

**UNIVERSIDAD NACIONAL AUTÓNOMA DE MÉXICO**  
**POSGRADO EN CIENCIAS BIOLÓGICAS**

**INSTITUTO DE ECOLOGÍA**  
**EVOLUCIÓN**

**De flores grandes a pequeñas en Solanum (Sección Androceras): una perspectiva  
multivariada**

**TESIS**

**(POR ARTÍCULO CIENTÍFICO)**

**From large to small flowers in Solanum (Section Androceras): a multivariate perspective**

**QUE PARA OPTAR POR EL GRADO DE:**

**MAESTRA EN CIENCIAS BIOLÓGICAS**

**PRESENTA:**

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OFICIO CPCB/380/2020  
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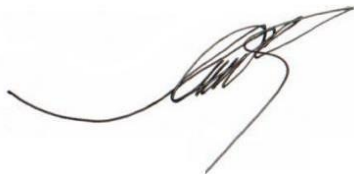
Me permito informar a usted, que el Subcomité de Ecología y Biología Evolutiva, del Posgrado en Ciencias Biológicas en su sesión ordinaria del día **23 de marzo de 2020**, aprobó el siguiente jurado para la presentación de su examen para obtener el de grado de **MAESTRA EN CIENCIAS BIOLÓGICAS** en el campo de conocimiento de Biología Evolutiva, de la estudiante **RUBINI PISANO MARÍA AIMÉ con número de cuenta: 517494013**, con la tesis en la modalidad de artículo científico, titulada: **"From large to small flowers in Solanum (Section Androceras): a multivariate perspective"**, que es producto del proyecto realizado en la maestría que lleva por título: **"De flores grandes a pequeñas en Solanum (Sección Androceras): una perspectiva multivariada"** bajo la dirección del **Dr. JUAN ENRIQUE FONONI AGNELLI**, quedando integrado de la siguiente manera:

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Sin otro particular, me es grato enviarle un cordial saludo.

**ATENTAMENTE**  
**"POR MI RAZA HABLARÁ EL ESPÍRITU"**  
Cd. Universitaria, Cd. Mx., a 18 de mayo de 2020

**COORDINADOR DEL PROGRAMA**



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## **Agradecimientos Institucionales**

Agradezco al Posgrado en Ciencias Biológicas de la Universidad Nacional Autónoma de México por las facilidades otorgadas para realizar este trabajo.

Agradezco al Consejo Nacional de Ciencia y Tecnología (CONACYT) por el apoyo económico n° 464273 otorgado para realizar los estudios de maestría y la Beca Mixta para la estancia de investigación en University of Stirling en Escocia que permitió la concreción de este proyecto. Además, esta investigación fue posible gracias al financiamiento a través del apoyo IN210617 por parte de PAPIIT-DGAPA-UNAM. Agradezco al programa de Becas CONACYT-OEA-AMEXCID por su apoyo económico por gastos de instalación.

Especialmente agradezco a mi tutor Dr. Juan Enrique Fornoni Agnelli por su constante apoyo, predisposición y enseñanzas brindadas a lo largo de este proyecto, y a los miembros de mi comité tutor Dra. Julieta Alejandra Rosell García y Dr. Daniel Ignacio Piñero Dalmau por su interés y valiosos aportes a este trabajo.

## **Agradecimientos Personales**

*“(...) —¿la vida, cuándo fue de veras nuestra?,  
(...) nunca la vida es nuestra, es de los otros,  
la vida no es de nadie, todos somos  
la vida -pan de sol para los otros,  
los otros todos que nosotros somos-,  
soy otro cuando soy, los actos míos  
son más míos si son también de todos,  
para que pueda ser he de ser otro,  
salir de mí, buscarme entre los otros,  
los otros que no son si yo no existo,  
los otros que me dan plena existencia,  
no soy, no hay yo, siempre somos nosotros (...)”*

*Piedra de sol, de Octavio Paz.*

Este trabajo no hubiera sido posible sin la constante, solidaria y desinteresada contribución de todas y todos mis compañeros del Laboratorio de Interacción Planta-Animal, con los que intercambié opiniones sobre aspectos teóricos y prácticos que fortalecieron las bases argumentales de la investigación y clarificaron ideas para escribir el manuscrito. La ciencia es una construcción colectiva.

