New Phytologist Supporting Information

**Article title:** Direct evidence supporting Darwin's hypothesis of cross-pollination promoted by sex organ reciprocity

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Methods S1. Methods flow chart for the analysis of mating patterns and phenotypic selection in experimental populations of Narcissus papyraceus.
Methods S2  Experimental setup, molecular procedures and paternity analyses in *Narcissus papyraceus*.

**Experimental setup and parental genotyping**

In December 2008, we collected 150 *N. papyraceus* individuals from a single large population in the centre of the distribution range of the species (36.1° N, 5.73° W). We moved these plants to a glasshouse at the University of Seville and looked after them until their massive flowering in the 2010-2011 flowering season. In November 2010, we collected leaf samples from each individual, which were immediately frozen at −80°C. We isolated DNA following Bernartzky and Tanksley’s (1986) protocol without mercaptoethanol and genotyped each individual with four specific microsatellite markers that showed high genetic variability and good amplification rates (A116, A121, B104, and B112; Simón *et al.*, 2010). We performed polymerase chain reactions (PCRs) in 25-μl reaction mixtures containing: 50 ng of template DNA, 1× PCR buffer, 1.5 mM MgCl2, 0.1 μM fluorescently labelled (6-FAM, VIC, NED, and PET dyes) forward primer, 0.1 μM reverse primer, 0.05 mM each dNTP, and 1.25 U Taq polymerase. PCRs programmes implemented in a Biometra Gradient Thermal Cycler (Biometra, Göttingen, Germany) were: initial denaturation at 94°C/5 min; 45 cycles at 94°C/30 sec, 58°C (A116 and A121) or 59°C (B104 and B112)/30 sec, and 72°C (30 sec); and final extension at 72°C/5 min. PCR products were analysed on an ABI 3130 × 1 Genetic Analyser and sized using GeneMapper version 4.0 (Applied Biosystems, Foster City, CA) and GeneScanTM 500 LIZ size standard.

We divided the individuals into two experimental populations and exposed them to natural pollinators at two sites with different pollination environments in the central and northern ranges of the species distribution (Finca de la Alcaidesa, 36.3° N, 5.4°W, long-tongued pollination environment, LTPE hereafter; and Pinares de Hinojos, 37.3° N, 6.4°W, short-tongued pollination environment, STPE hereafter), during the flowering period of natural populations at each region. In the LTPE, 64 individuals grouped in seven patches were maintained from 23 December 2010 until 21 January 2011. In the STPE, 50 individuals grouped in five patches were maintained from 11 until 31 January 2011. The means of maximum and minimum daily temperatures, and the mean daily rainfall during the experiment were within the normal ranges of climatic conditions in the flowering period of each region in a period of 50 years except for the
significantly higher rain rates in the LTPE (Spanish National Meteorological Agency 2012; Climatic Data from WorldClim database, Hijmans et al., 2005). Each experimental patch was composed of 8–12 individuals in a 2 m diameter circular pattern ensuring regular distances between individuals. The number of individuals (per morph) at each pollination environment and patch was variable, depending on flowering phenology. Plants in each patch were selected to ensure synchronic flowering and high multilocus microsatellite diversity for marker-based paternity assignment within patches. For each experimental patch, the number of alleles with the four markers ranged from 24 to 47. To avoid undesired pollen flow, the selected sites were 16 km and 8 km from the closest known naturally occurring population, respectively, and patches were located at least 300 m apart within each pollination environment (Barthelmess et al., 2006; Pérez-Barrales et al., 2018). Plants were removed from the field after flower withering and kept in the greenhouse until fruit maturation.

*Parental floral traits*

We used plants with their natural floral displays and included the number of flowers as a covariable in our analyses to avoid unpredictable effects on individual fitness due to the alteration of maternal resources. However, variable floral displays might have had undesirable effects on our experimental results. On one hand, floral display is likely to influence pollinator attraction in natural populations (Parachnowitsch & Kessler 2010; Agren et al., 2013). However, prior analyses (Simón-Porcar et al., 2015) showed negligible influence of floral display on mating patterns, and our analyses of phenotypic selection also showed the independence of female and male fitness from the number of flowers of individuals (see Results section). Thus, floral display did not seem to influence pollinator attraction in our experimental populations, composed of patches with relatively few and close individuals that may have had a magnet-effect as a whole (Torices et al., 2018). On the other hand, large floral displays may potentially deplete female fitness through geitonogamy due to late-acting self-incompatibility and ensuing ovule discounting. To test this hypothesis, we analysed the selfing rate detected from paternity analyses in each parental individual as a function of the number of flowers with a binomial GLM and found a negative and non-significant association between both variables (Estimate = -0.037
± 0.019; Z = -1.934; P > 0.05). Thus, we assume that the variable floral displays of experimental individuals did not influence our results.

We measured the first flower of each parental inflorescence, as it was not possible to measure the flowers subjected to pollinators. We tested the phenotypic representativeness of the first flower of *N. papyraceus* inflorescences by measuring the style length, upper and lower anthers height, corolla diameter and tube length in the first and fourth flowers in a control group of 71 inflorescences. This control group of inflorescences was originally from different populations because there was not enough available material from the population of origin of our experiment. The fourth flower position was selected because it was the most available at the time of collection. We calculated the Pearson r coefficient within inflorescences for each floral trait and found strong correlations (r > 0.9, d.f. = 66-69, P < 0.001). The lowest correlation was shown in floral tube (r = 0.9, d.f. = 67, P < 0.001) and the highest in style length (r = 0.95, d.f. = 69, P < 0.001). Linear models showed no effects of population of origin on the regression of any traits (F < 2.3913, d.f. = 4, P > 0.06), suggesting that the strong correlation of floral traits within inflorescences is constant for this species. Thus, we assumed there were equal floral traits for all flowers in each parental individual of our experiment.

