots with a mix of peat and perlite (3:1)
 2 and watered daily in the greenhouse of the University of Seville (Spain). Plants were
 3 randomly assigned to all combinations of treatments in a factorial design to assess the effects
 4 of floral morph (S- or L-styled plants), corona manipulation (intact or removed with fine
 5 scissors immediately after flower opening) and limited compatible pollen, with plants in
 6 solitary stands or in groups (assuming that the former do not have compatible pollen nearby,
 7 whereas the latter do). Groups included three different individual plants of the same morph to
 8 avoid a complex experimental design (the experiment was not designed to estimate assortative
 9 vs disassortative mating on seed production, see Pérez-Barrales & Arroyo 2010 and Simón-
 10 Porcar *et al.* 2014 for specific tests). The experiment in the dimorphic and monomorphic
 11 region started in December 2004 and January 2005 respectively (sites #2 and #1 in Fig. 1).
 12 Each treatment combination included three replicates, and replicates were placed randomly in
 13 the field at least 150 m distant apart from one another to reduce pollen flow among plots (this
 14 distance was presumed to be conservative, as shown for *Silene* species pollinated by a similar
 15 nocturnal and diurnal pollinator array, where pollen flow is negligible at distances longer than
 16 80 m, Barthelmess *et al.* 2006; Barluenga *et al.* 2011). A total of 48 plants were used per
 17 region. The areas selected for the study were inspected previously to the initiation of the
 18 experiment, to ensure that there were not naturally co-occurring populations within 2-3
 19 kilometres range, and avoid uncontrolled pollen flow. Experimental arrays were left in the
 20 field until all flowers withered, and plants were transported back to the greenhouse for fruit
 21 maturation to avoid fruit loss or uncontrolled damage. Previous experiments have shown that
 22 *N. papyraceus* plants are resistant to manipulations during their development (Arroyo *et al.*
 23 2002, Pérez-Barrales & Arroyo 2010). When ripen, fruits were collected, and the number of
 24 seeds and undeveloped ovules per flower were counted under a stereomicroscope.

25

1 *Experimental control of the effect of corona manipulation on seed production*

2 To control by the possible effects of corona manipulation on seed production, between
3 December 2005 and January 2006, 30 patches with L and S plants were randomly selected in
4 a large population in the dimorphic area (#3 in Fig. 1). Per patch, two plants per morph were
5 selected and the corona was removed in 1-3 flowers in one plant per morph, and left intact in
6 the other two plants. To control by the possible role of the corona on pollinator attraction, all
7 flowers were supplemented with pollen from other plants. After six weeks, fruits we
8 harvested and seeds counted as described above.

9

10 *Statistical analyses*

11 MANOVA was used to compare VOCs between treatments nested within plants, where the
12 morph was included as a fixed factor; however, due to sample size limitations, plants were not
13 nested within morph. To test for the effect of corona removal and limited compatible pollen
14 on seed production of L and S plants, generalized linear models (GLM) were performed on
15 the data collected from the experimental settings in the dimorphic and monomorphic regions,
16 respectively. In these analyses, the main effects (morph, corona manipulation and group size),
17 and the interaction effects morph x corona manipulation, morph x group size and corona
18 manipulation x group size were included. Posthoc analyses (least significance difference)
19 were conducted when a statistically significant interaction effect was detected. To evaluate the
20 possible effect of corona manipulation on seed production, a two-way full factorial GLM was
21 performed where corona manipulation and morph were included as fixed factors and seed
22 production as the response variable. For this analysis, a generalized estimating equation
23 (GEE) was used using the AR(1) working correlation structure, in which patch was set as
24 repeated measured to account for lack of independence in seed production of L- and S- plants
25 within each patch. Because overdispersion was detected in the response variable seed

1 production, a negative binomial error distribution was used to conduct the GLM analyses, and
2 the predicted means and standard errors were retrieved from the models. The analyses on
3 VOCs were conducted in R version 3.2.1 (R Core Team 2013), whereas the GLMs were run
4 in SPSS v. 23 for Mac (IBM. Corp. 2016).
5

1 **Results**

2 *Floral colour, floral organ for scent production and identification of floral volatile organic*
 3 *compounds (VOCs) in N. papyraceus*

4 Figure 2 shows the mean and s.e reflectance measured in 24 flowers calculated in 5 nm wide
 5 spectral intervals over the range of 300-700 nm. All the floral parts measured were reflective
 6 at around 425 nm. Reflectance was larger in the inner and outer tepals than the corona, with
 7 values around 60%. The measures taken in the external parts of the corona presented a peak at
 8 ca. 425 nm, with ca. 60% reflectance, after which reflectance declined, whereas the measures
 9 taken in internal part of the corona showed reflectance values below 50%.

10 A visual inspection of *N. papyraceus* flowers from the two populations subjected to
 11 the neutral red test revealed that, in cases, the corona stained the most, while staining was
 12 weaker in the rest of the floral (Fig. 3). A qualitative comparison showed that flowers from
 13 dimorphic population stained similarly, regardless the morph. With regards fragrance
 14 production, a total of ten VOCs were identified in *N. papyraceus* (Fig. 4). The two dominant
 15 compounds were the monoterpene cis-b-ocimene and the aromatic benzyl acetate (Fig. 4).
 16 There was not statistically significant effect of the morph and the corona removal treatment
 17 within plants (MANOVA: $F_1 = 0.64$, $P = 0.750$; $F_1 = 2.72$, $P = 0.295$, respectively).

18
 19 *Corona removal, availability of compatible pollen and seed production of L and S plants in*
 20 *experimental populations*

21 The analyses of seed production in L and S plants revealed similar patters between the
 22 dimorphic and monomorphic region, with few exceptions. In both regions, the terms morph,
 23 group and the interaction term morph x corona manipulation were statistically significant
 24 (Table 1). On average, L plants produced more seeds per flower than S flowers (estimated
 25 mean $\pm$ s.e in the dimorphic region, L: 9.19 ± 1.38 and S: 6.39 ± 1.24 ; monomorphic region,

1 L: 11.67 ± 1.38 and S: 8.16 ± 1.32). In addition, plants in groups produced on average more
 2 seeds per flower (dimorphic region: 15.61 ± 1.50 , $P < 0.001$, monomorphic region: $26.49 \pm$
 3 1.93) than plants in solitary stands (dimorphic region: 3.76 ± 0.91 , monomorphic region: 3.60
 4 ± 0.74). The results of the interaction term morph x corona manipulation were similar in the
 5 dimorphic and monomorphic region (Fig. 5). Flowers of L plants did not differ in seed
 6 production in relation to corona removal ($P = 0.97$ in dimorphic region, Fig. 5a; $P = 0.64$ in
 7 monomorphic region, Fig. 5b), whereas S plants did ($P = 0.043$ in dimorphic region, $P = 0.05$
 8 in monomorphic region). The comparisons between morphs revealed that the reduction in
 9 seed production with corona removal was stronger in S flowers than L flowers ($P < 0.001$ in
 10 dimorphic region and $P = 0.002$ in monomorphic region), with larger reduction in the
 11 monomorphic region (Fig. 5b). In contrast, both morphs produced similar seed number when
 12 the corona was left intact (Fig. 5a and b).