Pero sobre todo, este trabajo no hubiera sido posible sin el apoyo y amor incondicional de todas y todos mis seres amados, que a la distancia o muy cercanamente me dan plena existencia. No soy, no hay yo, siempre somos nosotros.

## Índice

Resumen.....	6
Abstract.....	7
Introducción general.....	8
From large to small flowers in Solanum (Section Androceras): a multivariate perspective .....	11
Introduction .....	11
Materials and Methods.....	14
Results .....	20
Discussion.....	28
Acknowledgements.....	31
References.....	31
Discusión general y Conclusiones .....	36
Supporting information.....	41

## Resumen

A pesar que las flores son concebidas como un módulo funcional compuesto por un conjunto de rasgos correlacionados, nuestro entendimiento de las transiciones evolutivas florales adaptativas rara vez se ha considerado desde una perspectiva multivariada. Algunos estudios indican que la transición entre sistemas de apareamiento y polinización afectan la estructura de relaciones entre rasgos de la flor. Las especies con autofertilización pueden tener covariaciones más intensas entre rasgos florales sexuales que las especies de entrecruza, y algunos síndromes de polinización expresan convergencia interna significativa en la relación señal-recompensa así como en la estructura de correlación entre los rasgos de la corola. En ambos casos, las transiciones evolutivas están marcadas por diferencias significativas en el tamaño de la flor, sugieren convergencia durante la transición entre sistemas de apareamiento y que las correlaciones entre caracteres florales juegan un rol funcional durante la reproducción. Además, la matriz floral de varianzas y covarianzas parece ser mucho más lábil durante la evolución de lo que se creía, como lo indican la aparente ausencia de señal filogenética significativa para la integración floral y la amplia variación en los valores de integración en angiospermas. Toda la evidencia parece señalar que las transiciones evolutivas que involucran el tamaño floral probablemente se encuentran relacionadas con ajustes en la estructura de correlación interna de la flor. Por lo tanto, si dichas correlaciones representan asociaciones funcionales, las transiciones evolutivas adaptativas probablemente afectan a todo el conjunto de correlaciones funcionales.

En este trabajo se utilizaron tres transiciones evolutivamente independientes de flores grandes a pequeñas dentro del género *Solanum*, Sección *Androceras*, presumiblemente asociadas con cambios del entrecruzamiento a la autofertilización, para examinar cambios en las correlaciones entre rasgos florales. Dos transiciones presentan un par de taxones hermanos (*S. citrullifolium* y *S. heterodoxum*; *S. rostratum* y *S. fructu-tecto*) y la última dos variedades (*S. grayi* var. *grandiflorum* y *S. grayi* var. *grayi*). Se emplearon 8 medidas lineares de la flor, incluyendo ancho y largo de la corola y los órganos sexuales (anteras y estilo). A partir de estos datos se construyó una matriz de varianza-covarianza de rasgos florales con el fin de evaluar la asociación entre las propiedades de esta matriz y el tamaño de la flor. A través de una aproximación morfoespacial se analizaron la integración (intensidad de la relación entre rasgos), modularidad (grupos de rasgos más asociados entre sí que con el conjunto), y estructura de la matriz floral.

Los resultados mostraron que, según lo previsto, los taxones de flores pequeñas ocuparon una

porción diferenciada del morfoespacio de valores medios pero convergieron en sus covarianzas en relación con los taxones de flores grandes. Asimismo, los taxones de flores pequeñas presentaron una reducción en el tamaño de su matriz floral consistente con la reducción esperada en la cantidad de variación genotípica promovida por la transición de entrecruza a autofertilización (mayor homocigosis). Finalmente y contrario a lo esperado, los taxones de flores grandes presentaron una integración fenotípica ligeramente mayor pero una modularidad similar a la de los taxones de flores pequeñas.

Por lo tanto, la transición a flores pequeñas fue marcada por una combinación de los efectos genéticos de la autofertilización reduciendo las combinaciones fenotípicas, y de la selección natural promoviendo la convergencia en valores medios de los rasgos y en la estructura de varianzas-covarianzas.