The individuals in the LTPE had significantly larger corolla, corona and floral tube diameters than the individuals in the STPE (F > 15.35, d.f. = 82, P < 0.001; Supporting Information Table S1). Our hypothesis is that this should be a subproduct of their different phenology, since early and late flowering individuals were selected for the LTPE and the STPE, respectively, to ensure synchronous flowering with other natural populations in their respective areas. This difference could not influence the results of the analyses of mating patterns, which was performed separately for each pollination environment and, given the equal reproductive success of plants at both pollination environments, could not influence the patterns of phenotypic selection either, since they are independent from absolute trait values. Remarkably, the pollination environment had no effect on either the calculated selection differentials or the selection gradients (see Results section and Supporting Information Tables S5 and S6).
Female fitness

We collected all matured fruits in March 2011 and counted the total number of produced seeds as maternal fitness measure. Because fruit and seed production were strongly dependent on the flower position, 92% of seeds were produced in the first four flowers of each plant, and since the plants varied in the number of flowers, we used the first four flowers per inflorescence to calculate maternal fitness, so that the values were comparable among individuals (Simón-Porcar et al., 2015).

Seedling genotyping and paternity analyses

Seeds were stored in darkness at 4ºC until sowed in November 2011. We grew them under homogeneous conditions (Simón-Porcar et al., 2015) until March 2012, when we selected three seedlings of every produced fruit (N = 760 seedlings). The three tallest seedlings were selected to ensure enough DNA material. Because germination percentage and maximum height did not differ between maternal style morphs or mating combinations (Simón-Porcar et al., 2015), sampling bias was regarded as negligible. The aboveground fractions of seedlings were dried in silica gel, and DNA was extracted using the DNeasy Plant Mini Kit (Qiagen, Chatsworth, CA). Seedling genotyping for the four SSR markers (A116, A121, B104, and B112; Simón et al., 2010) was performed under the same conditions as for the parents but with an annealing temperature of 50ºC, which allowed higher amplification rates without affecting the marker results. The genotyping error per locus was estimated from 40 individual replicates at 1.4 ± 0.2%.

We combined direct and probabilistic paternity exclusion analyses to determine the parental pair for each genotyped seedling. We first compared progeny and parental genotypes and directly assigned a single paternal parent to a total of 545 seedlings (72%). Autogamy was considered as some flowers set seeds by selfing (Arroyo et al., 2002). Seedlings that remained unassigned were subjected to exclusion analysis with the software CERVUS 3.0 (Marshall et al., 1998), which uses log-likelihood ratios (Meagher, 1986) to assign the most probable paternal parent to each seedling with 80–95% of confidence. We ran separate analyses for each patch, simulating 10,000 offspring with 100% candidate paternal parents, sampled and allowing for selfing. The mistyping error rate was set as the estimated genotyping error (0.014), and a
minimum of two loci typed was required. CERVUS assigned 68 and 38 seedlings to a single father with the 80% and 95% of confidence, respectively (total assigned seedlings 106, 14%). Hence, in this study we assigned a total of 651 seedlings (86%) with an overall assignment probability of 97.6%. Observed correlated paternity, calculated as the proportion of seedlings sired by the same individual, was 37 ± 23% (average ± SD) within maternal individuals and 22 ± 26% within fruits. It was not possible to distinguish whether two seeds in a maternal plant were sired by the same individual as a result of a single or of two different pollinator visits and, given that each seed has the same value for testing our hypothesis on pollen transfer efficiency, we treated each seed as an independent successful mating event in subsequent analyses.

References

Agren J, Hellstrom F, Torang P, Ehrlen J. 2013. Mutualists and antagonists drive among-population variation in selection and evolution of floral display in a perennial herb. *Proceedings of the National Academy of Sciences of the United States of America* **110**: 18202–18207.

Arroyo J, Barrett SCH, Hidalgo R, Cole WW. 2002. Evolutionary maintenance of stigma-height dimorphism in *Narcissus papyraceus* (Amaryllidaceae). *American Journal of Botany* **89**: 1242–1249.

Barthelmess EL, Richards CM, Mccauley DE. 2006. Relative effects of nocturnal vs. diurnal pollinators and distance on gene flow in small *Silene alba* populations. *New Phytologist* **169**: 689–698.

Bernartzky R, Tanksley S. 1986. Genetics of acting-related sequences in tomato. *Theoretical and Applied Genetics* **72**: 314–324.

Hijmans RJ, Cameron SE, Parra JL, Peter G, Jones AJ. 2005. Very high resolution interpolated climate surfaces for global land areas. *International Journal of Climatology: A Journal of the Royal Meteorological Society* **25**: 1965–1978.

Marshall TC, Slate J, Kruuk LEB, Pemberton JM. 1998. Statistical confidence for likelihood-based paternity inference in natural populations. *Molecular Ecology* **7**: 639–655.
Meagher TR. 1986. Analysis of paternity within a natural population of Chamaelirium luteum. 1. Identification of most-likely male parents. *American Naturalist* 128: 199–215.

Parachnowitsch AL, Kessler A. 2010. Pollinators exert natural selection on flower size and floral display in Penstemon digitalis. *New Phytologist* 188: 393–402.