13 Across regions, the term corona manipulation was statistically significant only in the
 14 monomorphic region (Table 1), and plants with corona produced more seeds (12.11 ± 2.29)
 15 than those where the corona had been removed (7.86 ± 0.83). In addition, the interaction term
 16 morph x group was statistically significant in the dimorphic region (Table 1, Fig. 6). Both L
 17 and S plants in groups produced more seeds than in solitary stands than in groups ($P = 0.021$
 18 for comparison between L flowers; $P < 0.001$ for comparisons between S flowers, Fig. 6a). In
 19 contrast, solitary stands of S plants produced less seeds per flower than solitary stands of L
 20 plants ($P < 0.0001$, Fig. 6a). In the monomorphic region, the patterns between L and S plants
 21 were similar, and seed production per flower was lower in solitary stands than in groups (Fig.
 22 6b). For both dimorphic and monomorphic regions, the term corona x group was not
 23 statistically significant (Table 1).

24

25

1 *Experimental control of the effect of corona manipulation on seed production*
2 The effect of corona removal on seed production was not statistically significant (Wald Chi-
3 square = 0.545, d.f. = 1, $P = 0.46$), but there was a significant effect of the term morph (Wald
4 Chi-square = 11.60, d.f. = 1, $P = 0.001$). On average, L plants produced more seeds per flower
5 (11.28 ± 1.03) than S plants (6.31 ± 0.87). The interaction term morph x corona was also
6 statistically significant (Wald Chi-square = 10, d.f. = 1, $P = 0.002$). Specifically, L flowers
7 with the corona removed produced less seeds than those where the corona was left intact (L
8 flowers with corona removed: 9.55 ± 1.09 ; L flowers with corona: 13.11 ± 1.71), whereas the
9 opposite trend was detected for S flowers (S flowers with corona removed: 8.09 ± 1.35 ; S
10 flowers with corona: 4.93 ± 0.80).
11
12
13

Discussion

Narcissus species display substantial morphological variation in the corona, including size, shape and colour (Blanchard 1990). Although this variation has been valuable for species classification (Webb 1980), and frequently assumed as important to attract pollinators (Vogel & Müller-Doblies 1975; Marques *et al.* 2007), its functional significance for pollination has been scarcely explored (but see Herrera 1995). The present study aimed at identifying the potential role of the corona of *N. papyraceus* for pollinator attraction, and testing its importance for female fitness.

Floral corona as visually and fragrantly attractive trait

Narcissus papyraceus flowers present a combination of traits typical of flowers with nocturnal pollination, with white and fragrant flowers (Dobson *et al.* 1997), and reflectance patterns similar to other species with nocturnal pollination (Raguso *et al.* 2003). The application of neutral red showed that the corona stained more intensively than other floral parts, without substantial differences between morphs or populations. These results suggest that the corona bears a relatively high concentration of osmophores compared to other floral parts, as found in the green-flowered *Narcissus viridiflorus* (Vogel & Müller Doblies 1975). Application of neutral red has been useful to localize osmophore tissue in order to identify scent producing organs (Stern *et al.* 1986; Wiemer *et al.* 2009). In our study, the characterization of VOCs in intact flowers showed that L and S produce similar compounds, as found in *Primula eliator* and *P. farinosa*, two distylous species with similar fragrance chemistry between morphs (Gaskett *et al.* 2005). However, VOCs identified in flowers with the corona removed were similar to those characterized in intact flowers. Although all floral parts in *N. papyraceus* produce similar compounds, our estimates of VOCs production did not allowed quantifying variation in the rate of production among different floral organs (Burdon

1 *et al.* 2015; Prieto-Benitez *et al.* 2016a), or whether emission of compounds followed a
 2 diurnal and nocturnal pattern, which could affect the attraction of nocturnal and diurnal
 3 visitors (Balao *et al.* 2011; Burdon *et al.* 2015; Prieto-Benitez *et al.* 2016a). In *N. papyraceus*,
 4 pollination by nocturnal insects is important for female fertility in dimorphic populations
 5 (Pérez-Barrales & Arroyo 2010), and it is possible that nocturnal fragrance production plays
 6 an important role in the attraction of insects. In this study, samples were collected at night and
 7 one of the most abundant VOCs was aromatic benzyl acetate, which has been found to elicit
 8 antennal responses in the hawkmoths *Sphinx perelegans* and *Hyles lineata* (Sphingidae:
 9 Lepidoptera), two pollinators of night-blooming species (Raguso *et al.* 1996; Raguso and
 10 Light, 1996). To better understand the role of floral scent for pollination in *Narcissus*, future
 11 research should incorporate assessment of circadian patterns in fragrance emission in relation
 12 to the attraction of diurnal and nocturnal pollinators (see Ruiz-Ramón *et al.* 2014 for diurnal
 13 and nocturnal VOCs production in cultivated daffodils), as well as the associated
 14 physiological response of different pollinators arrays.

