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Floral integration and pollinator diversity in the generalized plant-pollinator system of *Alstroemeria ligtu* (Alstroemeriaceae)

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Abstract Floral integration may result from the combined effects of pollinator-mediated selection, genetic correlations and abiotic factors. Thus, by sampling a set of populations in the field and examining their variation of floral correlations in relation to pollinators and abiotic factors, we intended to shed light on the ecological factors underlying the evolution of floral integration. In this study, floral integration patterns and the composition of pollinator assemblage were characterized across ten populations of *Alstroemeria ligtu* to test the following: (1) Whether the patterns of floral integration estimated at population level covary with the composition of local pollinator assemblage, climate and/or geographic distance. (2) Whether the magnitude of floral integration decreases with the number or the morphological variability of pollinators. (3) Whether the behavior of the three most widespread pollinators is affected by floral integration. Our results indicated that populations with similar patterns of integration showed greater similitude in pollinator assemblages, but that this was not related to climate or geographic distance. We also found that the most widespread pollinator, *Centris nigerrima*, invested lower handling time in populations with higher levels of floral integration. The magnitude of floral integration was not related to taxonomical diversity, yet, unexpectedly, correlated positively with the

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variability of pollinator proboscis length. We suggest that variation in the magnitude and pattern of floral integration across the species range was related to the composition of pollinator assemblage, and not to pollinator diversity per se, nor to climatic variables. *A. ligtu* was visited by numerous pollinators, but floral integration patterns are likely shaped by only a subset of them, such as *C. nigerrima*, which is sensitive to variations in floral architecture.

Keywords Floral evolution · Generalization · Natural selection · Phenotypic integration · Pollination

Introduction

Phenotypic integration refers to the coordinated variation of morphological traits, which result from genetic correlations, developmental constraints and the functional relationships between them (Cheverud 1982; Murren 2002; Pigliucci 2003; Pigliucci and Preston 2004). Flowers are suitable structures for assessing integration since they are morphologically complex, and their parts may covary, not only in relation to their development and spatial ‘proximity’ in the plant (Armbruster et al. 1999; Herrera et al. 2002), but also to their specific functions, such as pollinator attraction, pollen dispersal, and fruit development (Berg 1960; Bissell and Diggle 2008).

In the case of animal-pollinated plants, the arrangement of floral trait relationships (patterns of correlation), and the extent to which floral traits are correlated (magnitude of integration), are thought to result primarily from correlational pollinator-mediated selection (Stebbins 1974; Conner and Via 1993; Conner 1997; Armbruster et al. 1999, 2004; Anderson and Busch 2006; Ushimaro et al. 2006; but see Ordano et al. 2008). In a pioneer work, Berg (1960), suggested that plants pollinated by specific insect, floral traits display high correlation among themselves and become decoupled from vegetative traits, comprising a separate “correlation pleiades”, probably as a result of pollinator-mediated selection. Later, some studies showed that floral traits that have different functions in the pollination process, such as traits that enhance pollinator attraction (like petals) or those that favor a precise fit with them (like style and stamen length), can also comprise separate modules (Diggle 2014; Armbruster et al. 2014).

Different combinations of floral characteristics might be selected by different pollinator species given that they differ in body size, behavior, and size of mouthparts. Accordingly, it has been shown that floral integration is related to the diversity and the composition of pollinator assemblages (Pérez-Barrales et al. 2007, 2014; Pérez et al. 2007; but see Herrera et al. 2002; Nattero et al. 2010). Gómez et al. 2014 found a negative correlation between corolla-shape integration and pollinator richness in a comparative study with 37 species of *Erysimum*, probably because many pollinators impose conflicting selection, preventing the evolution of integrated flowers. The same trend was previously detected by Rosas-Guerrero et al. (2010) in *Ipomaea*, but no correlation could be observed once the phylogenetic effects were controlled. Differences in patterns of corolla integration among *Schizanthus* species pollinated by different functional groups of pollinators were reported by Pérez et al. (2007). Likewise, in two *Narcissus* species (*N. papyraceus* and *N. tazetta*), populations with long-tongued pollinators displayed greater phenotypic integration than those with shorter-tongued pollinators, supporting the idea that different pollinators can exert different selective pressures on floral traits depending on how they fit with flowers (Pérez-Barrales et al. 2007, 2014).