## **Abstract**

Despite flowers are accepted as functional modules composed by correlated traits, our understanding of adaptive floral evolutionary transitions has seldom taken a multivariate perspective. If correlations among floral traits represent functional associations, adaptive evolutionary transitions likely affects the whole set of functional correlations.

Here we used three independent evolutionary transitions from large to small flowers within the genus *Solanum*, to examine changes in covariation among floral traits presumably associated with shifts in mating system. A morphospace approximation combined with matrix structure and modularity analyses were performed to test for an association between flower size and the properties of the floral matrix (phenotypic integration, modularity, matrix structure).

Small-flowered taxa clearly occupied a differentiated portion of the morphospace of mean values but converged in their covariances relative to large-flowered taxa. Small-flowered taxa presented a reduction in their floral matrix size consistent with the expected reduction in the amount of genotypic variation promoted by the transition from outcrossing to selfing (higher homocigosity). Large-flowered taxa presented slightly higher phenotypic integration but similar modularity than small-flowered taxa.

Thus the transition to small flowers was labelled by the combination of selfing reducing phenotypic combinations and natural selection promoting convergence in mean trait values and the variance-covariance structure.



## Introducción general

En angiospermas, el tamaño floral es el rasgo reproductivo con mayor variabilidad que ha tenido consecuencias profundas para la adecuación (Harder y Johnson 2009). Insularidad, domesticación, condiciones físicas ambientales, dispersión, polinización y sistema de apareamiento son todos ejemplos de diferentes escenarios eco-evolutivos que promueven patrones convergentes de adaptación en diferentes taxones, afectando el tamaño individual y en particular el tamaño floral (Kliman 2016). Una de las transiciones más recurrentes es aquella relacionada al sistema de apareamiento, expresada como una reducción en el tamaño floral entre especies que se autofertilizan en relación a sus ancestros de entrecruza (Stebbins 1974; Grant 1981; Barrett 2002). La reducción en el tamaño floral en especies que se autofertilizan es usualmente acompañada por otros cambios simultáneos (esto es, reducción en la cantidad de polen, esencias, néctar y magnitud de la hercogamia, decrecimiento en el tamaño de la corola y en la relación polen:óvulo; Stebbins 1970, Sicard y Lenhard 2011). Esta transición evolutiva de la estrategia de apareamiento tiene consecuencias genéticas, reorganizando la variación, y también involucra un cambio drástico en el régimen selectivo sobre el fenotipo floral (Stebbins 1970; Charlesworth y Willis 2009; Sicard y Lenhard 2011). A pesar de que la flor es vista como un módulo funcional integrado que logra la reproducción (Berg 1960; Armbruster 1991; Armbruster *et al.* 2004), nuestro entendimiento de la transición entre flores de gran tamaño (entrecruza) a flores de menor tamaño (autofertilización) rara vez adopta una perspectiva multivariada. Dicho enfoque permite comprender los cambios evolutivos en los módulos florales, entendidos como el conjunto de rasgos florales semi-independientes de otros, interactuando e integrados gracias a un rol funcional común y a procesos ontogenéticos (Armbruster *et al.* 2004; Klingenberg 2008). Sin embargo, si la flor es considerada como un módulo funcional comprendido por un conjunto de rasgos correlacionados, la reducción evolutiva en el tamaño floral debería estar acompañada por otros ajustes que permitan la adaptación a diferentes entornos de polinización (por ej., una mayor autofertilización que implicaría una menor dependencia de los polinizadores). El presente estudio examina si la transición de flores grandes a pequeñas en taxones cercanamente emparentados, va más allá de cambios en los valores medios de los rasgos, afectando además tanto a las relaciones entre los mismos (covarianzas) como a sus varianzas.