15

16 *The effect of corona on seed production*

17 The natural geographic variation of floral visitors in *N. papyraceus* populations offers unique
 18 opportunities to indirectly test the response of different pollinator guilds to corona
 19 manipulation, and the consequences for seed production. The results of the experiment
 20 revealed similar patterns of variation in the dimorphic and monomorphic regions. Corona
 21 removal had a detrimental effect only in the pollination of S flowers, with 50% reduction in
 22 seed production in both regions (Fig. 5). Furthermore, the comparison between morphs
 23 revealed that seed production was similar in unmanipulated L and S flowers. S-flowers
 24 require the visit of long-tongued insects to successfully deposit pollen on the stigmas (Simón-
 25 Porcar *et al.* 2014). In addition, in dimorphic populations, nocturnal pollination by moths is

1 relatively more important for female fertility of S flowers than diurnal pollination (Pérez-
 2 Barrales & Arroyo 2010). In contrast, both long-tongued and short-tongued pollinators can
 3 deliver pollen to L stigmas (Simón-Porcar *et al.* 2014). If the corona is important in the
 4 attraction of nocturnal pollinators, our results are consistent with the hypothesis that a
 5 reduction in fragrance emission through corona removal would reduce the frequency of
 6 visitation by moths (Knudsen & Tollsten 1993; Raguso & Willis 2002; Balao *et al.* 2011;
 7 Burdon *et al.* 2015). The corona could also be involved in the visual attraction of pollinators
 8 (Borges *et al.* 2003; Armbruster *et al.* 2005). In fact, syrphid flies are important visitors in the
 9 monomorphic region, and they usually respond to floral patterns of white background with a
 10 central yellow spot (Dinkel & Lunau 2001; Lunau 2014, although fragrant stimuli is also
 11 important, particularly in non-yellow flowers, Primante & Dötterl, 2010). However, if the
 12 corona were important for visual attraction, L- flowers with trimmed coronas would have also
 13 experienced a reduction in fitness. Instead, the reduction occurred only in S-flowers, which
 14 suggests that the corona is visually unimportant (but different pollinators could provide
 15 different responses to corona manipulation, see Medel *et al.* 2003). As visual signalling, the
 16 corona might be relevant in species with contrasting colour patterns (Arroyo & Dafni 1995,
 17 Marques *et al.* 2007). In our study, we cannot rule out if the lower reflectance pattern of the
 18 internal part of the corona compared to the tepals could represent a contrast for insect
 19 approaching flowers frontally, as it occurs with hawkmoths. For insects approaching laterally,
 20 as hover flies do, the corona displays a similar colour pattern than the rest of the perianth. This
 21 deserves future investigations.

22 We conducted an experiment to assess the possible confounding effects of corona
 23 removal on seed production (e.g., reduction caused by damage, resource re-allocation), but the
 24 control experiment showed that this was not the case. Hence, the results obtained in the
 25 experimental plots are not an artefact of the corona manipulation. Although there were

1 differences between morphs in the control experiment, these did not contradict our findings.
 2 Instead, we think the results might be related to our unequal ability to conduct hand-
 3 pollinations in L and S flowers (e.g. stigmas in L-flowers are more exposed than those in S-
 4 flowers).

5 The purpose of grouping plants in experimental plots aimed at avoiding the possible
 6 confounding effects of the incompatibility system in *N. papyraceus* (Arroyo *et al.* 2002), and
 7 testing the effect of distance between compatible plants according to pollinator type. In
 8 general, solitary plants always produced fewer seeds than grouped plants (Fig. 6), probably
 9 due to a limitation of compatible pollen, and the reduction in fitness was stronger in the
 10 monomorphic than the dimorphic region. Grouped plants probably increase floral display and
 11 pollinator attraction (Harder & Barrett 1995; Harder *et al.* 2001; Harder & Johnson 2005;
 12 Bolstad *et al.* 2010). It was remarkable to find that the interaction effect group x morph was
 13 statistically significant only in the dimorphic populations. Specifically, differences in seed
 14 production between S plants in solitary stands and in groups were greater than those observed
 15 in L plants. The relative contribution of long-tongued vs. short-tongued visitors in the
 16 dimorphic region is more important than in the monomorphic region (Santos-Gally *et al.*
 17 2013b). It could be possible that the former were able to bring compatible pollen to solitary
 18 plots from nearby plots (ca. 150 m. distant apart). The reproductive success of S flowers relies
 19 on the prevalence of disassortative pollen transfer mediated by long-tongued pollinators
 20 (Thompson *et al.* 2003; Cesaro & Thompson 2004; Simón-Porcar *et al.* 2014), which might
 21 be more limited in solitary S stands. Female fitness of L plants can be achieved under
 22 different pollinator arrays, regardless the morph of the pollen donor (Pérez-Barrales & Arroyo
 23 2010; Simón-Porcar *et al.* 2014). Previous research has shown that pollen dispersal is more
 24 efficient with nocturnal pollinators, although it usually declines and becomes negligible
 25 within 100 m (Barthelmess *et al.* 2006; Barluenga *et al.* 2011). With diurnal pollinators,

pollen dispersal also declines with distance (Shulke & Waser 2001; Opedal *et al.* 2017). Our results indicate that solitary plots received visits that resulted in seed production, but the relatively low seed production suggests that visitation rate and the arrival of out-crossed pollen was low (Jennersten & Nilsson 1993; Engel & Irwin 2003). This was particularly evident in the monomorphic region, where visitation rates by syrphid flies are high, and usually pay visits among close flowers within the same or nearby plants (Pérez-Barrales R, personal observation).

It was surprising to find that experimental plots of S flowers produced seeds in the monomorphic region (but see Simón-Porcar *et al.* 2014). The main visitors in this region are short-tongued insects, which are inefficient in the pollination of S flowers (Arroyo *et al.* 2002, Pérez-Barrales *et al.* 2007, Pérez-Barrales & Arroyo 2010); instead, they promote self-pollination and a reduction of seed production (Arroyo *et al.* 2002; Simón-Porcar *et al.* 2014). Discordance with our expectations refers only to long-tongued pollinators. It is important to highlight that our data was collected in a single blooming season, and other *Narcissus* species have shown a strong between-year variability in pollination and female fertility, probably associated to availability of pollinators (Baker *et al.* 2000).

The role of the corona in the function of style polymorphism in Narcissus

The form and size of the corona, together with the floral tube, have been proposed as important traits for the evolution and maintenance of style polymorphism in *Narcissus* (Graham & Barrett 2004; Pérez *et al.* 2004; Barrett & Harder 2005; Pérez-Barrales *et al.* 2006; Santos-Gally *et al.* 2013a). Species pollinated by large bees usually present medium size cup-shaped coronas that allow bees to closely fit flowers, and increase the accuracy in the placement of pollen from different morphs on different parts of their bodies, as the Darwinian hypothesis on the evolution of heterostyly predicts (Lloyd & Webb 1992). Other species with