Geographic variation of flower traits may occur without shifts among pollinators (Herrera et al. 2006; Ellis and Johnson 2009). Phenotypic plasticity, genetic drift and divergent natural selection exerted by other biotic factors may promote floral variation (Herrera et al. 2006). Abiotic factors, including soil moisture, soil fertility and temperature are also strong regulators of floral phenotypes (Galen 1999; Caruso et al. 2003). All these factors that are known to affect floral traits can also affect floral trait correlations (Brock and Weining Brock and Weinig 2007; Brock et al. 2010; see also Waitt and Levin 1993; Caruso et al. 2003). Moreover, when they follow a clinal variation among populations, or at times change unpredictably, they might cause a geographically structured, or random variation of floral integration patterns.

Alstroemeria ligtu simsii Sprengel (Alstroemeriaceae) is a perennial herb, endemic to Central Chile, that grows between 32°S and 36°S, at elevations from sea level to 1,500 m. Flowers are visited by Dipterans, Hymenopterans and Lepidopterans (González et al. 2014), and are zygomorphic, with a perianth composed of two whorls of petaloid tepals. Experimental manipulation of tepals revealed that changes in floral architecture reduce pollinator assemblage diversity and visitation rates, increase the handling time of some pollinator species, and consequently decrease reproductive success (Botto-Mahan and Ojeda-Camacho 2000; Botto-Mahan et al. 2011). These results suggest that maintenance of floral architecture is a cue for some groups of pollinators, and, therefore, the magnitude and patterns of trait correlations might evolve in response to pollinator-mediated selection.

In this study, we examined the variation in floral integration among ten populations of *A. ligtu simsii*. We observed flower visitors and measured their visitation rate, body length, proboscis length, and handling time. We then estimated the species richness and the morphological variability of pollinator assemblage to assess whether the patterns of floral integration among populations of *A. ligtu simsii* are associated with pollinators. Specifically, we tested the following predictions: (1) Since pollinator species differ in both morphology and behavior, and can therefore select varying combinations of floral characteristics, populations with similar pollinator assemblage ought to display similar patterns of floral trait correlations. (2) Populations visited by a high number of pollinator species, or by those that are morphologically more diverse (in terms of body and proboscis length), are likely to experience less consistent selection, and should therefore display lower levels of floral integration. (3) Since the behavior of some pollinators is affected by floral architecture, they must invest lower handling time in populations with higher levels of floral integration, and probably visit more flowers in order to ensure the pollination service. This prediction was only tested for pollinator species present in more than five populations. Whilst floral variation within the geographic range of *A. ligtu simsii* can be influenced by climate or other geographically structured abiotic factors, we predict that populations with similar climatic conditions or geographic proximity might display similar patterns of floral trait correlations.

Materials and methods

Study species and sites

Alstroemeria ligtu simsii Sprengel (Alstroemeriaceae) is a hermaphroditic self-incompatible species (Botto-Mahan et al. 2011) with zygomorphic flowers. The perianth is composed of two whorls of six free tepals (petaloid organs): four tepals are orange and the others have a central yellow area streaked with brownish lines, and nectaries at the base (Fig. 1a). The flowering season extends from November to January, followed by the fruiting season that ends in February (Arroyo and Uslar 1993). This study was carried out during the summer season, specifically between November 2012 and February 2013. Ten populations of *A. ligtu simsii* were geo-referenced and sampled along the species' current latitudinal and altitudinal distribution range (Fig. 1b). All populations grow in a Mediterranean climate, with a mean annual precipitation ranging from 437 to 1,037 mm, and a mean annual temperature ranging from 7.7 to 14.6 °C (Table 1). Pollinator assemblage was previously characterized in six of these ten populations, and a marked variation in the visitation rate and the number of visitors per site was detected that did not follow any geographical pattern (González et al. 2014).

