Within-individual phenotypic plasticity in flowers fosters pollination niche shift

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Phenotypic plasticity, the ability of a genotype of producing different phenotypes when exposed to different environments, may impact ecological interactions. We study here how within-individual plasticity in *Moricandia arvensis* flowers modifies its pollination niche. During spring, this plant produces large, cross-shaped, UV-reflecting lilac flowers attracting mostly long-tongued large bees. However, unlike most co-occurring species, *M. arvensis* keeps flowering during the hot, dry summer due to its plasticity in key vegetative traits. Changes in temperature and photoperiod in summer trigger changes in gene expression and the production of small, rounded, UV-absorbing white flowers that attract a different assemblage of generalist pollinators. This shift in pollination niche potentially allows successful reproduction in harsh conditions, facilitating *M. arvensis* to face anthropogenic perturbations and climate change.
The angiosperm flower is the quintessential example of an adaptive-integrated structure formed by multiple, functionally related parts that fit tightly together in a coordinated way to attract efficient pollinators, disseminate pollen, and promote plant reproduction.1,2. Flowers may be plastic to attract certain pollinators or in response to some antagonists3–5. However, the environmental-induced modification of particular floral traits may imperil the correct functioning of the entire structure and diminish the fitness of the plastic phenotype6. In order to keep its functionality, the plasticity of flowers would thus require the concerted changes of all their parts and the production of a new fully integrated phenotype7,8. This abrupt multivariate plasticity is nevertheless unlikely, because the developmental requirements to produce any new complex structure constrain the ability to respond to immediate environmental changes7,8. Consequently, flowers tend to exhibit higher developmental canalization9 and express plasticity less frequently than other plant traits4,5.

Flowers have, in many cases, coevolved with pollinators2,9, resulting in a vast array of floral morphologies finely matching their behavioural and morphological traits1,10. Given the universal association between floral phenotype and pollinator diversity and identity, the environmental-driven modification of flowers may influence the preference and visitation rate of pollinators11–14. Therefore, floral plasticity will not only affect the performance of the plastic individuals but it may also reshape the structure and dynamics of their interaction networks and modify the identity and breadth of their pollination niches15,16. These ecological consequences of floral plasticity remain largely unexplored despite their great importance to understand how plants may potentially respond to changing environments16.

In this study, by combining ecological, physiological, and genetic approaches, we demonstrate both in the field and under controlled conditions the occurrence of abrupt multivariate within-individual floral plasticity in Moricandia arvensis that alter the structure of the pollinator network and cause a significant shift in pollination niches. Floral plasticity is expressed in this plant species at the level of the whole organ and as a consequence of the orchestrated response of multiple floral traits to the same environmental cues, causing the emergence of two radically different flowers in the same individual. This plasticity allows the same individual plants to exploit contrasting pollination niches by attracting different pollinators and reproduce successfully in a wide range of environments.

**Results and discussion**

Functional plasticity facilitates flowering in summer. *M. arvensis* (Brassicaceae) is a perennial herb relative to cabbage and radish that inhabits dry semiarid and arid ecosystems of the Western Mediterranean. In these types of environments, *M. arvensis* faces two contrasting climatic conditions, mild and wet during spring but extremely dry and hot during summer (Supplementary Table 1). A general strategy to cope with stressful environmental conditions is to express plasticity in traits essential for physiological and ecological functions4,17,18. Accordingly, *M. arvensis* was plastic for functional traits associated with resource acquisition, producing denser and thicker leaves with more structural carbon and higher water use efficiency during summer than during spring (Fig. 1a, Supplementary Table 2, Supplementary Fig. 1; see supplementary material for detailed methods). The peculiar C3–C4 photosynthetic path of *M. arvensis*, a type of photosynthesis considered an intermediate step in the evolution from C3 to C4 photosynthesis19, may also help this species to face warm climates4,20. The CO2 compensation point of C3–C4 species lies between the values of C3 and C4 species20. However, we found that the *M. arvensis* CO2 compensation point shifted from typical C3–C4 values in spring conditions to values closer to C4 in summer conditions (Fig. 1b). Photosynthetic and vegetative plasticity may help to prolong the activity of *M. arvensis* into the hotter and drier season. Consequently, unlike most co-occurring species, *M. arvensis* extends its flowering well into summertime, starting to flower in early spring (late February to early March) and keeping blooming throughout the summer and even during autumn (Supplementary Table 1).