1 larger and wider coronas and tubes provide basking sites due to higher temperatures inside the
2 flower, where insects move freely (Herrera 1995), although these species are style
3 monomorphic (*Narcissus* sect. *Pseudonacissi* and *Bulbocodii*). Experimental manipulations
4 have helped to quantify the value of flower traits in the attraction of pollinators (Borges *et al.*
5 2003; Armbruster *et al.* 2005). To our knowledge, the present study provides the first
6 experimental evidence on the adaptive value of the corona in a style dimorphic species by
7 measuring its effects on seed production. It was unpractical to obtain data on insect visitation
8 in the experimental plots due to the low visitation rates and the difficulties of observing
9 nocturnal pollinators (Pérez-Barrales *et al.* 2007). Although VOCs production in the corona
10 was similar to those produced in the rest of the flower, the relatively high concentration of
11 osmophores suggests that fragrance emission is greater. Hence, it is likely that the floral
12 corona is important for the attraction of nocturnal moths, which largely contribute to the
13 maintenance of style polymorphism (Pérez-Barrales & Arroyo 2010). A reduction in the
14 visitation rate of long-tongued pollinator could reduce the fertility of S-plants, which would
15 explain their absence in monomorphic populations. Surprisingly, our results in the
16 monomorphic area do not support this prediction. We think that these results might be related
17 to particular mild weather conditions during the study period (monomorphic populations
18 usually flower during colder periods with freezing nocturnal temperatures than dimorphic
19 populations), which can change dramatically the composition of pollinator fauna. Long-term
20 studies are required to evaluate yearly fluctuations in pollinators and implications for
21 pollination (Herrera 1987; 1988; Fabina *et al.* 2010). Nevertheless, one of the key results of
22 our study, i.e. corona removal affected seed production of S flowers dramatically (Fig. 5), can
23 only be explained in terms pollination by long-tongued pollinators (mostly moths and
24 hawkmoths), most likely through olfactory quantitative stimuli or a subtle visual stimuli.
25 Corona was less important for seed production in L-flowers. Probably, opportunistic short-

tongued insects (mostly syrphid flies) use more floral cues and pollinate equally L-flowers with or without coronas (see discussion above). Here we provided a mechanistic explanation for former results (Pérez-Barrales & Arroyo 2010; Simón-Porcar *et al.* 2015b) on the role of contrasting pollinator groups.

The diverse pollinator arrays and shifts are present in other *Narcissus* species, both across populations (*N. tazetta*, Arroyo & Dafni 1995) and species (*N. rupicola*, *N. watieri*, Pérez-Barrales *et al.* 2006; *Narcissus* subgenera *Hermione*, Santos-Gally *et al.* 2013a). While pollinator shifts have been associated to perianth changes (Pérez-Barrales *et al.*, 2006, 2007, 2009; Santos-Gally *et al.* 2013a; Pérez-Barrales *et al.* 2014), it remains unknown if it also involves the appearance of fragrance ecotypes. The high variability of chemical composition of fragrances in these *Narcissus* species, including among-population variation, (Dobson *et al.* 1997) offers a promising avenue for further investigating the role of floral fragrances in the attraction of different functional pollinators and their consequences in the maintenance of stylar polymorphism.

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4 **References**

- 5 Andersson, S. (2003). Foraging responses in the butterflies *Inachis io*, *Aglais urticae*
6 (Nymphalidae), and *Gonopteris rhamni* (Pieridae) to floral scents. *Chemoecology*, **13**, 1-
7 11.
- 8 Armbruster, W.S., Antonsen, L., Pélabon, C. (2005) Phenotypic selection on *Dalechampia*
9 blossoms: honest signaling affects pollination success. *Ecology* **86**, 3323-3333.
- 10 Armbruster, W. S., Hansen, T. F., Pélabon, C., Pérez-Barrales, R., Maad, J. (2009). The
11 adaptive accuracy of flowers: measurement and microevolutionary patterns. *Annals of*
12 *Botany*, **103**, 1529-1545.
- 13 Armbruster, W.S., Pérez-Barrales, R., Arroyo, J., Edwards, M.E., Vargas, P. (2006) Three-
14 dimensional reciprocity of floral morphs in wild flax (*Linum suffruticosum*): a new twist
15 on heterostyly. *New Phytologist*, **171**, 581-590.
- 16 Arroyo, J., Dafni, A. (1995) Variations in habitat, season, flower traits and pollinators in
17 dimorphic *Narcissus tazetta* L. (Amaryllidaceae) in Israel. *New Phytologist* **129**, 135-
18 146.
- 19 Arroyo, J., Barrett, S.C.H., Hidalgo, R., Cole, W.W. (2002) Evolutionary maintenance of
20 stigma-height dimorphism in *Narcissus papyraceus* (Amaryllidaceae). *American*
21 *Journal of Botany*, **89**, 1242-1249.
- 22 Ashman, T. L. (2009) Sniffing out patterns of sexual dimorphism in floral scent. *Functional*
23 *Ecology*, **23**, 852-862
- 24 Ashman, T. L., Bradburn, M., Cole, D.H., Blaney, B.H., Raguso, R. A. (2005) The scent of a
25 male: the role floral volatiles in pollination of a gender dimorphic plant. *Ecology* **86**,
26 2009-2105.
- 27 Balao, F., Herrera, J., Talavera, S., Dotterl, S. (2011). Spatial and temporal patterns of floral
28 scent emission in *Dianthus inoxianus* and electroantennographic responses of its
29 hawkmoth pollinator. *Phytochemistry*, **72**, 601-609.
- 30 Barrett, S. C. H. (2002) The evolution of plant sexual diversity. *Nature Review Genetics*, **3**,
31 274-284