Algunos estudios empíricos indican que la transición entre sistemas de apareamiento y polinización afectan la estructura de correlación de la flor (Armbruster *et al.* 2004; Fornoni *et al.* 2016). Primero, las especies de entrecruza deberían manifestar altos niveles de integración en sus caracteres

de atracción, pero también en sus órganos sexuales debido a los requerimientos de ajuste preciso entre éstos y los polinizadores (Armbruster *et al.* 2004). Sin embargo, una revisión reciente encontró que las especies que son capaces de autofertilizarse podrían tener correlaciones más intensas (es decir, mayor integración fenotípica) entre rasgos florales sexuales que las especies de entrecruza (Fornoni *et al.* 2016), sugiriendo cambios convergentes de las propiedades de las matrices florales durante la transición entre sistemas de apareamiento. Más aún, modificaciones importantes en las presiones selectivas, como la pérdida o disminución de los polinizadores, podrían promover mayor integración de los órganos sexuales posibilitando una transferencia de polen más eficiente dentro de la flor. Segundo, los síndromes de polinización por abejas y polinización por colibríes en el género *Salvia* expresaron convergencia interna significativa en la correlación señal-recompensa (Benitez-Vieyra *et al.* 2014), así como en la estructura de correlación entre los rasgos de la corola (Benitez-Vieyra *et al.* 2019). En ambos casos, las transiciones evolutivas fueron marcadas por diferencias significativas en el tamaño de la flor. Así, estos ejemplos sugieren que las correlaciones entre caracteres florales juegan un rol funcional durante la reproducción. Tercero, la aparente ausencia de señal filogenética significativa para la integración floral (Gómez *et al.* 2014; Benitez-Vieyra *et al.* 2019; Fornoni *et al.* 2016, etc.) y la amplia variación en los valores de integración a lo largo de las plantas con flores (Ordano *et al.* 2008), también sugiere que la matriz floral es mucho más lábil durante la evolución de lo que previamente se creía. Por sobre todo, la evidencia indica que las transiciones evolutivas que involucran el tamaño floral probablemente se encuentren relacionadas con ajustes en la estructura de correlación interna de la flor (Wagner y Altenberg 1996; ver por ejemplo Conner y Sterling 1995; Armbruster *et al.* 1999; Pérez *et al.* 2007; Benitez-Vieyra *et al.* 2019).

Estudios previos con el género *Solanum* en la Sección monofilética *Androceras* (*Solanaceae*) describen tres transiciones independientes de taxones de flores grandes a pequeñas (Whalen 1978, 1979, Vallejo-Marín *et al.* 2014). Estas transiciones en tamaño floral son acompañadas por algunas de las características clave y distintivas de los cambios de entrecruza a autofertilización, incluyendo una separación reducida entre el estigma y la antera, una baja relación polen:óvulo y una pérdida del dimorfismo entre anteras, todas condiciones que disminuyen la dependencia de los polinizadores al momento de la reproducción (Vallejo-Marín *et al.* 2014). Aunque las estimaciones genéticas de las tasas de entrecruzamiento se encuentran disponibles sólo para una de las especies de entrecruza (*S. rostratum*,  $t = 0.70-0.94$ ; Vallejo-Marín *et al.* 2013, Mora-Carrera *et al.* 2019), los caracteres morfológicos

que distinguen entre taxones de flores grandes o pequeñas, y la mayor producción de frutos por parte de los taxones de flores pequeñas por autofertilización en el invernadero, apoyan fuertemente la diferencia en el sistema de apareamiento entre estos taxones. Si la evolución de flores pequeñas durante la transición hacia la autofertilización involucra ajustes funcionales en la totalidad de la matriz floral impulsados por un nuevo régimen selectivo, serán observados tanto cambios en las medias como en las covarianzas de los rasgos en relación a sus parientes de tamaño floral mayor. Las matrices florales de los taxones de flores grandes, al interactuar estos últimos con diferentes polinizadores, mostrarán mayor divergencia entre sí que sus parientes de flores pequeñas, los que presumiblemente se encuentren sujetos a la misma presión de selección, dada su mayor independencia de vectores bióticos de polen. Si la corola y los caracteres sexuales interactúan promoviendo una transferencia eficiente de polen en taxones de flores grandes (entrecruza; Berg 1960; Armbruster 1991; Pérez-Barrales *et al.* 2014), éstas mostrarán una mayor integración que sus parientes de flores pequeñas. A su vez, los caracteres sexuales en taxones de flores pequeñas estarán más integrados, proveyendo seguridad reproductiva, y estarán menos conectados con los rasgos de la corola que respecto a sus ancestros de flores grandes (es decir, los taxones de flores pequeñas mostrarán una mayor modularidad entre la corola y los rasgos sexuales que los taxones de flores grandes; Fornoni *et al.* 2016). Como alternativa a nuestra expectativa previa, si la evolución de flores pequeñas reduce principalmente la asignación de recursos a flores individuales, estos taxones mostrarán un tamaño floral reducido sin cambios en la estructura de covarianzas de la flor. Si los taxones de flores pequeñas caracterizan la transición a la autofertilización, el esperado incremento en el desequilibrio de ligamiento también afectará varianzas y covarianzas (Phillips *et al.* 2001; Charlesworth y Charlesworth 1995).