Diversity and composition of pollinator assemblages

From each population, flower visitors were observed within plots measuring 2 m^2 during intervals of 15 min, with the observation plot being changed after each interval. Observations were carried out by 6–8 observers between 11:00 a.m. to 16:00 p.m. on 3 sunny days per population, rendering a total observation time of 1,050 h (5 h × 3 days × 7 observers × 10 populations). A total of 1,961 visits were recorded, whilst only species coming into contact with the reproductive structures were considered legitimate pollinators. Pollinators were identified at the highest taxonomical resolution possible, and the handling time and visitation rate of each species was estimated. We quantified the number of pollinator taxa (S′) and the Shannon–Wiener proportional diversity index (H′), which considers the relative abundance of each pollinator taxa using the PAST software (Hammer et al. 2001). We also measured the body size and proboscis length of one to 20 individuals per species, and estimated the coefficient of variation of body size (CV_b) and proboscis length (CV_p), using the mean values of each pollinator, weighted by its relative abundance (Herrera et al. 2002). Finally, we calculated the proportional similarity index (PS) between each pair of populations (Schemske and Brokaw 1981). This index ranges between 0 (indicating no shared pollinators) and 1 (high similitude), taking into account both the

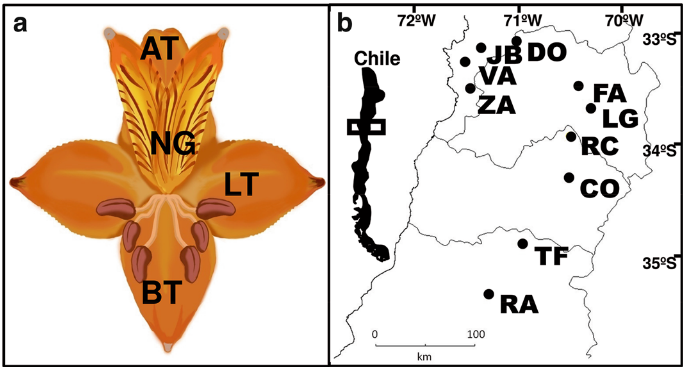

Fig. 1 **a** Flower of *A. ligtu simsii* showing the four tepals measured: apical tepal (*AT*), nectar guide tepal (*NG*), lateral tepal (*LT*), and basal tepal (*BT*). **b** Geographic location of the ten populations analyzed in this study: La Dormida (*DO*), Jardín Botánico (*JB*), Lo Vásquez (*VA*), Farellones (*FA*), Zapata (*ZA*), Lagunillas (*LG*), Reserva Nacional Río Clarillo (*RC*), Coya (*CO*), Termas del Flaco (*TF*), and Radal Siete Tazas (*RA*)

Table 1 Floral integration, climatic conditions, and pollinator diversity indices of *A. ligtu simsii* populations

Pop	INT	Tmin	Tmax	Pp	Taxonomical diversity		Morphological diversity		Handling time (s)		
					S′	H′	CV_b	CV_p	*C. n*	*A. g*	*M. g*
DO	2.15 (1.78–2.59)	7.4	23.2	437	5	1.32 ± 0.12	0.45	0.98	2.9	–	9.2
JB	3.34 (2.96–3.74)	9.3	21.2	594	9	1.33 ± 0.03	1.00	1.32	3.3	4.0	5.5
VA	1.73 (1.41–2.14)	9.8	23.1	566	4	0.97 ± 0.09	0.19	0.60	5.2	–	3.0
FA	1.90 (1.55–2.33)	4.7	21	537	6	1.38 ± 0.05	1.02	0.80	4.3	3.5	2.7
ZA	3.74 (3.33–4.19)	10.7	24.9	478	11	1.42 ± 0.08	1.26	1.54	2.0	4.0	15.1
LG	2.62 (2.17–3.14)	3.0	19.0	564	9	1.47 ± 0.03	2.05	1.85	5.3	4.1	–
RC	2.31 (1.91–2.77)	4.5	20.8	683	7	1.56 ± 0.01	0.97	1.45	3.6	3.9	14.3
CO	1.82 (1.55–2.15)	9.7	24.7	789	10	1.72 ± 0.07	1.00	1.01	5.0	1.8	5.5
TF	1.98 (1.65–2.45)	7.5	23.7	1,037	8	1.43 ± 0.09	0.99	1.10	4.3	3.0	–
RA	2.86 (2.44–3.30)	9.8	26.3	836	8	0.91 ± 0.08	1.01	1.13	3.3	2.3	2.0

Handling time of the most widespread pollinators: *Centris nigerrima* (*C. n*), *Alloscirtetica gayi* (*A. g*), *Manuelia gayi* (*M. g*) is also shown. See population abbreviations in Fig. 1

INT: floral integration values ± 95 % confidence intervals; Tmin: minimum temperature of flowering season; Tmax: maximum temperature of flowering season; Pp: mean annual precipitation; S′: pollinator species richness; H′: pollinator Shannon–Wiener diversity index; CV_b: coefficient of variation of pollinator body lengths; CV_p: coefficient of variation of proboscis pollinator lengths

identity of pollinators and their relative abundance. We made pairwise comparisons using all pollinator species, and then constructed a single 10 × 10 matrix of similarity in the composition of local pollinator assemblages (M_{PS}).