- 1 Barrett, S. C. H. (2010). Darwin's legacy: the form, function and sexual diversity of flowers.
2 *Philosophical transactions of the Royal Society B- Biological Sciences*, **365**, 351-368.
- 3 Baker, A. M, Barrett, S.C.H., Thompson, J. D. (2000) Variation of pollen limitation in the
4 early flowering Mediterranean geophyte *Narcissus assoanus* (Amaryllidaceae).
5 *Oecologia*, **124**, 529–535.
- 6 Barrett, S.C.H., Harder, L.D. (2005) The evolution of polymorphic sexual systems in
7 daffodils (*Narcissus*). *New Phytologist*, **165**, 45-53.
- 8 Barrett, S.C.H., Jesson, L. K., Baker, A.M. (2000) The evolution and function of stylar
9 polymorphisms in flowering plants. *Annals of Botany* **85** (suppl. A), 253-265.
- 10 Barluenga, M., Austerlitz, F., Elzinga, J. A., Teixeira, S., Goudet, J., Bernasconi, G. (2011).
11 Fine-scale spatial genetic structure and gene dispersal in *Silene latifolia*. *Heredity*, **106**,
12 13-24.
- 13 Barthelmess, E. L., Richards, C. M., McCauley, D. E. (2006). Relative effects of nocturnal vs
14 diurnal pollinators and distance on gene flow in small *Silene alba* populations. *New*
15 *Phytologist*, **169**, 689–698.
- 16 Blanchard, J. W. (1990) *Narcissus: a guide to wild daffodils*. Alpine Garden Society, Woking,
17 UK.
- 18 Bolstad, G. H., Armbruster, W. S., Pélabon, C., Pérez-Barrales R., Hansen, T. F. (2010)
19 Direct selection at the blossom level on floral reward by pollinators in a natural
20 population of *Dalechampia schottii*: full-disclosure honesty? *New Phytologist*, **188**, 370-
21 384.
- 22 Borges, R. M., Gowda, V., Zacharias, M. (2003) Butterfly pollination and high-contrast visual
23 signals in a low-density distylous plant. *Oecologia*, **136**, 571–573.
- 24 Brys, R., Jacquemyn, H. (2010) Floral display size and spatial distribution of potential mates
25 affect pollen deposition and female reproductive success in distylous *Pulmonaria*
26 *officinalis* (Boraginaceae). *Plant Biology*, **12**, 597-603.
- 27 Brys, R., Jacquemyn, H., Endels, P., van Rossum, F., Hermy, M., Triest, L., De Bruyn L.,
28 Blust, G. D. E. (2004). Reduced reproductive success in small populations of the self-
29 incompatible *Primula vulgaris*. *Journal of Ecology*, **92**, 5-14.
- 30 Brys, R., Jacquemyn, H., Hermy, M. (2007). Impact of mate availability, population size, and
31 spatial aggregation of morphs on sexual reproduction in a distylous, aquatic plant.
32 *American Journal of Botany*, **94**, 119-127.
- 33 Burdon, R. C. F., Raguso, R. A., Kessler, A., Parachnowsitsch, A. L. (2015). Spatiotemporal
34 floral scent variation of *Penstemon digitalis*. *Journal of Chemical Ecology*, **41**, 641-650.

- 1 Cesaro, A. C., Thompson, J. D. (2004). Darwin's cross-promotion hypothesis and the
2 evolution of stylar polymorphism. *Ecology Letters*, **7**, 1209-1215.
- 3 Chittka, L., Menzel, R. (1992). The evolutionary adaptation of flower colors and the insect
4 pollinators' color vision systems. *Journal of Comparative Physiology A*, **171**:171-181.
- 5 De Vega, C., Herrera, C. M., Dotterl, S. (2014). Floral volatiles play a key role in specialized
6 and pollination. *Perspectives in Plant Ecology, Evolution and Systematics*, **16**, 32-42.
- 7 Dafni, A. (1992) *Pollination ecology: A practical approach*. Oxford University Press, Oxford.
- 8 Darwin, C. (1862). *On the various contravariations by which British and foreign orchids are*
9 *fertilised by insects*. John Murray, London.
- 10 Darwin, C. (1876). *The effects of cross and self-fertilization in the vegetable kingdom*. John
11 Murray, London.
- 12 Darwin, C. (1877) *The different forms of flowers on plants of the same species*. John Murray,
13 London.
- 14 Dinkel, T., Lunau, K. (2001) How drone flies (*Eristalis tenax* L., Syrphidae, Diptera) use
15 floral guides to locate food sources. *Journal of Insect Physiology*, **47**, 1111–1118.
- 16 Dobson, H. E. M. (2006) Relationship between floral fragrance composition and type of
17 pollinator. In: Dudareva, N., Pichersky, E. (Eds.). *Biology of floral scent*. CRC Press,
18 Taylor and Francis, Boca Raton, FL, pp. 147-198.
- 19 Dobson, H. E. M., Arroyo, J., Bergsröm, G., Groth, I. (1997) Interspecific variation in floral
20 fragrances within the genus *Narcissus* (Amaryllidaceae). *Biochemical Systematics and*
21 *Ecology* **25**, 685-706.
- 22 Effmert, U., Buss, D., Rohrbeck, D. & Piechulla, B. (2006) Localization of the synthesis and
23 emission of scent compounds within the flower. In: Dudareva, N., Pichersky, E. (Eds.).
24 *Biology of floral scent*. CRC Press, Taylor and Francis, Boca Raton, FL, pp. 105-124.
- 25 Engel, E. C., Irwin, R. E. (2003) Linking pollinator visitation rate and pollen receipt.
26 *American Journal of Botany*, **90**, 1612-1618
- 27 Fabina, N.S, Abbott, K.C., Gilman, R.T. (2010) Sensitivity of plant-pollinator-herbivore
28 communities to changes in phenology. *Ecological Modelling*, **221**, 453-458
- 29 Faegri, K. & van der Pijl, L. (1979) *The Principles of Pollination Ecology*, 3rd. ed. Pergamon
30 Press, Oxford.
- 31 Fenster, C. C., Ambruster, W.S., Wilson, P., Dudash, M.R. & Thompson, J.D. (2004)
32 Pollination syndromes and floral specialization. *Annual Review in Ecology and*
33 *Systematics*, **35**, 375-403.