Pérez-Barrales R, Abarca CA, Santos-Gally R, Schiestl FP, Arroyo J. 2018. The function of the floral corona in the pollination of a Mediterranean style dimorphic daffodil. *Plant Biology* 20: 118–127.

Simón VI, Picó FX, Arroyo J. 2010. New microsatellite loci for Narcissus papyraceus (Amaryllidaceae) and cross-amplification in other congeneric species. *American Journal of Botany* 97: e10–e13.

Simón-Porcar VI, Meagher TR, Arroyo J. 2015. Disassortative mating prevails in style-dimorphic Narcissus papyraceus despite low reciprocity and compatibility of morphs. *Evolution* 69: 2276–2288.

Spanish National Meteorological Agency. 2011. URL http://www.aemet.es/. [accessed 15 March 2011].

Torices R, Gómez JM, Pannell JR. 2018. Kin discrimination allows plants to modify investment towards pollinator attraction. *Nature Communications* 9. DOI: 10.1038/s41467-018-04378-3.
**Fig. S1** Within-mating pair correlation of any given trait when all possible outcrosses are allowed in a population. Excluding selfing implies a slightly negative slope on the regression line.
Fig. S2 Percentage of variance explained by the dimensions resulting from a Principal Components Analysis of the seven floral traits, measured in the parental individuals of *Narcissus papyraceus*. 
Fig. S3 Graphical representation of the significant results of mismatch for each level of sex organs and pollination environment in experimental populations of *Narcissus papyraceus*. Individuals with a lower mismatch in the lower-level sex organs and upper-level sex organs had greater mating success under the long-tongued pollination environment (a) and short-tongued pollination environment (b), respectively.
Table S1 (a) Results of ANOVAs testing for differences in the seven floral traits measured in the parental individuals of *Narcissus papyraceus* between floral morphs and pollination environments. Significance: ***P < 0.001. (b) Mean and sd values of each trait for the whole dataset and the groups with significant differences. LTPE: long-tongued pollination environment; STPE: short-tongued pollination environment; L: long-styled morph; S: short-styled morph.

| Trait       | df | F   | P    | df    | F    | P     |
|-------------|----|-----|------|-------|------|-------|
| Corolla width | 82 | 0.596 | 0.442 | 82 | 19.38 | <0.001 *** |
| Corona width  | 82 | 0.795 | 0.375 | 82 | 15.35 | <0.001 *** |
| Tube width    | 82 | 0.135 | 0.715 | 82 | 17.64 | <0.001 *** |
| Corona height | 81 | 1.213 | 0.274 | 81 | 0.354 | 0.553 |
| Tube length   | 82 | 0.167 | 0.683 | 82 | 0.003 | 0.956 |
| Upper anthers height | 82 | 0.224 | 0.637 | 82 | 0.021 | 0.884 |
| Lower anthers height | 82 | 0.456 | 0.502 | 82 | 0.913 | 0.342 |
| Style length  | 82 | 196.74 | <0.001 *** | 82 | 0.101 | 0.751 |

| Trait       | mean (sd) |
|-------------|-----------|
| Whole dataset (n = 118) | |
| Corolla width | 33.913 (4.415) |
| Corona width  | 6.516 (0.961) |
| Tube width    | 2.542 (0.385) |
| Corona height | 3.839 (1.187) |
| Tube length   | 15.845 (1.506) |
| Upper anthers height | 17.059 (1.55) |
| Lower anthers height | 13.652 (1.528) |
| Style length  | 12.779 (4.344) |
| LTPE (n = 68) | |
| Corolla width | 35.2 (4.05) |
| Corona width  | 6.78 (0.941) |
| Tube width    | 2.65 (0.344) |
| STPE (n = 50) | |
| Corolla width | 31.1 (3.87) |
| Corona width  | 5.97 (0.758) |
| Tube width    | 2.31 (0.367) |
| L (n= 55) | |
| Style length  | 16.5 (2.77) |
| S (n=63) | |
| Style length  | 9.24 (1.91) |
**Table S2** Pearson correlation coefficients between the seven floral traits measured in the parental individuals of *Narcissus papyraceus*. Significance: ***P < 0.001, **P < 0.01, *P < 0.05.

|                   | Corolla width | Corona width | Tube width | Corona height | Tube length | Upper anthers height |
|-------------------|---------------|--------------|------------|---------------|-------------|---------------------|
| Corona width      | 0.41 ***      |              |            |               |             |                     |
| Tube width        | 0.27 *        | 0.48 ***     |            |               |             |                     |
| Corona height     | 0.08          | -0.03        | 0.07       |               |             |                     |
| Tube length       | 0.26 *        | -0.03        | -0.04      | 0.14          |             |                     |
| Upper anthers height | 0.34 **      | -0.02        | 0.06       | 0.02          | 0.82 ***    |                     |
| Lower anthers height | 0.39 ***      | 0.07         | -0.06      | 0.08          | 0.66 ***    | 0.65 ***            |
Table S3  Best fitting models (ΔAICc < 2.0) for observed mating success of *Narcissus papyraceus* individual pairs as a function of number of flowers, spatial distance between individuals, sex organs mismatch, pollinator environment (PE), floral tube width and their interactions, for each level of sex organs. Each column presents the coefficient for the different explanatory variables included. The cross indicates the variable was present in the model.