**Floral plasticity as a response to flowering in summer.** By flowering in two contrasting environmental conditions, the same individuals produced flowers differing radically in phenotype across seasons (Fig. 2a). During spring, plants produced large,
cross-shaped, bright lilac UV-reflecting flowers (Fig. 2a, b), similar to the archetypal Moricandia flower21–23. During summer, in contrast, plants produced small, rounded, UV-absorbing white flowers (Fig. 2a, b) resembling the flowers of species belonging to different clades of the genus or even to different genera and taxonomic tribes in the Brassicaceae family (Fig. 2c, Supplementary Table 3)21–23. The colour of the flower during spring was caused in *M. arvensis* by the accumulation of anthocyanin derivatives (Supplementary Table 4), although other flavonoids absorbing in the UV range, such as the flavonols quercetin, kaempferol, and isorhamnetin, were also present in the petals (Supplementary Table 4). As a consequence of this change in phenotype, individuals exhibited significant within-individual plasticity for all floral traits: corolla diameter, tube length, shape, and anthocyanin and flavonol concentration (Fig. 3a–e, Supplementary Table 5). This plasticity in floral traits was a widespread phenomenon since any individual from any of the studied populations that happened to flower during summer expressed it (Fig. 3, Supplementary Tables 5 and 6). This seasonal phenotypic change in the *M. arvensis* flower was coordinated across floral traits, since there were significant correlations between the plasticity's magnitudes of corolla diameter, tube length, shape, and flavonols (Supplementary Table 7). Consequently, both types of flowers were similarly integrated (8.5 ± 0.9% vs. 14.6 ± 3.3%;
Wilcoxon rank test $= 3.0, p = 0.2; N = 4$ populations, 5 traits, 117 individuals) (see supplementary material for detailed methods). Likewise, both types of flowers were fully functional and contributed to the overall reproductive success of the plants, since they produced a similar amount of seeds in field conditions (49.2 ± 2.1 vs. 56.7 ± 1.9 seeds/flower, respectively, $N = 4$ populations, 117 individuals; Supplementary Table 8). Finally, both types of flowers required pollen vectors for full seed set, since flowers excluded from pollinators and experimentally self-fertilized did not produce seeds (Supplementary Fig. 2).

The floral plasticity exhibited by M. arvensis in the field was triggered by changes in temperature and photoperiod, since it was reproduced in controlled conditions by modifying these two environmental factors (Fig. 3f–j) (see supplementary material for detailed methods). All floral traits, except petal flavonols, were plastic when plants growing in experimental spring conditions were afterward exposed to mild summer or hot summer conditions (Fig. 3f–j, Supplementary Table 9). There was an increase in the magnitude of plasticity with the severity of the experimental summer conditions. The individual slopes were lower in mild summer conditions than in hot summer conditions, and the reaction norms of plants from the hot summer condition were always below the reaction norms of the mild summer condition (Fig. 3f–j, Supplementary Table 9). Control treatment (plants submitted to two rounds of spring conditions) did not elicit floral plasticity (Supplementary Table 9), indicating that these changes in floral traits were a consequence of changes in the experimental abiotic conditions rather than an artefact of the time that the plants were in chambers. Floral plasticity was, nevertheless, stronger in field conditions than in experimental conditions (Supplementary Fig. 3), suggesting that although temperature and photoperiod prompt floral plasticity in M. arvensis, other environmental factors are also involved in the change.

We checked whether the change in M. arvensis floral phenotype, rather than being a plastic response to environmental conditions, was a consequence of the whole-plant ontogenetic changes (i.e., heteroblasty in plants) by quantifying, both observationally and experimentally, the potential reversibility of floral phenotype (see supplementary material for detailed methods). All floral traits were reversible in both natural and experimental plants, and the flowers produced after summer, when facing again milder conditions, resembled the spring flowers (Supplementary Fig. 4, Supplementary Tables 10 and 11). This outcome shows that this within-individual change in floral phenotype is indeed a consequence of phenotypic plasticity rather than a consequence of ontogeny.

Altogether, our findings demonstrate that M. arvensis produces two types of functional, well-integrated, animal-pollinated flowers. Floral plasticity usually expresses as subtle quantitative changes, such as variations in flower or petal size, colour, nectar production, floral volatiles, and number of reproductive structures or changes in the proportion of self-pollinating cleistogamous flowers. In contrast, such an extreme multivariate within-individual floral plasticity as the one reported here, stronger even than plasticity in key functional foliar traits (Supplementary Fig. 3) and causing the appearance of two sets of animal-pollinated flowers that radically differ in their phenotype, has not been previously found.