- 1 Fenster, C. C., Reynolds, R. J., Williams C. W., Makowsky, R., Dudash, M. R. (2015).
2 Quantifying hummingbird preference for floral trait combinations: the role of selection
3 of trait interactions in the evolution of pollination syndromes. *Evolution*, **69**, 1113-1127.
- 4 Galen, C., Newport, M. E. A. (1988) Pollination quality, seed set, and flower traits in
5 *Polemonium viscosum*: complementary effects of variation in flower scent and size.
6 *American Journal of Botany*, **75**, 900-905.
- 7 Galen, C., Zimmer, K. A., Newport, M. E. (1987) Pollination in floral scent morphs of
8 *Polemonium viscosum*: A mechanisms for disruptive selection on flower size. *Evolution*,
9 **41**, 599-606.
- 10 García-Robledo, C., Mora, F. (2007). Pollination biology and the impact of floral display,
11 pollen donors, and distyly on seed production in *Arcytophyllum lamarum* (Rubiaceae).
12 *Plant Biology*, **9**, 453-461.
- 13 Gaskett, A. C., Conti, E., Schiestl, F. (2005) Floral odor variation in two heterostylous species
14 of *Primula*. *Journal of Chemical Ecology*, **31**, 1223-1228.
- 15 Graham, S.W., Barrett, S.C.H. (2004) Phylogenetic reconstruction of the evolution of stylar
16 polymorphism in *Narcissus* (Amaryllidaceae). *American Journal of Botany*, **91**, 1007-
17 1021.
- 18 Harder, D. H., Barrett, S. C. H. (1995). Mating cost of large floral displays in hermaphrodite
19 plants. *Nature*, **373**, 512-515.
- 20 Harder, D. H., Johnson, S. D. (2005) Adaptive plasticity of floral display in animal-pollinated.
21 *Proceeding of the Royal Society B Biological Sciences*, **272**, 2651-2657.
- 22 Herrera, C. M. (1987) Components of pollinator “quality”: comparative analysis of a diverse
23 insect assemblage. *Oikos*, **50**, 79-90.
- 24 Herrera, C. M. (1988) Variation in mutualisms: the spatio-temporal mosaic of a pollinator
25 assemblage. *Biological Journal of the Linnean Society*, **35**, 95-125.
- 26 Herrera, C. M. (1995) Floral biology, microclimate, and pollination by ectothermic bees in an
27 early-blooming herb. *Ecology*, **76**, 218-228.
- 28 IBM Corp. Released 2016. IBM SPSS Statistics for Mac, Version 23.0. Armonk, NY: IBM
29 Corp.
- 30 Jennersten, O., Nilsson, S. G. (1993) Insect flower visitation frequency and seed production in
31 relation to patch size of *Viscaria vulgaris* (Caryophyllaceae). *Oikos*, **68**, 283-292.
- 32 Kearns, C.A., Inouye, D.W. (1993) *Techniques for pollination biologists*. Colorado University
33 Press, Colorado.

- 1 Knight, T.M., Steets, J.A., Vamosi, J.C., Mazer, S.J., Burd, M., Campbell, D.R., Dudash,
2 M.R., Johnston, M.O., Mitchell, R.J., Ashman, T.L. (2005). Pollen limitation of plant
3 reproduction: pattern and process. *Annual Review of Ecology, Evolution and*
4 *Systematics*, **36**, 467-497.
- 5 Knudsen, J.T., Eriksson, R., Gershenzon, J., Stahl, B. (2006). Diversity and distribution of
6 floral scent. *The Botanical Review*, **72**, 1-120.
- 7 Knudsen, J.T., Tollsten, L. (1993) Trends in floral scent chemistry in pollination syndromes:
8 floral scent composition in moth-pollinated taxa. *Botanical Journal of the Linnean*
9 *Society*, **113**, 263-284.
- 10 Larue, A. A. C., Raguso, R. A., Junker, R. R. (2016). Experimental manipulation of floral
11 scent bouquets restructures flower-visitor interactions in the field. *Journal of Animal*
12 *Ecology*, **85**, 396-408.
- 13 Lau, P., Bosque, C. (2003) Pollen flow in distylous *Palicourea fendleri* (Rubiaceae): an
14 experimental test of the disassortative pollen flow hypothesis. *Oecologia*, **135**, 593-600.
- 15 Lloyd, D. G., Webb, C. J. (1986) The avoidance of interference between the presentation of
16 pollen and stigmas in angiosperms. I. Dichogamy. *New Zealand Journal of Botany*, **24**,
17 135-162.
- 18 Lloyd D. G., Webb C. J. (1992) The selection of heterostyly. In: Barrett S.C.H. (Ed.),
19 Evolution and function of heterostyly. Springer-Verlag, Berlin, Germany, pp 179–207.
- 20 Lunau, K. (2014) Visual ecology of flies with particular preference to colour vision and
21 colour preference. *Journal of comparative physiology a sensorial and neural*
22 *behavioural physiology*, **200**, 497-512.
- 23 Marques, I., Rossello-Graell, A., Draper, D., Iriondo, J. M. (2007). Pollination patterns limit
24 hybridization between two sympatric species of *Narcissus* (Amaryllidaceae). *American*
25 *Journal of Botany*, **94**, 1352-1359.
- 26 Massinga, P. H., Johnson, S. D., Harder, L. D. (2005). Heteromorphic incompatibility system
27 and efficiency of pollination in two distylous *Pentstemon* species (Rubiaceae). *Annals of*
28 *Botany*, **95**, 389-399.
- 29 Medel, R., Botto-Mahan, C., Kalin-Arroyo, K. (2003) Pollinator-mediated selection on the
30 nectar guide phenotype in the Andean monkey flower, *Mimulus luteus*, *Ecology*, **84**,
31 1721-1732.
- 32 Meeus, S., Honnay, O., Brys, R., Jacquemyn, H. (2012). Biased morph ratios and skewed
33 mating success contribute to loss of genetic diversity in the distylous *Pulmonaria*
34 *officinalis*. *Annals of Botany*, **109**, 227-235.

- 1 Milet-Pinheiro, P., Ayasse, M., Schindwein, C., Dobson, H. E. M., Dotterl, S. (2012). Host
2 location by visual and olfactory floral clues in an oligolectic bee: innate and learned
3 behaviour. *Behavioral Ecology*, **23**, 531-538.
- 4 Nettancourt D. de (1997) Incompatibility in angiosperms. *Sexual Plant Reproduction*, **10**,
5 185–199.
- 6 Odell, E., Raguso, R. A., Jones, K. N. (1999) Bumblebee Foraging Responses to Variation in
7 Floral Scent and Color in Snapdragons (*Antirrhinum*: Scrophulariaceae). *The American*
8 *Midland Naturalist*, **142**, 257-265.
- 9 Opedal, Ø. H., Falahati-Anbaran, M., Albertsen, E., Armbruster, W. S., Pérez-Barrales, R.,
10 Stenøien, H. K., Pélabon, C. (2017). Euglossine bees mediate only limited long-distance
11 dispersal gene flow in a tropical vine. *New Phytologist*, **213**, 1898-1908.
- 12 Parachnowitsch, A. L., Kessler, A. (2010) Pollinators exert selection on flower size and floral
13 display in *Penstemon digitalis*. *New Phytologist*, **188**, 393-402.
- 14 Parachnowitsch, A. L., Raguso, R. A., Kessler, A. (2012). Phenotypic selection to increase
15 floral scent emission, but no flower size or colour in bee-pollinated *Penstemon digitalis*.
16 *New Phytologist*, **195**, 667-675.
- 17 Pérez-Barrales, R., Arroyo, J. (2010). Pollinator shifts and the loss of style polymorphism in
18 *Narcissus papyraceus* (Amaryllidaceae). *Journal of Evolutionary Biology*, **23**, 1117-
19 1128.
- 20 Pérez-Barrales, R., Arroyo, J., Armbruster, W.S. (2007). Differences in pollinator faunas may
21 generate geographic differences in floral morphology and integration in *Narcissus*
22 *papyraceus* (Amaryllidaceae). *Oikos*, **116**, 1904-1918.
- 23 Pérez-Barrales, R., Pino, R., Albaladejo, R.G., Arroyo, J. (2009). Geographic variation of
24 flower traits in *Narcissus papyraceus* (Amaryllidaceae): do pollinators matter? *Journal*
25 *of Biogeography*, **36**, 1411-1422.
- 26 Pérez, R., Vargas, P., Arroyo, J. (2004) Convergent evolution of flower polymorphism in
27 *Narcissus* (Amaryllidaceae). *New Phytologist*, **161**, 235-252
- 28 Pérez-Barrales, R., Vargas, P., Arroyo, J. (2006) New evidence for the Darwinian hypothesis
29 of heterostyly: breeding systems and pollinators in *Narcissus* sect. Apodanthi. *New*
30 *Phytologist*, **171**, 553–567.
- 31 Pérez-Barrales, R., Simón-Porcar V. I., Santos-Gally, R., Arroyo, J. (2014). Phenotypic
32 integration in style dimorphic daffodils (*Narcissus*, Amaryllidaceae) with different
33 pollinators. *Philosophical Transactions of the Royal Society B-Biological Sciences*, **369**,
34 article number 20130258.