|          | Flowers | Distance | Mismatch | PE | Tube | Mismatch: PE | Mismatch: Tube | PE: Tube | Mismatch: PE:Tube | df | logLik   | AICc   | ΔAICc | weight |
|----------|---------|----------|----------|-----|------|--------------|---------------|----------|------------------|----|----------|--------|-------|--------|
| **Upper** |         |          |          |     |      |              |               |          |                  |    |          |        |       |        |
| sex organs | 0.476   | -0.213   | +        | -0.316 | +    | -0.303       | +             | -0.299   | -0.316           | 6  | -249.73 | 511.8  | 0     | 0.114  |
|           | 0.47    | -0.277   | +        | -0.303 | +    | -0.316       | +             | -0.316   | -0.303           | 7  | -249.14 | 512.7  | 0.93  | 0.072  |
|           | 0.501   |          | +        | -0.324 |      |              |               |          |                  | 5  | -251.31 | 512.8  | 1.08  | 0.067  |
|           | 0.476   | -0.103   |          | -0.299 | +    | -0.316       | +             | -0.299   | -0.316           | 7  | -249.26 | 512.9  | 1.16  | 0.064  |
|           | 0.487   |          | -0.219   | +        | -0.255 | +             |               |          |                  | 7  | -249.54 | 513.5  | 1.73  | 0.048  |
|           | 0.47    | -0.114   |          | -0.276 | +    | -0.283       | +             | -0.276   | -0.283           | 8  | -248.57 | 513.7  | 1.91  | 0.044  |
|           | 0.477   |          | 0.267    |          | -0.257 |              | -0.496        |          |                  | 7  | -249.67 | 513.7  | 1.98  | 0.042  |
| **Lower** |         |          |          |     |      |              |               |          |                  |    |          |        |       |        |
| sex organs | 0.319   | -0.209   |          |      |      |              |               |          |                  | 4  | -313.45 | 635    | 0     | 0.216  |
|           | 0.323   | -0.214   |          |      |      |              |               |          |                  | 5  | -313.09 | 636.4  | 1.33  | 0.111  |
|           | 0.337   |          |          |      |      |              |               |          |                  | 5  | -313.23 | 636.6  | 1.62  | 0.096  |
|           | 0.325   | -0.19    |          |      |      |              |               |          |                  | 5  | -313.15 | 636.7  | 1.63  | 0.096  |
|           | 0.321   | -0.206   | -0.049   |      |      |              |               |          |                  | 5  | -313.24 | 636.7  | 1.65  | 0.095  |
|           | 0.327   | -0.185   | -0.074   |      |      |              |               |          |                  | 8  | -310.68 | 637.8  | 2.78  | 0.054  |
Table S4 Comparison of the female ($\lambda_F$), male ($\lambda_M$) and absolute ($\lambda_A$) fitness of Narcissus papyraceus individuals. (a) Pearson correlations between the female, male and absolute individual fitness. Results for each pair of measures in the whole dataset and subsets for each pollination environment (LTPE: long-tongued pollination environment; STPE: short-tongued pollination environment) and morph (L: long-styled morph; S: short-styled morph). (b) Results of Levene’s test of homogeneity comparing the variance of female and male fitness. Significance: ***$P < 0.001$, **$P < 0.01$, *$P < 0.05$, ·$P < 0.1$.

| a           | r      | t      | df | P       |
|-------------|--------|--------|----|---------|
| $\lambda_F - \lambda_M$ |        |        |    |         |
| Whole dataset | 0.078  | 0.821  | 110| 0.414   |
| LTPE        | 0.137  | 1.069  | 60 | 0.289   |
| STPE        | -0.019 | -0.131 | 48 | 0.896   |
| L           | 0.158  | 1.133  | 50 | 0.263   |
| S           | -0.023 | -0.174 | 58 | 0.863   |
| LTPE - L    | 0.052  | 0.319  | 38 | 0.752   |
| LTPE - S    | 0.258  | 1.195  | 20 | 0.246   |
| STPE - L    | 0.023  | 0.109  | 22 | 0.914   |
| STPE - S    | -0.058 | -0.284 | 24 | 0.779   |
| $\lambda_F - \lambda_A$ |        |        |    |         |
| Whole dataset | 0.834  | 15.874 | 110| < 0.001 *** |
| LTPE        | 0.849  | 12.427 | 60 | < 0.001 *** |
| STPE        | 0.809  | 9.544  | 48 | < 0.001 *** |
| L           | 0.861  | 11.966 | 50 | < 0.001 *** |
| S           | 0.799  | 10.125 | 58 | < 0.001 *** |
| LTPE - L    | 0.754  | 7.084  | 38 | < 0.001 *** |
| LTPE - S    | 0.914  | 10.065 | 20 | < 0.001 *** |
| STPE - L    | 0.833  | 7.048  | 22 | < 0.001 *** |
| STPE - S    | 0.784  | 6.183  | 24 | < 0.001 *** |
| $\lambda_M - \lambda_A$ |        |        |    |         |
| Whole dataset | 0.615  | 8.173  | 110| < 0.001 *** |
| LTPE        | 0.64   | 6.453  | 60 | < 0.001 *** |
| STPE        | 0.572  | 4.832  | 48 | < 0.001 *** |
| L           | 0.639  | 5.867  | 50 | < 0.001 *** |
| S           | 0.583  | 5.461  | 58 | < 0.001 *** |
| LTPE - L    | 0.695  | 5.951  | 38 | < 0.001 *** |
| LTPE - S    | 0.628  | 3.611  | 20 | 0.002 ** |
| STPE - L    | 0.573  | 3.281  | 22 | 0.003 ** |
| STPE - S    | 0.575  | 3.44   | 24 | 0.002 ** |
| b            | Var $\lambda_F$ | Var $\lambda_M$ | df | $F$  | $P$  |
|--------------|-----------------|-----------------|----|------|------|
| Whole dataset| 0.012           | 0.006           | 222| 10.779 | 0.001 ** |
| LTPE         | 0.013           | 0.006           | 122| 4.8298 | 0.029 * |
| STPE         | 0.01           | 0.005           | 98 | 6.4464 | 0.012 * |
| L            | 0.015           | 0.007           | 102| 3.6187 | 0.059 · |
| S            | 0.009           | 0.005           | 118| 5.951 | 0.016 * |
Table S5 Selection differentials (a) and selection gradients (b) resulting from the phenotypic selection analyses on the seven measured floral traits, based on female (λF), male (λM) and absolute (λA) individual fitness in *Narcissus papyraceus*. Significance: ***P < 0.001, **P < 0.01, *P < 0.05, · P < 0.1. In (a), bold values are significant after Bonferroni correction.