Floral gene expression changes in summer conditions. We analysed the floral transcriptomes of five experimental individuals consecutively exposed to spring and summer conditions (Supplementary Table 12; see “Methods” section). Overall gene expression was different between these two experimental conditions, with 256 genes expressed significantly more in spring conditions and 371 in summer (Fig. 4a, b). These differentially expressed genes (DEG) were enriched in GO terms related to responses to stress, temperature, radiation, light, and other environmental stimuli (Fig. 4c; Supplementary Data 1). In particular, summer flowers showed higher expression of genes coding for heat shock proteins (Fig. 4c; Supplementary Data 1). The higher expression of genes encoding heat shock proteins could help to maintain other proteins’ functionality in summer. Heat shock proteins, particularly Hsp90 and their co-chaperones, have also been associated with the perception and transduction of signals related to programmed plasticity, that is, the directional shift in phenotype in response to environmental changes.

Not only buffering genes changed their expression in the experimental flowers, but genes potentially associated with the observed phenotypic changes in size, shape, and colour did. For
example, the *M. arvensis* homologous of RADIALIS, a transcription factor associated with flower asymmetry in *Antirrhinum majus*, was expressed more in summer flowers (Supplementary Data 2). Likewise, several homologous to *Arabidopsis thaliana* genes associated with growth-promoting processes, such as *EXO*, related to cell expansion, *SAUR10*, related to cell elongation, *ARF5* and *ARF8*, involved in auxin-response, and *BON2*, related to cell death, were differentially expressed in spring and summer flowers of *M. arvensis* (Supplementary Data 2). Finally, there was a reduction in the expression of several genes of the anthocyanin biosynthetic pathway, including both structural genes such as *PAL*, *4CL*, *CHS*, *DFR*, and several UDP-glucosyl transferases (*U78D2* and *U75C1*), as well as transcriptional factors such as *MYB90* and *TTG1* (Supplementary Data 2, Supplementary Fig. 5). These changes in structural and regulatory genes suggest a coordinated response that could potentially drive flower colour changes in response to high temperatures and long photoperiods.

Floral plasticity affects the interaction with pollinators. The display of two different animal-pollinated flowers during spring and summer caused the same *M. arvensis* plant to attract different pollinators with different preferences and effectiveness. *M. arvensis* flowers were visited mostly by long-tongued large bees in spring (Fig. 5a), but mostly by short-tongued small bees, butterflies, and beetles in summer (Fig. 5b, Supplementary Table 13). We explored whether this change in pollinators results in a pollination niche shift by determining the modularity of the network describing the frequency of visits of main pollinator functional groups to each studied population (see supplementary material for detailed methods). This pollinator network was significantly modular (modularity $= 0.46, p < 0.0001$), suggesting the occurrence of several pollination niches in *M. arvensis* (Fig. 5c). Most important, all studied plant populations changed between modules from spring to summer (Fig. 5c), indicating that they changed seasonally between pollination niches. By displaying two contrasting flowers, the same plant is attracting two different
Fig. 5 Seasonal changes in pollination niche. a Main pollinator of spring flowers: long-tongued bee (Anthophora sp.). b Main pollinators of summer flowers: small bee (Lasio glossum sp.), small beetle (Mordellisten sp.) and butterfly (Pieris sp.) (photographs taken by F. Perfectti). Outcome of the modularity analysis of the pollination networks built upon the visitation rate of the main pollinator functional groups c to three M. arvensis populations during summer and spring; d to experimental plants displaying spring flowers and summer flowers during spring in the field; e to experimental plants displaying spring flowers and summer flowers during summer in the field. Pollinator Functional Groups: 1. short-tongued small bees; 2. short-tongued medium-sized bees; 3. short-tongued large bees; 5. long-tongued large bees; 6. ants; 7. small wasps; 8. small hoverflies; 9. large hoverflies; 10. bees; 11. large flies; 12. long-tongued small flies; 13. small butterflies; 14. large butterflies; 16. hawkmoths; 17. beetles; 18. trips. Insect silhouettes drawn by Divulgare (www.divulgare.net) under a Creative Commons license (http://creativecommons.org/licenses/by-nc-sa/3.0).
pollinator assemblages and exploits two different pollination niches across seasons.

To assess how profound was this seasonal change in the pollination niche, we determined their position within the niche space of those plant species phylogenetically related to M. arvensis (Supplementary Data 3–6). This network was again significantly modular (modularity = 0.42, p < 0.0001), with the niche space divided into five different pollination niches (Supplementary Fig. 6). Interestingly, during spring, when M. arvensis plants display flowers similar to other Moricandia species, M. arvensis belongs to the canonical Moricandia pollination niche dominated by long-tongued large bees (Supplementary Fig. 6). During summer, by displaying a completely different flower, M. arvensis jumps to a contrasting and more diverse pollination niche, including short-tongued bees, butterflies, and beetles. This new niche is shared with only one species of Moricandia, M. foetida, which displays small rounded whitish flowers, like M. arvensis during summer. This finding suggests that the pollination niche shift between M. arvensis spring and summer flower is severe.