El conjunto de tres transiciones replicadas mencionado anteriormente fue utilizado para evaluar si (1) los taxones con flores grandes y pequeñas divergieron en sus valores florales medios o matrices florales de varianza-covarianza utilizando una aproximación morfométrica, (2) los taxones con flores grandes tienen mayores y más disímiles matrices que los taxones con flores pequeñas, y (3) si los taxones con flores pequeñas tienen mayor modularidad entre la corola y los órganos sexuales que los taxones con flores grandes. Para examinar estas expectativas, una aproximación morfoespacial y un análisis de Random Skewers fueron realizados con el fin de comparar las propiedades matriciales y sus diferencias (por ejemplo, como en Benitez-Vieyra *et al.* 2019). La integración floral fue estimada utilizando un

análisis de componentes principales (Herrera *et al.* 2001), y la modularidad utilizando el índice CR (Adams 2016).

## **From large to small flowers in *Solanum* (Section *Androceras*): a multivariate perspective**

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**Keywords:** flower size, mating system, morphospace, phenotypic integration, self-fertilization, *Solanum*, outcrossing.

### **Introduction**

Across flowering plants, flower size is the most variable reproductive trait that has deep consequences for fitness (Harder & Johnson 2009). Insularity, domestication, environmental physical conditions, dispersal, pollination and mating are all examples of different eco-evolutionary scenarios promoted convergent patterns of adaptation in different taxa affecting individual size and in particular flower size (Kliman 2016). One of the most recurrent transitions is that related to mating expressed as a reduction in flower size among species that self-fertilize relative to their outcrossing ancestors (Stebbins

1974; Grant 1981; Barrett 2002). Reduction in flower size in self-fertilizing species is usually accompanied by other concurrent changes (i.e., reduction in the amount of pollen, scents, nectar, and magnitude of herkogamy, a decrease in corolla size and the pollen:ovule ratio; Stebbins 1970, Sicard & Lenhard 2011). This evolutionary transition of the mating strategy has genetic consequences reorganizing genetic variation and also involve a drastic change in the selective regime upon the floral phenotype (Stebbins 1970; Charlesworth & Willis 2009; Sicard & Lenhard 2011). Despite the flower being viewed as an integrated functional module for reproduction (Berg 1960; Armbruster 1991; Armbruster *et al.* 2004), our understanding of the transition from large (outcrossing) to small (selfing) flowers seldom take a multivariate perspective. Such approach allows to comprehend the evolutionary shifts in floral modules, i.e. floral traits semi-independent from others, interacting and integrated by a common functional role and onto-genetic processes (Armbruster *et al.* 2004; Klingenberg 2008). If the flower is considered as a functional module comprised of a set of correlated traits, the evolutionary reduction in flower size should be accompanied by other adjustments to fit different pollination environments (e.g., higher selfing, reduced reliance on pollinators). The present study examines whether the transition from large to small flowers in closely related taxa, goes beyond changes in mean floral trait values, and also affects both the relations among traits (covariances) and their variances.

Empirical studies indicates that transitions between mating and pollination systems affect the correlation structure of the flower (Armbruster *et al.* 2004; Fornoni *et al.* 2016). First, outcrossing species should manifest high integration levels in their attraction traits, but also in sexual organs due to requirements of accurate fit among them and pollinators (Armbruster *et al.* 2004). However, a recent review found that species that are able to self-fertilize could have more intense correlations (i.e., higher phenotypic integration) among floral sexual traits than outcrossing species (Fornoni *et al.* 2016), suggesting convergent shifts of floral matrices properties during the transition between mating systems. Moreover, major changes in selective pressures as loss or decrease of pollinators could promote higher sexual organs integration, enabling an efficient transfer of pollen within the flower. Second, bee- and hummingbird-pollination syndrome in the genus *Salvia* expressed significant within-syndrome convergence on the signal-reward correlation (Benitez-Vieyra *et al.* 2014) and on the correlation structure among corolla traits (Benitez-Vieyra *et al.* 2019). In both cases, significant differences in flower size labeled the evolutionary transitions. These two cases suggest that correlations between floral traits