| a | λF Coefficient (SE) | t | P  | λM Coefficient (SE) | t  | P  | λA Coefficient (SE) | t  | P  |
|---|---------------------|---|----|---------------------|---|----|---------------------|---|----|
| No. Flowers | 0.011 (0.01) | 1.03 | 0.305 | 0.011 (0.007) | 1.62 | 0.108 | 0.011 (0.006) | 1.663 | 0.099 |
| Corolla width | 0.037 (0.011) | **3.369** | **0.001** | 0.003 (0.008) | 0.351 | 0.727 | 0.019 (0.008) | 2.576 | 0.012 |
| Tube width | 0.006 (0.012) | 0.465 | 0.643 | -0.004 (0.008) | -0.475 | 0.636 | 0 (0.008) | -0.042 | 0.967 |
| Tube length | -0.011 (0.012) | -0.911 | 0.365 | 0 (0.008) | 0.062 | 0.951 | -0.006 (0.008) | -0.729 | 0.468 |
| Corona width | 0.017 (0.012) | 1.449 | 0.151 | -0.006 (0.008) | -0.816 | 0.417 | 0.005 (0.008) | 0.648 | 0.519 |
| Corona height | 0.006 (0.012) | 0.506 | 0.614 | -0.006 (0.008) | -0.824 | 0.412 | -0.001 (0.008) | -0.084 | 0.933 |

| b | λF Coefficient (SE) | t  | P  | λM Coefficient (SE) | t  | P  | λA Coefficient (SE) | t  | P  |
|---|---------------------|---|----|---------------------|---|----|---------------------|---|----|
| Intercept | 1.169 (1.144) | 1.021 | 0.311 | 2.234 (0.802) | 2.787 | 0.007 | 1.646 (0.758) | 2.173 | 0.033 |
| No. Flowers | 0.089 (0.05) | 1.789 | 0.078 | 0.042 (0.035) | 1.183 | 0.241 | 0.064 (0.033) | 1.922 | 0.059 |
| Corolla width | -0.065 (0.159) | -0.411 | 0.683 | -0.056 (0.112) | -0.5 | 0.619 | -0.057 (0.106) | -0.544 | 0.588 |
| Corona width | -0.026 (0.128) | -0.201 | 0.841 | 0.036 (0.09) | 0.403 | 0.688 | 0.005 (0.085) | 0.053 | 0.958 |
| Tube width | -0.047 (0.129) | -0.361 | 0.719 | 0.179 (0.091) | 1.966 | 0.053 | 0.069 (0.085) | 0.807 | 0.423 |
| Corona height | 0.055 (0.052) | 1.059 | 0.293 | -0.008 (0.036) | -0.23 | 0.819 | 0.022 (0.034) | 0.642 | 0.523 |
| Tube length | 0.289 (0.187) | 1.546 | 0.127 | 0.303 (0.129) | 2.358 | **0.021** | 0.281 (0.124) | 2.269 | **0.027** |
| No. Flowers ^2 | -0.001 (0.001) | -1.326 | 0.189 | 0 (0) | -0.825 | 0.412 | -0.001 (0) | -1.399 | 0.167 |
| Corolla width ^2 | 0.001 (0.001) | 0.649 | 0.519 | 0 (0.001) | 0.56 | 0.577 | 0.001 (0.001) | 0.755 | 0.453 |
| Corona width ^2 | 0.003 (0.019) | 0.161 | 0.873 | -0.008 (0.014) | -0.597 | 0.552 | -0.002 (0.013) | -0.188 | 0.852 |
| Tube width ^2 | 0.055 (0.126) | 0.431 | 0.668 | -0.174 (0.089) | -1.951 | 0.055 | -0.063 (0.084) | -0.752 | 0.454 |
| Corona height ^2 | -0.006 (0.006) | -0.985 | 0.328 | 0 (0.004) | -0.097 | 0.923 | -0.003 (0.004) | -0.76 | 0.45 |
| Tube length ^2 | -0.013 (0.008) | -1.663 | 0.101 | -0.013 (0.005) | -2.378 | **0.02** | -0.012 (0.005) | -2.378 | **0.02** |
Table S6 Results of the ANCOVAs regressing female (λF), male (λM) or absolute (λA) individual fitness of *Narcissus papyraceus* on each (a) or all (b) floral traits and their interactions with floral morph and pollination environment (PE). Significance: ***P < 0.001, **P < 0.01, *P < 0.05, · P < 0.1.