The diversity and composition of the pollinator fauna change seasonally in the Mediterranean area. Accordingly, the observed change in pollination niches could be a consequence of M. arvensis flowers being visited by the pollinators available each season. To know whether the exploitation of this new pollination niche was just a mere response to the seasonal replacement in floral visitors or was a real consequence of floral plasticity, we experimentally offered spring and summer flowers to pollinators, both during spring and summer (see supplementary material for detailed methods). During spring, experimental spring flowers received more visits than experimental summer flowers (1.7 ± 0.2 vs. 0.6 ± 0.1 visits flower⁻¹ h⁻¹, respectively, mean ± 1 s.e.m, F = 46.7, df = 1,118, p < 0.0001). During summer, both types of experimental flowers received similar number of visits (2.6 ± 0.5 vs. 2.7 ± 0.3 visits flower⁻¹ h⁻¹, respectively; F = 0.07, df = 1, 25, p = 0.80, ANOVA). The experimental networks were modular in both seasons (spring modularity = 0.33, summer modularity = 0.18, all p < 0.0001), indicating that the two floral phenotypes were visited by different pollinator faunas and thereby belonged to different niches (Fig. 5d, e). Experimental spring flowers belonged to the same pollination niche, associated with long-tongued large bees, in both spring and summer seasons (Fig. 5d, e). In contrast, most experimental summer flowers belonged to pollination niches dominated by butterflies and other generalist pollinators in both seasons (Fig. 5d, e). These experiments indicate that the observed shift in pollination niche was due to the change in floral phenotype rather than a consequence of a change in the availability of different pollinators in each season. In brief, our study shows that the discrete within-individual floral plasticity allows M. arvensis to jump to a different region of the floral phenotypic space and exploit alternative pollination niches.

Consequences of plasticity-mediated niche shifts. Phenotypic plasticity may have important consequences for M. arvensis. By being able to flower and attract efficient pollinators even in summer, this species produced viable seeds during this harsh season, when most other co-occurring plants are inactive and dormant. Plasticity is thus beneficial itself since it provides the plastic phenotype with an extra amount of seeds. Furthermore, floral plasticity and the subsequent shift in the pollination niche allow M. arvensis to reproduce successfully in a broader range of environments, a property facilitating the colonization of new territories and the ability to withstand the adverse effects of global change. This may explain the rapid expansion of M. arvensis geographic range in historical times.

Our study suggests that the effect of phenotypic plasticity in the structure and dynamics of biotic niches and ecological networks may help plants to respond to present-day anthropogenic perturbations and future climate change scenarios.

Methods
Field sampling design. To determine if there was within-individual plasticity in floral traits between spring and summer conditions, 50 plants of each of four populations from SE Spain (Supplementary Table 1) were marked at the onset of the flowering period in late February–early March 2018. The phenotype of two flowers per individual was quantified (see below). We revisited each population during summer (June 2018) and the same floral traits were quantified in the summer flowers of those plants still flowering (117 plants; Supplementary Table 1).

Experimental design. We performed an experiment testing the effect of temperature and photoperiod in floral plasticity. It included three treatments: (1) Treatment 1, where 30 plants flowered first in conditions mimicking the spring temperature and photoperiod of Mediterranean Spain (day/night = 10/14 h, temperature = 20/10 °C, average daily temperature = 14.2 °C, see Supplementary Table 1), and afterwards in conditions mimicking a mild summer (day/night = 16/8 h, temperature = 30/20 °C, average daily temperature = 23.8 °C). (2) Treatment 2, where 30 plants flowered first in spring conditions and afterwards in hot summer conditions (day/night = 16/8 h, temperature = 35/25 °C, average daily temperature = 28.8 °C). (3) Treatment 3 (control) where 15 plants flowered first in spring conditions, and afterwards they flowered again in spring conditions. For all treatments, we removed flowers before starting the second round of flowering.

We experimentally tested the occurrence of reverse plasticity by performing a Treatment 4 in which 15 plants from Treatment 1 that flowered both during spring and summer conditions were again submitted to a period mimicking spring conditions (Supplementary Table 1).

Floral traits. We measured, both in field and experimental conditions, three floral traits during spring (or under experimental spring conditions) and in summer (or under experimental hot summer conditions). These traits were corolla size, corolla shape and corolla colour.