Geographic distribution and climatic variables

We estimated the distance in kilometers between each population pair using the geo-referenced data, and constructed a 10 × 10 geographic distance matrix (M_G). The climatic variables for each population were then compiled from WorldClim Global Climate GIS data (available in http://www.worldclim.org). We restricted the climatic analysis to the period comprising the flowering season of *A. ligtu simsii* (from November to December). The variables considered were: minimal temperature in flowering season (Tmin), maximum temperature in flowering season (Tmax), and mean annual precipitation (Pp) (Table 1). These variables were used to construct a climatic similarity matrix (M_{CLIM}) employing a multivariate analysis with Euclidean distance in the PAST software.

Floral integration measurements

We collected one flower in 200 plants per population (n = 2,000) to estimate floral integration at population level. Flowers were dissected and immediately photographed in the field. The length and width of the apical tepal (AT), nectar guide tepal (NG), lateral tepal (LT), and basal tepal (BT) were recorded from digital images to minimize measurement errors using the software ImageJ 1.46r (available at: http://rsb.info.nih.gov/ij/; Fig. 1a). Errors were therefore too small to bias the overall integration values. To reduce the potential effects of fluctuating asymmetry on floral integration, we consistently measured the same side of paired structures. All measurements were log-transformed for statistical analyses. To estimate the pattern of floral integration, we constructed an 8 × 8 character phenotypic correlation matrix for each population (M_C) using Pearson's correlation coefficients between every pair of traits. The magnitude of floral integration (INT) for each population was estimated from the variance of eigenvalues in each correlation matrix (Var [λ_i]) (Wagner 1984; Cheverud et al. 1989). A confidence interval of INT at 95 % was estimated by bootstrapping the original log-transformed data. We also estimated the correlation coefficient between M_C matrices for every pair of populations before constructing a 10 × 10 matrix of similarity among correlation matrices of floral traits (M_{CP}).

Relationship between floral integration and pollinators

To estimate whether populations with similar pollinator assemblages show similar patterns of floral trait correlations, we calculated the correlation between M_{CP} versus M_{PS} using the Mantel test in the PAST software and running for 10,000 iterations. Secondly, to test whether floral integration decreases with the diversity of pollinator assemblage, we estimated Spearman partial correlations between INT values versus S', H', CV_b and CV_p, using the pcor package of R (R development Core team 2013). Thirdly, to assess whether the behavior of pollinators is affected by floral integration, we estimated the Pearson correlation coefficients between INT values versus handling times and visitation rates of the three widespread pollinator species (shared by more than 5 populations), using the PAST software.

Relationship between floral integration, climate and geographic distance

To assess the influence of climate or population geographic distribution on patterns of floral trait correlations, we correlated M_{CP} against M_{CLIM} and M_G using the Mantel test in the PAST software and running this for 10,000 iterations. Finally, we estimated Spearman partial correlations between INT value versus Tmin, Tmax and Pp using the pcor package of R (R development Core team 2013).

Results

Diversity and composition of local pollinator assemblages

Flowers of *A. ligtu simsii* were visited by 23 pollinator species belonging to the following orders: Hymenoptera (61.5 % of visits), Diptera (37 % of visits) and Lepidoptera (1.4 % of visits). 50 % of the total visits recorded (N = 984) were performed by only three species: *Lasia aenea*, *Ruizantheda proxima*, *Alloscirtetica gayi* (Table S1). The most widespread species was *Centris nigerrima* shared by all localities, followed by *Manuelia gayi* and *A. gayi*, which were present in 8 of the 10 localities (Table S1). Pollinator species richness (S') varied from four to eleven species per population, whereas Shannon's diversity indices ranged from 0.91 in RA to 1.72 in CO (Table 1).