| a             | λF df | λF F  | λF P  | λM df | λM F  | λM P  | λA df | λA F  | λA P  |
|---------------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| No. Flowers   | 1     | 1.038 | 0.311 | 1     | 2.57  | 0.112 | 1     | 2.724 | 0.102 |
| Morph         | 1     | 0.023 | 0.881 | 1     | 1.341 | 0.249 | 1     | 0.74  | 0.392 |
| PE            | 1     | 0.246 | 0.621 | 1     | 0.014 | 0.905 | 1     | 0.312 | 0.577 |
| No. Flowers : Morph | 1 | 0.203 | 0.653 | 1 | 0.03  | 0.863 | 1 | 0.097 | 0.756 |
| No. Flowers : PE | 1 | 1.187 | 0.278 | 1 | 0.251 | 0.617 | 1 | 1.225 | 0.271 |
| Residuals     | 106   |       |       | 108   |       |       | 106   |       |       |
| Corolla width |       | 11.082| 0.001 |       | 1     | 0.12  | 0.73  |       | 6.481 | 0.013 |
| Morph         |       | 0.078 | 0.781 |       | 1     | 0.479 | 0.491 |       | 0.35  | 0.556 |
| PE            |       | 0.783 | 0.379 |       | 1     | 0.269 | 0.606 |       | 0.056 | 0.814 |
| Corolla width : Morph | 1 | 1.005 | 0.319 | 1 | 1.298 | 0.258 | 1 | 1.521 | 0.221 |
| Corolla width : PE | 1 | 0.291 | 0.591 | 1 | 0.081 | 0.777 | 1 | 0.202 | 0.655 |
| Residuals     | 75    |       |       | 78    |       |       | 75    |       |       |
| Tube width    |       | 0.206 | 0.651 |       | 1     | 0.223 | 0.638 |       | 0.002 | 0.967 |
| Morph         |       | 0     | 0.999 |       | 1     | 0.468 | 0.496 |       | 0.139 | 0.711 |
| PE            |       | 0.242 | 0.624 |       | 1     | 0.902 | 0.345 |       | 1.125 | 0.292 |
| Tube width : Morph | 1 | 0.111 | 0.74  | 1 | 1.311 | 0.256 | 1 | 0.151 | 0.699 |
| Tube width : PE | 1 | 0.019 | 0.89  | 1 | 0.28  | 0.598 | 1 | 0.367 | 0.547 |
| Residuals     | 75    |       |       | 78    |       |       | 75    |       |       |
| Tube length   |       | 0.829 | 0.365 |       | 1     | 0.004 | 0.952 |       | 0.53  | 0.469 |
| Morph         |       | 0.011 | 0.917 |       | 1     | 0.426 | 0.516 |       | 0.205 | 0.652 |
| PE            |       | 0.477 | 0.492 |       | 1     | 0.405 | 0.527 |       | 0.882 | 0.351 |
| Tube length : Morph | 1 | 1.661 | 0.202 | 1 | 0.015 | 0.903 | 1 | 1.304 | 0.257 |
| Tube length : PE | 1 | 1.851 | 0.178 | 1 | 0.008 | 0.929 | 1 | 1.362 | 0.247 |
| Residuals     | 75    |       |       | 78    |       |       | 75    |       |       |
| Corona width  |       | 2.058 | 0.156 |       | 1     | 0.658 | 0.42  |       | 0.408 | 0.525 |
| Morph         |       | 0.016 | 0.9    |       | 1     | 0.347 | 0.558 |       | 0.179 | 0.673 |
| PE            |       | 0.007 | 0.935 |       | 1     | 1.045 | 0.31  |       | 0.481 | 0.49  |
| Corona width : Morph | 1 | 0.09  | 0.765 | 1 | 1.511 | 0.223 | 1 | 0.102 | 0.75  |
| Corona width : PE | 1 | 2.37  | 0.128 | 1 | 0.03  | 0.863 | 1 | 1.064 | 0.306 |
| Residuals     | 75    |       |       | 78    |       |       | 75    |       |       |
| Corona height |       | 0.257 | 0.614 |       | 1     | 0.666 | 0.417 |       | 0.007 | 0.933 |
| Morph         |       | 0.005 | 0.943 |       | 1     | 0.728 | 0.396 |       | 0.28  | 0.598 |
| PE            |       | 0.53  | 0.469 |       | 1     | 0.626 | 0.431 |       | 1.104 | 0.297 |
| Corona height : Morph | 1 | 0.001 | 0.978 | 1 | 1.129 | 0.291 | 1 | 0.237 | 0.628 |
| Corona height : PE | 1 | 3.806 | 0.055 | 1 | 0.001 | 0.983 | 1 | 2.205 | 0.142 |
| Residuals     | 74    |       |       | 77    |       |       | 74    |       |       |
|                  | $\lambda_F$ |   | $\lambda_M$ |   | $\lambda_A$ |   |
|------------------|-------------|---|-------------|---|-------------|---|
|                  | df | $F$ | $P$ | df | $F$ | $P$ | df | $F$ | $P$ | df | $F$ | $P$ |
| No. Flowers      | 1  | 2.139 | 0.149 | 1  | 2.364 | 0.129 | 1  | 3.389 | 0.071 |  |
| Corolla width    | 1  | 12.391 | 0.001 | 1  | 0.113 | 0.738 | 1  | 6.897 | 0.011 |  |
| Tube width       | 1  | 0.009 | 0.923 | 1  | 0.178 | 0.675 | 1  | 0.124 | 0.726 |  |
| Tube length      | 1  | 4.309 | 0.042 | 1  | 0    | 0.995 | 1  | 2.609 | 0.112 |  |
| Corona width     | 1  | 0.014 | 0.906 | 1  | 0.735 | 0.395 | 1  | 0.344 | 0.56  |  |
| Corona height    | 1  | 0.622 | 0.433 | 1  | 0.52  | 0.474 | 1  | 0.019 | 0.892 |  |
| Morph            | 1  | 0.988 | 0.324 | 1  | 1.077 | 0.304 | 1  | 1.911 | 0.172 |  |
| PE               | 1  | 1.704 | 0.197 | 1  | 0.876 | 0.353 | 1  | 0.054 | 0.817 |  |
| No. Flowers : Morph | 1  | 0.02  | 0.889 | 1  | 0.003 | 0.961 | 1  | 0.033 | 0.857 |  |
| Corolla width : Morph | 1  | 1.331 | 0.253 | 1  | 0.177 | 0.676 | 1  | 0.86  | 0.358 |  |
| Tube width : Morph | 1  | 0.065 | 0.8   | 1  | 2.016 | 0.161 | 1  | 0.281 | 0.598 |  |
| Tube length : Morph | 1  | 3.871 | 0.054 | 1  | 0.001 | 0.977 | 1  | 2.256 | 0.138 |  |
| Corona width : Morph | 1  | 0.007 | 0.934 | 1  | 0.159 | 0.692 | 1  | 0.05  | 0.823 |  |
| Corona height : Morph | 1  | 1.711 | 0.196 | 1  | 1.296 | 0.26  | 1  | 2.373 | 0.129 |  |
| No. Flowers : PE | 1  | 3.176 | 0.08  | 1  | 1.211 | 0.276 | 1  | 3.068 | 0.085 |  |
| Corolla width : PE | 1  | 0.709 | 0.403 | 1  | 0.079 | 0.78  | 1  | 0.396 | 0.532 |  |
| Tube width : PE  | 1  | 0.254 | 0.616 | 1  | 0.199 | 0.657 | 1  | 0.001 | 0.982 |  |
| Tube length : PE | 1  | 0.208 | 0.65  | 1  | 0.018 | 0.895 | 1  | 0.113 | 0.738 |  |
| Corona width : PE | 1  | 3.725 | 0.058 | 1  | 0.01  | 0.922 | 1  | 1.756 | 0.19  |  |
| Corona height : PE | 1  | 0.24  | 0.626 | 1  | 0.041 | 0.84  | 1  | 0.07  | 0.792 |  |
| Residuals        |      | 59  |     |      | 60  |     |      | 59  |     |
Table S7 Selection coefficients (a) and ANCOVAs (b) regressing style length with either female (λF), male (λM) or absolute (λA) individual fitness in *Narcissus papyraceus* (and its interaction with pollination environment, PE) for each floral morph (L: long-styled morph; S: short-styled morph).