Corolla size of each studied flower was estimated by means of two traits: (1) corolla diameter, estimated as the distance in mm between the edge of two opposite petals. (2) Corolla tube length, the distance in mm between the corolla tube aperture and the base of the sepals. These variables were measured by using a digital caliper with ±0.1 mm of error.

Corolla shape variation was studied using geometric morphometric tools based on a landmark-based methodology. For this, in each of the two selected flowers per individual plant studied in each of the four populations, we took a digital photo.
of the front view and planar position. We defined 32 co-planar landmarks covering the corolla shape and using midrib, primary and secondary veins and petal extremity and connections.42,43 From the corolla to leaf area landmarks, we extracted shape information and computed the generalized orthogonal least-squares Procrustes averages using the generalized procrustes analysis (GPA) superposition method. Due to the intrinsic symmetry pattern exhibited by Brassicaceae flowers, we did the analyses considering the symmetry and analysing all flowers and petal couples in both directions.44–47 We computed the area of a principal component analysis (PCA) on the GPA-aligned specimens, and afterwards, we did a canonical variate analysis (CVA) to explore the difference in shape between season and populations.45,46 Geometric morphometric analyses were performed using packages Geomorph48 and Shape49,50 To explore the relative position of the corolla shape of spring and summer flowers in the morphospace created by the species most related phylogenetically with M. arvensis, we performed a phylomorphospace. This analysis creates a plot of the main principal dimensions (the three first principal components in this case) of a tangent space for the Procrustes shape variables of the pool of species considered in the analysis and superimposed the phylogenetic tree relating these species in this plot.51,52 By doing this, this analysis reveals how the shape evolves. To perform this analysis, we collected information on the corolla shape of 72 additional species belonging to the Brassicaceae tribe Brassicaceae, the tribe to which M. arvensis belongs (Supplementary Table 3). We followed the same procedure as with M. arvensis for the same number of landmarks and computed the generalized orthogonal least-squares Procrustes averages using GPA superposition method. In this analysis, we kept separate the spring and summer flowers of M. arvensis. The phylogenetic relationship between these 72 species was obtained by making a super树 using Brassicaceae trees hosted in the repository TreeBASE Web (TreeBASE.org)31. We downloaded individual phylogenetic trees from TreeBASE. Second, we concatenated all these individual trees and made a skeleton super-tree. Finally, we pruned this super-tree, keeping only the species included in the geometric morphometric analysis, and insert the two ‘pseudospecies’ of M. arvensis (spring and summer) as sister species. Afterwards, we projected the value of the three PCs of each species on the phylogenetically explicit plot. The phylogenetic analysis was performed in the R packages ‘treemadon’54, ‘phangorn’55,56 ‘phytools’57 and ‘treebase58,59’ whereas the phylomorphospace analysis was performed in the R package ‘geomorph’60.

The corolla colour of M. arvensis is produced by the accumulation of flavonoids.28 Anthocyanins and non-anthocyanin flavonoids present in the petals of M. arvensis were analysed by ultra-performance liquid chromatography (UPLC) (ACQUITY System I-Class, Waters) coupled with quadrupole time-of-flight mass spectrometry (SYNAPT G2 HDMS-Q-TOF, Waters). Analytical separation of flavonoids was performed on an Acuity HST33 analytical column (150 mm × 2.1 mm internal diameter, 1.8 μm). A mobile phase with a gradient programme combining deionized water with 0.5% of acetic acid as solvent A and acetonitrile with 0.5% of acetic acid as solvent B was used. The initial conditions were 95% A and 5% B and a linear gradient was then established to reach 95% (v/v) B. The total run time was 15 min and the post-run time was 5 min. The mobile phase flow rate was 0.4 ml min−1. After chromatographic separation, a high-resolution mass spectrometry analysis was carried out in positive electrospray ionization (ESI+) mode. The ionization source parameters using high-purity nitrogen were set at 600 L h−1. The analysis was carried out in positive electrospray ionization (ESI+) mode. We measured responses of CO2 assimilation rate (A) versus calculated substomatal or intercellular CO2 concentration (Ci) (henceforth, A–Ci curves) to determine the instantaneous photosynthetic metabolism of plants of the intermediate C3–C4 species M. arvensis on plants grown under the two experimental conditions (N= 22 plants, spring and hot summer conditions). Gas exchange measurements were performed on one expanded leaf. We analysed 10 expanded leaves per plant and experimental condition using a LICOR 6400 (LI-COR Biosciences, Lincoln, USA) and following the standard recommendations to correct leakage errors.61–63 Curve fittings were maintained at a constant photosynthetic photon flux density (PPFD) of 1500 μmol m−2 s−1, a vapour pressure deficit (VPD) that ranged from 1.0 to <2.4 kPa and a cuvette temperature that was either 20 or 30 °C (see below). A–Ci curves were measured at a series of ambient (i.e., reference) CO2 concentrations (Ci) starting at 400 ppm. The Ci was then lowered stepwise from 400 to 40 ppm and then increased again to 1600 ppm, all on 21 steps: 400, 300, 200, 150, 100, 50, 40, 40, 40, 60, 100, 200, 400, 600, 800, 1200, 1400, 1600 ppm of CO2.