play a functional role during reproduction. Third, the apparently absence of significant phylogenetic signal for floral integration (Gómez *et al.* 2014; Benitez-Vieyra *et al.* 2019; Fornoni *et al.* 2016, etc.) and the wide variation in integration values across flowering plants (Ordano *et al.* 2008) also suggest that the matrix of floral traits is much more labile during evolution than previously thought. Overall, the evidence indicate that evolutionary transitions involving flower size are likely related to adjustments in the internal correlation structure of the flower (Wagner & Altenberg 1996; see for example Conner & Sterling 1995; Armbruster *et al.* 1999; Pérez *et al.* 2007; Benitez-Vieyra *et al.* 2019).

Previous studies with the genus *Solanum* in the monophyletic Section *Androceras* (Solanaceae) described three independent transitions from large to small flowered taxa (Whalen 1978, 1979, Vallejo-Marín *et al.* 2014). These flower-size transitions are accompanied by some of the other key hallmarks of outcrossing to selfing shifts including reduced anther-stigma separation, lower pollen:ovule ratios, and loss of anther dimorphism, all conditions that decrease the reliance on pollinators for reproduction (Vallejo-Marín *et al.* 2014). Although genetic estimates of outcrossing rates are only available for one of the outcrossing taxa (*S. rostratum*,  $t = 0.70-0.94$ ; Vallejo-Marín *et al.* 2013, Mora-Carrera *et al.* 2019), morphological traits distinguishing large- and small-flowered taxa, and the higher fruit set of small flowered taxa by autonomous self-pollination in the glasshouse strongly support the difference in mating system between large- and small-flowered taxa.

If the evolution of small flowers during the evolution of selfing involves functional adjustments on the whole floral matrix driven by a new selection regime, both changes in trait means and covariances between floral traits will be observed relative to their large flowered relatives. As large-flowered taxa interact with different pollinators, floral matrices will show more divergence among them than their small-flowered relatives which are presumably subject to the same selection pressure given their much more independence from pollen vectors. If corolla and sexual traits interact promoting an efficient pollen transference in large flowered taxa (outcrossers) (Berg 1960; Armbruster 1991; Pérez-Barrales *et al.* 2014), these will show higher integration than their small flowered relatives. In turn, sexual traits in small-flowered taxa will be more integrated to provide reproductive assurance and will be less connected with corolla traits than in their large-flowered ancestors (i.e., small flowered taxa will show higher modularity between the corolla and sexual traits than large flowered taxa) (Fornoni *et al.* 2016). As an alternative to our previous expectations, if the evolution of small flowers mainly reduces allocation

of resources to individual flowers, these taxa will show a reduced flower size without changes in the covariance structure of the flower. If small-flowered taxa characterized transition to selfing, the expected increment in linkage disequilibrium will also affect variances and covariances (Phillips *et al.* 2001; Charlesworth & Charlesworth 1995).

The above mentioned set of three replicated transitions was used to test whether (1) taxa with small and large flower size diverged in their means and/or variance-covariance floral matrices using a morphometric approximation, (2) large-flowered taxa have larger and more dissimilar matrices than small-flowered taxa, (3) small-flowered taxa have higher modularity between corolla and sexual traits than large-flowered taxa. To examine these expectations a morphospace approximation and random skewer analyses were performed to compare matrices properties and their dissimilarities (e.g, Benitez-Vieyra *et al.* 2019). Floral integration was estimated using principal component analysis (Herrera *et al.* 2001) and modularity using the CR index (Adams 2016).