### a: Coefficient (SE) t P

|   | Coefficient (SE) | t   | P   |
|---|------------------|-----|-----|
| λF |                   |     |     |
| L  | 0.016 (0.022)    | 0.701 | 0.488 |
| S  | -0.014 (0.014)   | -1.007 | 0.32  |
| λM |                   |     |     |
| L  | 0.02 (0.012)     | 1.683 | 0.1   |
| S  | -0.012 (0.01)    | -1.206 | 0.235 |
| λA |                   |     |     |
| L  | 0.016 (0.015)    | 1.065 | 0.145 |
| S  | -0.013 (0.009)   | -1.486 | 0.294 |

### b: ANCOVAs

|   | λF | λM | λA |
|---|----|----|----|
| df | F  | P  | F  | P  | F  | P  |
| L-morph |     |     |     |     |     |     |
| Style length | 1   | 0.468 | 0.499 | 1   | 2.78 | 0.104 | 1   | 1.091 | 0.303 |
| PE    | 1   | 0.085 | 0.772 | 1   | 1.064 | 0.309 | 1   | 0.566 | 0.457 |
| Style length : PE | 1 | 0.113 | 0.739 | 1 | 0.231 | 0.634 | 1 | 0.01 | 0.92 |
| Residuals | 35 |     |     | 37 |     |     | 35 |     |     |
| S-morph |     |     |     |     |     |     |
| Style length | 1   | 0.99 | 0.326 | 1   | 1.474 | 0.232 | 1   | 2.133 | 0.152 |
| PE    | 1   | 0.705 | 0.407 | 1   | 0.004 | 0.949 | 1   | 0.453 | 0.505 |
| Style length : PE | 1 | 0.31 | 0.581 | 1 | 2.568 | 0.117 | 1 | 0.188 | 0.667 |
| Residuals | 38 |     |     | 39 |     |     | 38 |     |     |
Table S8 Results of the ANCOVAs regressing *Narcissus papyraceus* individual fitness on each (a) or all (b) floral traits and their interactions with sex function. Models are for each pair of measures of female ($\lambda_F$), male ($\lambda_M$) and absolute ($\lambda_A$) fitness. Significance: $***P < 0.001$, $**P < 0.01$, $*P < 0.05$, $\cdot \ P < 0.1$.