Table 2 Similarity matrices and geographic distance (km) among ten populations of *A. ligtu simsii*

	JB	VA	FA	ZA	LG	RC	CO	TF	RA
A) Matrix of similarity in the composition of local pollinator assemblages M_{PS}									
DO	0.04	0.57	0.25	0.62	0.33	0.13	0.19	0.09	0.06
JB		0.37	0.21	0.18	0.07	0.41	0.22	0.36	0.12
VA			0.1	0.77	0.06	0.39	0.14	0.17	0.08
FA				0.13	0.35	0.34	0.39	0.1	0.12
ZA					0.16	0.22	0.18	0.2	0.07
LG						0.38	0.48	0.4	0.04
RC							0.27	0.31	0.16
CO								0.62	0.13
TF									0.07
B) Matrix of similarity in climatic conditions M_{CLIM}									
DO	0.85	0.88	0.90	0.95	0.87	0.79	0.72	0.61	0.70
JB		0.98	0.95	0.89	0.97	0.93	0.86	0.74	0.83
VA			0.97	0.92	0.99	0.90	0.84	0.72	0.81
FA				0.94	0.97	0.88	0.81	0.69	0.78
ZA					0.91	0.82	0.77	0.64	0.74
LG						0.91	0.83	0.71	0.80
RC							0.92	0.80	0.90
CO								0.87	0.97
TF									0.89
C) Matrix of similarity among correlation matrices of floral traits M_{CP} *(all data with statistical significance* $p < 0.01$*)*									
DO	0.88	0.82	0.85	0.93	0.90	0.82	0.83	0.92	0.92
JB		0.90	0.65	0.86	0.80	0.84	0.64	0.66	0.77
VA			0.72	0.92	0.72	0.82	0.65	0.73	0.76
FA				0.84	0.86	0.81	0.94	0.90	0.85
ZA					0.80	0.90	0.80	0.78	0.82

Table 2 continued

	JB	VA	FA	ZA	LG	RC	CO	TF	RA
LG						0.87	0.89	0.84	0.91
RC							0.77	0.72	0.87
CO								0.87	0.84
TF									0.81
D) Matrix of geographic distance M_G									
DO	19.60	41.30	71.60	54.50	91.30	101.80	144.50	196.70	244.80
JB		22.50	75.80	34.60	89.50	97.80	130.20	183.50	226.90
VA			77.50	14.50	84.10	88.40	119.60	162.70	203.40
FA				88.70	27.70	45.70	97.10	158.40	216.50
ZA					93.90	95.10	120.40	160.00	197.40
LG						19.60	70.40	133.40	193.00
RC							51.40	114.20	173.20
CO								63.30	126.00
TF									63.80

See population abbreviations in Fig. 1

Relationship between floral integration and pollinators

The patterns of floral correlations differed among populations, with pairwise similarity indices among phenotypic correlation matrices ranging from 0.64 to 0.94 (Table 2, M_{CP}). As expected, populations with similar patterns of integration showed greater similitude in pollinator assemblages (Mantel test: M_{CP} vs M_{PS}: $r_m = 0.42$, $p = 0.009$). The magnitude of floral integration (INT) also differed among populations, as revealed by the non-overlapping, 95 % confidence intervals (Table 1). INT values ranged from 1.73 and 3.74 representing 22 and 46 % of the maximum possible integration. INT values followed a positive correlation with the variability of pollinator proboscis length (INT vs CV_p: $r_\rho = 0.72$, $p = 0.006$, Fig. 2a) and also a marginally significant, positive correlation with the number of pollinators (INT vs S': $r_\rho = 0.59$, $p = 0.08$). No relation with Shannon–Wiener proportional diversity (INT vs H': $r_\rho = -0.49$, $p = 0.17$) nor with the variability of pollinator body length (INT vs CV_b: $r_\rho = 0.11$, $p = 0.77$) was detected. The magnitude of floral integration correlated significantly, and negatively, with the handling time of the most widespread pollinator, *C. nigerrima* ($r = -0.70$, $p = 0.02$, Fig. 2b), but not with the handling time of the other two widespread pollinators, *M. gayi* ($r = 0.42$, $p = 0.29$) and *A. gayi* ($r = 0.5$, $p = 0.21$), nor with their visitation rates (VR) (INT vs VR: *C. nigerrima* $r = 0.30$, $p = 0.38$; *M. gayi* $r = -0.31$, $p = 0.45$; *A. gayi* $r = -0.54$, $p = 0.16$).