The CO2 compensation point (substantial CO2 concentration value at which A is zero) and initial slope of each A–Ci curve were calculated performing a linear regression of 10 different Ci values (all below 300 ppm). Ci of 300 ppm was used for the calculations as we had some technical issues with the LICOR in one of the two measurement phases. This fact might overestimate absolute results for CO2 compensation points, but the relative differences (if any) between the two experimental conditions (spring and summer) remain. Measurements of gas exchange were made at two different LICOR cuvette temperatures (20 and 30 °C) for plants growing in spring and hot summer experimental conditions, respectively. Although the CO2 compensation point can increase with cuvette temperature, we measured the compensation points of six plants grown in spring chamber conditions and at these two contrasting cuvette temperatures, and results did not differ (CO2 compensation point ± s.e.m. = 29.72 ± 4.03 vs. 31.42 ± 4.03 ppm at 20 and 30 °C bulk temperature of the LICOR chamber, respectively; GLM results, F1,6 = 0.09; P-value = 0.7796; N= 6 individuals). Moreover, mean CO2 compensation points of plants grown in spring chamber conditions were significantly higher (not lower) than values for the same plants grown in hot-summer conditions (see main text; mean ± s.e.m. = 25.0 ± 0.21 ppm in spring versus 14.7 ± 0.16 ppm in hot summer conditions).

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between individual plants. Each survey was done at least by two researchers. We were able to obtain information in three populations (Malaha, Quesada, Spring and summer pollinators of *M. arvensis* setting fruits, collected the fruits that were taken to the lab for counting their seeds with own pollen; (2) outcrossing, where the flower was pollinated with pollen from one different conspecific individual from the same season. Consequently, the number of flowers of the treatment varied across individual, but was always higher than two, with a mean ± 1 s.e.m. equal to 6.1 ± 0.8 flowers per plant. Because all plants were located inside the experimental chambers, they were excluded from the visit of any pollinator. At the end of the fruiting period, we counted all flowers setting fruits, collected the fruits that were taken to the lab for counting their seeds as explained in the previous section.

**Spring and summer pollinators of *M. arvensis***. We identified the pollinators visiting the flowers of the studied populations both during spring and summer. For this, we conducted flower visitor counts in each population season combination. We were able to obtain information in three populations (Malaha, Quesada, Tabernas). We visited each of these populations both during spring and during summer, always between 11:00 a.m. and 5:00 p.m. In these visits, we recorded the insects visiting the flowers of the plants for two hours without differentiating between individual plants. Each survey was done at least by two researchers simultaneously, sampling each population for at least 8–9 h person⁻¹. We only recorded those insects contacting anthers or stigma and doing legitimate visits at least during part of their foraging at flowers. We did not record those insects only eating petals or thieving nectar without doing any legitimate visit. Previous studies using the methodology carried out with similar Brassicaceae species and performing rarefaction analysis indicate that a sample of 130–150 insects provides an accurate estimate of the diversity of the pollinator assemblages.[21,33,69,70] To ensure that our sampling was representative, we recorded 471 insects in Malaha population, 334 insects in Quesada population and 300 insects in Tabernas population (Supplementary Table 13).