| a                  | $\lambda_F - \lambda_M$ |        |        |        | $\lambda_M - \lambda_A$ |        |        |        | $\lambda_F - \lambda_A$ |        |        |
|--------------------|--------------------------|--------|--------|--------|--------------------------|--------|--------|--------|--------------------------|--------|--------|
|                    | df | F       | P   | df | F       | P   | df | F       | P   | df | F       | P   |
| No. Flowers        | 1  | 3.149   | 0.077 · | 1  | 5.369   | 0.021 * | 1  | 3.083   | 0.081 · |
| $\lambda$          | 1  | 0.121   | 0.729 | 1  | 0.082   | 0.774 | 1  | 0.017   | 0.897 |
| No. Flowers : $\lambda$ | 1  | 0.006   | 0.939 | 1  | 0.007   | 0.932 | 1  | 0     | 0.991 |
| Residuals          | 222 | 222 | 220 | 222 | 222 | 220 | 222 | 222 | 220 | 222 | 220 |
| Corolla width      | 1  | 8.737   | **0.004** | 1  | 4.121   | **0.044** * | 1  | 17.92   | < **0.001** *** |
| $\lambda$          | 1  | 0.374   | 0.541 | 1  | 0.212   | 0.646 | 1  | 0.063   | 0.803 |
| Corolla width : $\lambda$ | 1  | 6.607   | **0.011** * | 1  | 2.36    | 0.126 | 1  | 1.819   | 0.179 |
| Residuals          | 161 | 161 | 158 | 161 | 161 | 158 | 161 | 161 | 158 | 161 | 161 |
| Tube width         | 1  | 0.016   | 0.899 | 1  | 0.131   | 0.718 | 1  | 0.133   | 0.716 |
| $\lambda$          | 1  | 0.355   | 0.552 | 1  | 0.22    | 0.64  | 1  | 0.056   | 0.814 |
| Tube width : $\lambda$ | 1  | 0.428   | 0.514 | 1  | 0.09    | 0.765 | 1  | 0.169   | 0.682 |
| Residuals          | 161 | 161 | 158 | 161 | 161 | 158 | 161 | 161 | 158 | 161 | 161 |
| Tube length        | 1  | 0.501   | 0.48  | 1  | 0.208   | 0.649 | 1  | 1.35    | 0.247 |
| $\lambda$          | 1  | 0.365   | 0.547 | 1  | 0.215   | 0.643 | 1  | 0.056   | 0.813 |
| Tube length : $\lambda$ | 1  | 0.656   | 0.419 | 1  | 0.322   | 0.571 | 1  | 0.129   | 0.72  |
| Residuals          | 161 | 161 | 158 | 161 | 161 | 158 | 161 | 161 | 158 | 161 | 161 |
| Corona width       | 1  | 0.568   | 0.452 | 1  | 0.015   | 0.904 | 1  | 2.449   | 0.12  |
| $\lambda$          | 1  | 0.358   | 0.551 | 1  | 0.214   | 0.644 | 1  | 0.057   | 0.812 |
| Corona width : $\lambda$ | 1  | 2.779   | **0.097** · | 1  | 1.071   | 0.302 | 1  | 0.719   | 0.398 |
| Residuals          | 161 | 161 | 158 | 161 | 161 | 158 | 161 | 161 | 158 | 161 | 161 |
| Corona height      | 1  | 0.001   | 0.973 | 1  | 0.415   | 0.52  | 1  | 0.142   | 0.707 |
| $\lambda$          | 1  | 0.421   | 0.518 | 1  | 0.245   | 0.621 | 1  | 0.067   | 0.796 |
| Corona height : $\lambda$ | 1  | 0.774   | 0.38  | 1  | 0.274   | 0.601 | 1  | 0.22    | 0.64  |
| Residuals          | 159 | 159 | 156 | 159 | 159 | 156 | 159 | 159 | 156 | 159 | 159 |
| b             | $\lambda_F - \lambda_M$ |                  | $\lambda_M - \lambda_A$ |                  | $\lambda_F - \lambda_A$ |                  |
|--------------|--------------------------|------------------|--------------------------|------------------|--------------------------|------------------|
|              | df | F      | P    | df | F      | P    | df | F      | P    |
| No. Flowers  | 1  | 4.434  | 0.037 * | 1  | 5.973  | 0.016 * | 1  | 4.909  | 0.028 * |
| Corolla width| 1  | 8.851  | 0.003 **| 1  | 4.206  | 0.042 * | 1  | 18.645 | < 0.001 ***|
| Tube width   | 1  | 0.12   | 0.729 | 1  | 0.327  | 0.569 | 1  | 0.076  | 0.783 |
| Tube length  | 1  | 2.736  | 0.1   | 1  | 1.245  | 0.266 | 1  | 6.676  | 0.011 * |
| Corona width | 1  | 0.388  | 0.534 | 1  | 1.123  | 0.291 | 1  | 0.181  | 0.671 |
| Corona height| 1  | 0.031  | 0.86  | 1  | 0.212  | 0.646 | 1  | 0.508  | 0.477 |
| $\lambda$    | 1  | 0.391  | 0.533 | 1  | 0.197  | 0.658 | 1  | 0.078  | 0.781 |
| No. Flowers : $\lambda$ | 1  | 0.045  | 0.833 | 1  | 0.007  | 0.934 | 1  | 0.021  | 0.885 |
| Corolla width : $\lambda$ | 1  | 6.551  | 0.011 * | 1  | 2.338  | 0.128 | 1  | 1.858  | 0.175 |
| Tube width : $\lambda$ | 1  | 0.03   | 0.862 | 1  | 0.006  | 0.941 | 1  | 0.015  | 0.904 |
| Tube length : $\lambda$ | 1  | 2.713  | 0.102 | 1  | 1.239  | 0.267 | 1  | 0.588  | 0.445 |
| Corona width : $\lambda$ | 1  | 0.19   | 0.663 | 1  | 0.066  | 0.797 | 1  | 0.055  | 0.815 |
| Corona height : $\lambda$ | 1  | 1.139  | 0.288 | 1  | 0.419  | 0.519 | 1  | 0.314  | 0.576 |
| Residuals    | 147 | 147    | 146   |