Relationship between floral integration, geographic distance and climate

No significant correlation between INT values and climatic variables was detected, neither with the temperature in the flowering season (Tmin: $r_\rho = -0.04$, $p = 0.99$; Tmax: $r_\rho = 0.09$, $p = 0.81$), nor with mean annual precipitation (Pp: $r_\rho = -0.18$, $p = 0.66$). Likewise, the patterns of integration neither correlated with climatic conditions (Mantel test: M_{CP} vs M_{CLIM}: $r_m = -0.04$, $p = 0.52$), nor with geographic distance (Mantel test: M_{CP} vs M_G: $r_m = 0.06$, $p = 0.32$).

Discussion

In this study, we examined whether the variation in floral integration among populations of *A. ligtu simsii* was associated with pollinators, testing three predictions: (1) populations

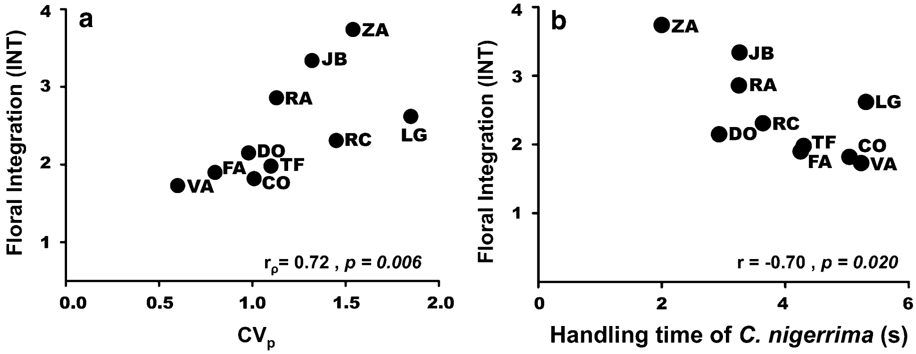

Fig. 2 Floral integration (INT values) versus the coefficient of variation of pollinator proboscis length (CV_p), and the handling time of the most widespread pollinator, *Centris nigerrima*

with similar pollinator assemblage ought to display similar patterns of floral trait correlations; (2) populations visited by a high number of pollinator species, or by those that are morphologically more diverse should display lower levels of floral integration; (3) the behavior of the pollinators should be affected by the magnitude of floral integration. As expected, we found that populations of *A. ligtu simsii* with similar pollinator assemblage showed similar patterns of floral trait correlations, independently of the similarity in climatic conditions or geographic distance among populations; moreover the most widespread pollinator (*C. nigerrima*) invested lower handling time in populations with higher levels of floral integration. However, we failed to detect a negative correlation between the magnitude of floral integration and the diversity of pollinator assemblage.

The magnitude of floral integration in *A. ligtu simsii* varied from 22 to 46 % of the maximum possible integration. These values were twice as high as those reported by Ordano et al. (2008), who in a comparative study on 36 angiosperms found that most integration values fell between 5 and 25 %. These differences might be related to the development affinities, or functions, of the floral traits assessed. We measured integration among tepals from two whorls corresponding to the calyx (external tepals) and corolla (inner tepals). In *Alstroemeria* (similar to other Monocots), external tepals are petaloids, and probably work together with inner tepals as a functional unit, or module, for pollinator attraction and rewards signaling. In contrast, Ordano et al. (2008) included floral traits from four whorls, which can have different functions during pollination (e.g. pollinator attraction, pollen transfer, bud protection, among others). Therefore, these traits may comprise different functional units or modules (Diggle 2014, Armbruster et al. 2014). In addition, it is known that floral traits from different whorls are less constrained as a whole in the developmental process (Herrera et al., 2002), thus reducing floral integration.