- 1 Prieto-Benitez, S., Dotterl, S., Gimenez-Benavides, L. (2016a) Circadian rhythm of a *Silene*
2 species favours nocturnal pollination and constrains diurnal visitation. *Annals of Botany*,
3 **118**, 907-918.
- 4 Prieto-Benitez S, Millanes, A. M., Dotterl, S., Gimenez-Benavides, L. (2016b) Comparative
5 analyses of flower scent in *Silene* reveal a contrasting phylogenetic signal between night
6 and day emissions. *Ecology and Evolution*, **21**, 7869-7881.
- 7 Primante, C., Dotterl, S. (2010). A syrphid fly uses olfactory cues to find a non-yellow flower.
8 *Journal of Chemical Ecology*, **36**, 1207-1210.
- 9 R Core Team (2016). *R: A language and environment for statistical computing*. R Foundation
10 for Statistical Computing, Vienna, Austria. URL <http://www.R-project.org/>.
- 11 Raguso R. A. (2008). Start making scents: the challenge of integrating chemistry into
12 pollination ecology. *Entomologia experimentalis et applicata*, **128**, 196-207
- 13 Raguso, R. A., Hensel, C., Buchmann, S. L., Nabhan, G. P. (2003). Trumpet flowers of
14 Sonoran Desert: floral biology of *Peniocereus cacti* and sacred *Datura*. *International*
15 *Journal of Plant Science*, **164**, 877-892.
- 16 Raguso, R. A., Light, D. M. (1998) Electroantennogram responses of male *Sphinx perelegans*
17 hawkmoths to floral and green-leaf volatiles. *Entomologia experimentalis et applicata*,
18 **86**, 287-293.
- 19 Raguso, R. A., Light, D. M., Pichersky, E. (1996) Electroantennogram responses of *Hyles*
20 *lineata* (Sphingidae: Lepidoptera) to volatile compounds from *Clarkia breweri*
21 (Onagraceae) and other moth pollinated flowers. *Journal of Chemical Ecology*, **22**,
22 1735–1766.
- 23 Raguso, R.A., Willis, M. A. (2002) Synergy between visual and olfactory cues in nectar
24 feeding by naïve hawkmoths, *Manduca sexta*. *Animal Behavior*, **64**, 685-695.
- 25 Riffel, J. A., Lei, H., Abrell, L., Hildebrand, J. G. (2013). Neural basis of a pollinator's buffet:
26 olfactory specialization and learning in *Manduca sexta*. *Science*, **339**, 200-204.
- 27 Ruíz-Ramón, F., Aguila, D. J., Egea-Cortines, M., Weiss, J. (2014). Optimization of fragrance
28 extraction: daytime and flower age affect scent emission in simple and double narcissi.
29 *Industrial crop and products*, **52**, 671-678.
- 30 Santos-Gally, R., González-Voyer, A., Arroyo, J. (2013a). Deconstructing heterostyly: the
31 evolutionary role of incompatibility system, pollinators, and floral architecture.
32 *Evolution*, **67**, 2072-2082.

- 1 Santos-Gally, R., Pérez-Barrales, R., Simón, V.I., Arroyo, J. (2013b). The role of short-
2 tongued insects in floral variation across the range of a style-dimorphic plant. *Annals of*
3 *Botany*, **111**, 317-328.
- 4 Schemske, W. S. (1980) Evolution of floral display in the orchid *Brassalova nodosa*.
5 *Evolution*, **34**, 489-493.
- 6 Schiestl, F.P. (2010) The evolution of floral scent and insect chemical communication.
7 *Ecology Letters*, **13**, 643-656.
- 8 Schiestl, F. P. (2015) Ecology and evolution of volatile-mediated information transfer in
9 plants. *New Phytologist*, **206**, 571-577.
- 10 Schiestl, F. P., Dotterl, S. (2012). The evolution of floral scent and olfactory preferences in
11 pollinators: coevolution or pre-existing bias? *Evolution*, **66**, 2042-2055.
- 12 Schiestl, F. P., Huber F.K., Gómez, J. M. (2011). Phenotypic selection on floral scent: trade-
13 off between attraction and deterrence? *Evolutionary Ecology*, **25**, 237-248.
- 14 Simón-Porcar, V. I., de Castro, A., Herrero, M., Arroyo, J. (2015a). Ovarian self-
15 incompatibility in *Narcissus papyraceus* (Amaryllidaceae) is the result of a pre-zygotic
16 response. *Botanical Journal of the Linnean Society*, **177**, 629-643.
- 17 Simón-Porcar, V. I., Meagher, T. R., Arroyo, J. (2015b) Dissasortative mating prevails in
18 style dimorphic *Narcissus papyraceus* despite low reciprocity and compatibility of
19 morphs. *Evolution*, **69**, 2276-2288.
- 20 Simón-Porcar, V. I., Santos-Gally, R., Arroyo J. (2014). Long-tongued insects promote
21 disassortative pollen transfer in style-dimorphic *Narcissus papyraceus*
22 (Amaryllidaceae). *Journal of Ecology*, **102**, 116-125.
- 23 Schulke, B., Waser, N. M. (2001). Long-distance pollinator flights and pollen dispersal
24 between populations of *Delphinium nuttallianum*. *Oecologia*, **127**, 239-245.
- 25 Sprengel, C. K. (1793). *Das entdeckte Geheimniss der Natur im Bau und in der Befruchtung*
26 *der Blumen*. Vieweg, Berlin.
- 27 Sprengel, C. K. (1996) Discovery of the secret of nature in the structure and fertilization of
28 flowers. In: Lloyd, D. G. & Barrett, S. C. H. (Eds.), *Floral Biology: Studies on Floral*
29 *Evolution in Animal-Pollinated Plants*, transl. P. Hasse, Chapman & Hall, New York,
30 pp. 3-43.
- 31 Stehlik, I., Caspersen, J. P., Barrett, S. C. H. (2006). Spatial ecology of mating success in a
32 sexually polymorphic plant. *Proceedings of the Royal Society B-Biological Sciences*,
33 **273**, 387-394.