**Pollinators of the relative species**. We compiled information on 308,096 insect visits belonging to 38 functional groups and more than 5000 morphospecies visiting 114 plant species belonging to the same tribe than *M. arvensis*, the Brassicaceae (Supplementary Data 3). We use our data and data from the literature. In those species studied in the field (74 species), we conducted flower visitor counts in 1–16 populations per plant species. We visited the populations during the peak of the bloom, always at the same phenological stage and between 11:00 a.m. and 5:00 p.m. In these visits, we recorded the insects visiting the flowers of the plants for two hours without differentiating between individual plants. Insects were identified in the field, and some specimens were captured for further identification in the laboratory. We only recorded those insects contacting anthers or stigma and doing legitimate visits at least during part of their foraging at flowers. We did not record those insects only eating petals or thieving nectar without doing any legitimate visit. In addition, we included information on pollinators obtained from the literature to supplement our data (Supplementary Data 3 and 6). In this case, we use the information provided in the primary literature in terms of pollinator species and abundance at flowers. In this case, the plant species included in the network do not coexist, implying that this is a clade-oriented network rather than an ecological network, those including species with information coming from disparate and contrasting sources.[21,33,40] We thereby grouped all pollinators visiting *M. arvensis* and the other Brassicaceae species using criteria of similarity in body length, proboscis length, morphological match with the flower, foraging behaviour, and feeding habits.[21] Supplementary Table 5 describes the 38 functional groups used in this study and Supplementary Data 4 shows the distribution of these functional groups among the studied Brassicaceae taxa.

We determined the occurrence of different pollination niches in our studied populations and seasons using bipartite modularity, a complex-network metric. Modularity has proven to be a good proxy of interaction niches both in ecological networks, those included coexisting species or population, as well as in clade-oriented network, those including species with information coming from disparate and contrasting sources.[22] We constructed a weighted bipartite network, including pollinator data of four populations both during spring and summer flowering period. In this network, we pooled the data from the different individuals in a population and did not consider the time difference involved in sampling across different species. We removed all plant species with <20 visits. We subsequently determined the modularity level in this weighted bipartite network by using the QuanBiMo algorithm.[52] This method uses a simulated annealing Monte-Carlo approach to find the best division of populations into modules. A maximum of 10⁸ Monte-Carlo steps with a tolerance level = 10⁻¹⁰ were used in 100 iterations, retaining the iterations with the highest likelihood value as the optimal modular configuration. We tested whether our network was significantly more modular than random networks by running the same algorithm in 100 random networks, with the same linkage density as the empirical one.[21] Modularity significance was tested for each module with a classical chi-squared test. We calculated the topological indices using a z-score test.[71] After testing the modularity of our network, we determined the number of modules[72] We subsequently identified the pollinator functional groups defining each module and the plant species that were ascribed to each module. Modularity analysis was performed using R package bipartite 2.0.[22]

**Pollinator preference experiments**. We carried out two experiments, one in late June 2019 and the other in late February/early March 2020, where 10 plants displaying spring-type flowers and 10 plants displaying summer-type flowers were offered at the same time to pollinators in a natural *M. arvensis* population. Plants were experimentally grown in chambers under each of the levels of the Treatment 2 conditions (spring conditions and hot summer conditions) and taken to the field in pots. Plants with each type of flowers were randomly distributed in a 5 × 4 grid with plants separated one metre. We performed ten 2-h trials in 2019 and nine 2-h trials in 2020, where the abundance and identity of the insects visiting the flowers of each experimental plant were recorded. Each trial was done by two researchers simultaneously, totalling 40 h of observation in 2019 and 36 h in 2020. Trials were performed between 11:00 and 13:00 local time in 2019 and between 12:00 and 14:00 local time in 2020. We counted each day the number of open flowers per plant, and, to control per between-plant differences in number of open flowers, insect abundance was expressed in number of visits per flower per hour. Afterwards, we followed the approach explained in the previous section to build up the bipartite interaction network, determine modularity and obtain the different modules, using only those plants having more than 10 visits per flower per hour (18 plants in the 2019 experiment and 20 plants in the 2020 experiment).

**De novo transcriptome analysis**. In five plants from Treatment 1 (labelled MAR-70, MAR-81, MAR-83, MAR-98, and MAR-120), we sampled one starting-to-open flower bud during period 1 (experimental spring conditions) and another flower bud during period 2 (experimental mild summer conditions). We introduced the flower buds in liquid N₂ and conserved them at −80 °C until RNA extraction. We used the RNAeasy Plant Mini Kit (Qiagen) for total-RNA extraction. We checked the quality and quantity of the extracted RNA with a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, Wilmington, DE, USA). The 10 RNA samples (five individuals, two conditions) were sequenced by Macrogen Inc. (Seoul, South Korea) after treatment with RiboZero to remove ribosomal RNA.[72] Libraries were produced using the TruSeq Stranded Total RNA LT Sample Prep Kit (Plant) and sequenced in an Illumina NovaSeq6000 platform run (paired-end 150 bp) for a minimum of 40M reads/sample. See Supplementary Table 12 for information on the total number of bases read and the total number of reads. RNA-Seq raw reads were submitted to Sequence Read Archive with the project accession number PRJNA604514. We retrieved 171,210 trinity genes (Supplementary Table 12 and Supplementary Data 1), but only 47,440 passed the criteria for inclusion in the analyses (at least 10 counts per million in spring or summer conditions). We calculated the quality of the raw sequences with FastQC.[73] After that, we used cutadapt (v. 1.15) and sickle (v. 1.33) to quality trim and remove adapters. To produce a reference transcriptome, we concatenated the libraries (only paired sequences) and submitted them to RNA-Seq de novo assembly using Trinity v2.8.1.[74] A total of more than 372 Mbp were assembled in 424,981 transcripts (171,210 trinity ‘isoforms’), with a median contig length of 499 bp, an average length of 876.97 bp, and 10% of the assembled nucleotides appearing in transcripts of more than 4257 bp. We used trinity add-on scripts to compute contigs statistics with the help of Bowtie2 (v. 2.3.4.1)[40] A total of 97.24% of reads aligned to the reference, with >80% aligned more than one time. We performed a BLASTX search of the assembled transcriptome to the SwissProt database. We introduced that 10,696 proteins of this database mapped in more than 90% of their sequences.