Reduction in floral integration might hamper short-distance orientation and landing of *C. nigerrima* and other pollinators that use nectar guides and floral architecture as visual cues, increasing the time taken to access the nectar and pollen rewards. Botto-Mahan et al. (2011), for example, showed that when 20 % of the nectar guide tepal of *A. ligtu simsii* was removed, the pollinator spends more time handling flowers. Pollinator foraging should be sensitive, not only to the cost of acquiring high-energy rewards, but also to the time and energy taken to access them (Leonard and Papaj 2011). If pollinator fitness depends upon maximizing the rate of nectar collection, pollinators should select flowers that require shorter handling times. An increment in handling time might also negatively affect plant fitness. In a self-incompatible species such as *A. ligtu simsii*, a higher handling time might reduce the likelihood of pollinators visiting different plants, thereby decreasing the number of compatible pollen grains deposited in the stigmas. In addition, a higher handling time may result in pollen wastage, thereby reducing male plant fitness. However, larger handling times may culminate in greater pollen deposits on the stigma and/or the ability of pollinators to collect larger amounts of pollen (Ohashi 2002), thus increasing plant fitness.

We found that *C. nigerrima*, as opposed to the other two widespread pollinators (*M. gayi* and *A. gayi)*, invested higher handling times in populations with lower levels of floral integration. This result is consistent with the experimental evidence found by Botto-Mahan et al. (2011), who observed that, whereas removing 20 % of the nectar guide tepal area (NG) in *A. ligtu simsii* led to a two-fold increase in the handling time of *C. nigerrima* compared to non-manipulated flowers, the same experiment did not affect the handling time of *M. gayi* and *A. gayi*. The same experimental data showed that *Lassia corvina*, a congeneric species of *L. aenea*, was sensitive to variations in floral architecture. Our study revealed that these two species, together with *C. nigerrima*, represented 42 % of total recorded visits; hence, they might play a major role on floral integration.

Since the pioneer work of Berg (1960), several studies have investigated the relation between floral integration and specialization (Armbruster et al. 1999; Herrera et al. 2002; Rosas-Guerrero et al. 2010). Rosas-Guerrero et al. (2010) documented in *Ipomoea*, that floral integration is higher in species pollinated by one functional group than generalist plant species, while Pérez-Barrales et al. 2014 evidenced in *N. papyraceus* and *N. tazetta,* that populations visited by specific insects (long-tongued pollinators) displayed greater phenotypic integration than those pollinated by generalist insects (shorter-tongued pollinators). In the same way, in a comparative study on the genus *Erysimum,* Gómez et al. (2014) documented that the magnitude of floral integration increased when the pollinator assemblage was dominated by a particular functional group, irrespective of its identity or pollinator efficiency. In our study, we failed to detect a significant and negative correlation between floral integration and the number of flower visitor species (S'), or between floral integration and the Shannon diversity index (H'). Furthermore, we detected an unexpected, positive relationship between floral integration and the variation coefficient of pollinator proboscis length (CV_p). The relationship between CV_p and INT, however, might result from a confounding effect of proboscis length on floral integration. In our data set, floral integration was correlated positively with the visitation rate of pollinators with the longest proboscis (length > 7 cm; $r_\rho = 0.69$, $p = 0.02$), such as *L. corvina* and *C. nigerrima*, which are known to be sensitive to changes in the floral architecture (Botto-Mahan et al. 2011), and might be selecting plants with higher floral integration.

Floral variation within a species range can be influenced by several factors including natural selection mediated by both biotic and abiotic factors, genetic drift, and gene flow (Galen 1999; Caruso et al. 2003; Cosacov et al. 2014). In our study, we were enable to detect a significant relationship between the patterns of floral trait correlations and geographic distance that would account for a significant role of gene flow, or a geographically structured abiotic factor on floral integration. Neither did we find a significant effect from climatic variables on the magnitude and patterns of floral trait correlations, suggesting that pollinators themselves are the main selective agents for floral integration.

Overall, our study suggests that variation in the magnitude and patterns of floral integration across the species range was correlated to the composition of pollinator assemblage, and not to pollinator diversity, nor to climatic conditions. *A. ligtu simsii* was visited by a large number of pollinators, including Hymenopterans, Dipterans, and Lepidopterans, but floral integration was probably shaped by the most frequent floral visitors, which are insects with long proboscises that are sensitive to changes in floral architecture. Consequently, for some species, the reduction in floral integration might signify a cost in terms of fitness as the handling time increases during flower visits. Under this scenario, it is possible that *A. ligtu simsii* may engage in a more specialist, rather than generalist, plant-pollinator interaction as was previously thought. Future studies should set about evaluating pollinator efficiency and the impact of floral integration on reproductive success and fitness.

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