- 1 Stern, W. L., Curry, K. J., Whitten, W. M. (1986) Staining fragrance glands in orchid flowers.
2 *Bulletin of the Torrey Botanical Club*, **113**, 288-297.
- 3 Stone, J.L., Thomson, J.D. (1994) The evolution of distyly: pollen transfer in artificial
4 flowers. *Evolution*, **48**, 1595-1606.
- 5 Thompson, J. D., Barrett, S. C. H., Baker, A. M. (2003). Frequency-dependent variation in
6 reproductive success in *Narcissus*: implications for the maintenance of stigma- eight
7 dimorphism. *Proceedings of the Royal Society B-Biological Sciences*, **270**, 949-953.
- 8 Vogel, S., Müller-Doblies, D. (1975) Eine nachtblütige herbst-Narzisse. Zwiebelblau und
9 blütenökologie von *Narcissus viridiflorus* Schousboe. *Botanische Jahrbücher*
10 *Systematische*, **96**, 427-447.
- 11 Webb, C. J., Lloyd, D.G. (1986) The avoidance of interference between the presentation of
12 pollen and stigmas in angiosperms. II. Herkogamy. *New Zealand Journal of Botany*, **24**,
13 163-178.
- 14 Webb, D. A. (1980) *Narcissus*. In: Tutin, T.G., Heywood, V.H., Burges, N.A., Moore, D.M.,
15 Valentine, D.H., Walters, S.M., Webb, D.A (Eds.), *Flora europaea*, vol. 5. Cambridge
16 University Press, Cambridge, pp. 78-84.
- 17 Wiemer, A. P., More, M., Benítez-Vieyra, S., Cocucci, A. A., Raguso, R. A., Sersic, A. N.
18 (2009). A simple floral fragrance and unusual osmophore structure in *Cyclopogon elatus*
19 (Orchidaceae). *Plant Biology*, **11**, 506-514.
20

Table 1. Results of the generalized linear models in the effect of morph (L- and S-styled), corona manipulation (removed and intact) and group size (one plant and three plants) on seed production in *Narcissus papyraceus* in a natural area region mainly long-tongued pollinators (dimorphic region) and short-tongued pollinators (monomorphic region) (see Material and Methods for analytical details)

Fixed factors	Dimorphic region			Monomorphic region		
	Wald Chi-square	d.f.	<i>P</i>	Wald Chi-square	d.f.	<i>P</i>
Morph	1.649	1	0.002	3.687	1	0.055
Corona	5.322	1	0.113	3.862	1	0.049
Group	27.708	1	<0.001	81.329	1	<0.001
Morph x Corona	4.370	1	0.046	5.199	1	0.023
Morph x Group	14.303	1	<0.001	0.598	1	0.439
Corona x Group	1.341	1	0.247	0.025	1	0.875

1 Table 2. Results of the generalized linear models to assess pollen limitation in a dimorphic and a monomorphic population of *Narcissus papyraceus*. The
2 estimates from the GLM were power transformed to show the results in seeds. (see Material & Methods)

Region		Wald Chi-square	d.f.	P	Supplemented		Control	
					estimate	s.e.	estimate	s.e.
Dimorphic	L-styled plants	0.038	1	0.85	19.00	5.03	17.67	4.69
	S-styled plants	0.176	1	0.67	14.07	3.76	12.00	3.23
Monomorphic	L-styled plants	<0.001	1	0.99	26.55	8.15	26.44	8.12

Figure legends

Figure 1. Geographic range of populations of *Narcissus papyraceus* with areas of long-styled monomorphism (L), anisoplethic dimorphism (L>S) and isoplethic dimorphism (L=S) depicted by dashed lines. Experimental populations: 1, Aznalcázar (Sevilla prov.); 2, La Alcaidesa (Cádiz prov.); 3, Bolonia (Cádiz prov).

Figure 2. Spectral reflectance of *Narcissus papyraceus* flowers through UV and human vision wavelengths. The internal (grey circles) and external (white circles) tepals, and the external part of the corona (white squares) all peaked at ca. 425 nm with a reflectance around 60%, while the internal part of the corona (grey squares) peaked at the same wavelength but at ca. 45% reflectance.

Figure 3. A *Narcissus papyraceus* flower stained with neutral red (left). The floral corona is deeply stained, whereas the tepals are only slightly stained. A natural inflorescence of a long-styled plant photographed in the field (right; photograph by Víctor Fernández-Pasquier).

Figure 4. Mean $\pm$ s.d. of VOCs emitted by long-styled (L) and short-styled (S) morphs of *N. papyraceus*. Experimental treatment refers to flowers where the crown was removed (treatment) while in the other remained intact (control).

Figure 5. Predicted mean seed production $\pm$ s.e. from the generalized linear model to evaluate the effect of corona removal on the seed production of long-styled (L) and short-styled (S) flowers of *Narcissus papyraceus* in a natural dimorphic region (A) and a monomorphic region (B)

1
2 Figure 6. Predicted mean seed production $\pm$ s.e. from the generalized linear model to assess
3 the effect of group size on seed production of long-styled (L) and short-styled (S) flowers of
4 *Narcissus papyraceus* in a natural dimorphic region (A) and a monomorphic region (B)
5

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254x190mm (72 x 72 DPI)

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254x190mm (150 x 150 DPI)

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Figure 6. Predicted mean seed production $\pm$ s.e. from the generalized linear model to assess the effect of group size on seed production of long-styled (L) and short-styled (S) flowers of *Narcissus papyraceus* in a natural dimorphic region (A) and a monomorphic region (B)

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