**Analysis of DEG**. We estimated the abundance of transcripts and genes using the Trinity abundance_estimates_to_matrix.pl script. Since the objective was to compare the within-individual expression of genes, we produced a matrix of raw gene counts as the input for the following analyses. We normalized (TMM)[54] and log2-transformed (conserving genes with at least 10 transcripts) the matrix using edgeR.[88] A total of 47,870 genes passed this criterion. We analysed this matrix using a design of repeat samples with two conditions (spring as control and summer) and fit a negative binomial generalized log-linear model to the read counts for each gene. We selected as DEG the genes with false discovery rate adjusted P values < 0.05.

**Annotation and GO-enrichment analysis**. We used Trinotatev3.[53] to annotate the 47,870 genes using the uniprot_sprot and Pfam-A databases, and mapping the longest isoform of each of those genes. Additionally, we used sma3s[56] with similar results. For the gene ontology (GO) enrichment analyses, we used the
 Bayesian generalized multivariate multilevel models. Plasticity integration was determined by quantifying the correlations between the plasticities of pair of traits. For this, we used Bayesian generalized multivariate multilevel models. The structure of these models was similar to that explained previously for random regression, including individuals as random intercepts and slopes. As the dependent variable, we used a composite variable including all the five floral traits analysed independently in the previous analyses. We scaled and centred both the independent and dependent variables and used weakly informative prior with normal distribution and centred on zero. We ran four chains with 2000 iteration each, burning 1000 samples per chain. In total, we analysed 400 post-warmup samples. We got in all chains the potential scale reduction factor on split chains to be 1 or very close to 1 at convergence. Significance was obtained for each effect by means of the posterior distribution of the 95% credible interval of its mean estimate. Bayesian analyses were performed in the R packages \textit{brm} 

\textbf{Data availability}

Source data are provided with this paper. All raw sequence reads have been deposited in the NCBI SRA database under BioProject accession number PRJNA604514. Source data are provided with this paper.

\textbf{Received:} 22 June 2020; \textbf{Accepted:} 20 July 2020; \textbf{Published online:} 11 August 2020

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Acknowledgements Authors thank Raquel Sánchez, Ángel Caravante, Isabel Sánchez Almazo, Tatiana López Pérez, Samuel Cantarero, María José Jordà and Germán Fernández for helping us during several phases of the study and to José Miguel Ángel de Arroyo for drawing the insect silhouettes. This research is supported by grants from the Spanish Ministry of Science, Innovation and Universities (CGL2015-67129-P, CGL2015-67129-C2-1-P, CGL2017-86622-C2-1-P, CGL2017-86622-C2-2-P, UGR15-CE-3315, including EU FEDER funds), Junta de Andalucía (PIF-18R-3641), Xunta de Galicia (CITACBA), BBVA Foundation (PR17_ECO_0021), and a postdoctoral grant to C.A. from the former Spanish Ministry of Economy and Competitiveness (RYC-2012-12277). This is a contribution to the Research Unit Modeling Nature, funded by the Consejería de Economía, Conocimiento, Empresas y Universidad, and European Regional Development Fund (ERDF), reference SOMMI176109/UGR.
Author contributions
The study was conceived, performed and written by J.M.G., F.P., C.A., E.N., A.G.-M., L.N., L.D.S. and R.T.

Competing interests
The authors declare no competing interests.

Additional information
Supplementary information is available for this paper at https://doi.org/10.1038/s41467-020-17875-1

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Peer review information Nature Communications thanks the anonymous reviewer(s) for their contribution to the peer review of this work.

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