## **Materials and Methods**

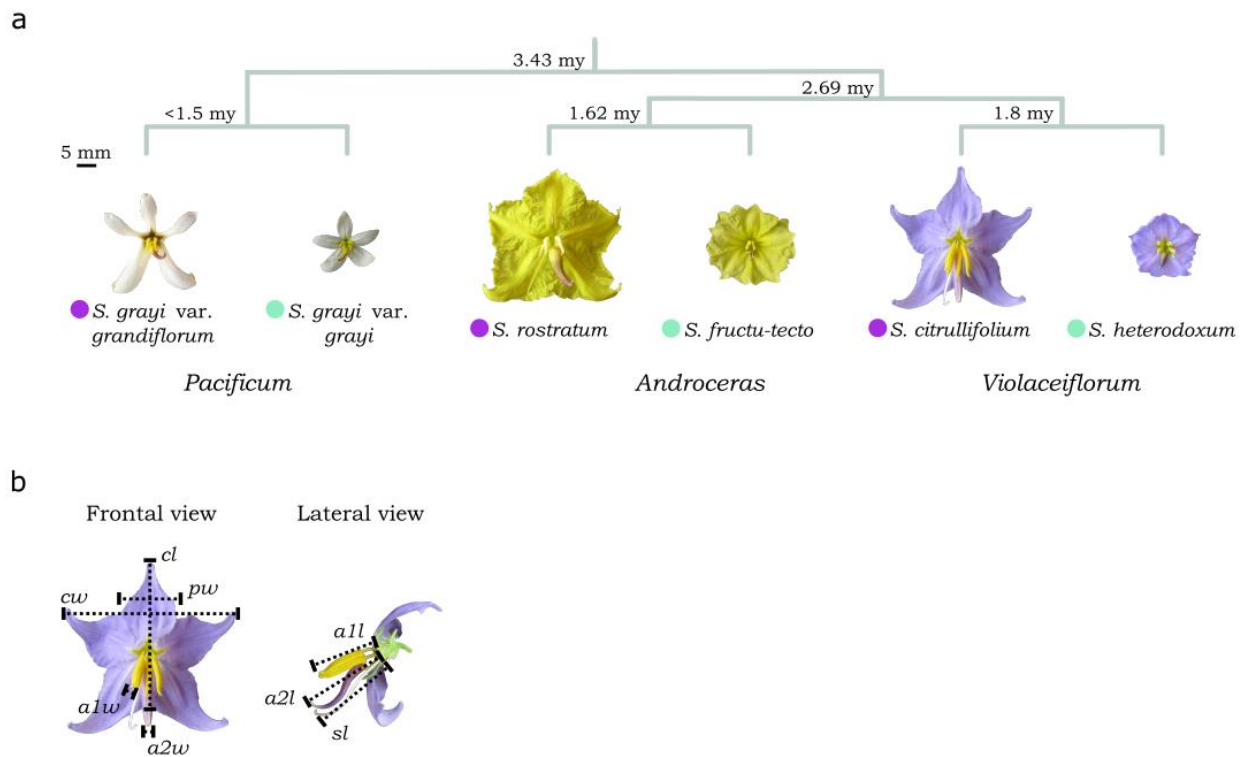
### *Study system*

*Solanum* Section *Androceras* is a monophyletic clade of approximately 12 taxa (Whalen 1979). Taxa in Section *Androceras* are annual or perennial herbs native to Mexico and the southern USA, and are classified in three taxonomic groups: series *Violaceiflorum* (four taxa) with purple corollas, the yellow-flowered series *Androceras* (five taxa), and series *Pacificum* (three taxa) with yellow or white corollas (Whalen 1979). As other species of *Solanum*, flowers of Section *Androceras* are hermaphroditic, pentamerous, nectarless, and with anthers that open through small apical pores (poricidal anthers; Harris 1905). Their flowers are buzz-pollinated (Arroyo-Correa *et al.* 2019, Vallejo-Marín 2019) and visited by bees, including species in Halictidae, Colletidae, and Apidae (Bowers 1975, Solis-Montero, Vergara & Vallejo-Marín 2015). However, unlike most other *Solanum*, Section *Androceras* is distinguished by having two or more sets of morphologically distinct stamens in the same flower (heteranthery; Todd 1882, Whalen 1979, Vallejo-Marín *et al.* 2010). Heteranthery is often accompanied by a bilaterally symmetric corollas, and in some cases enantiostyly, where anthers and styles are deflected to opposite sides of the flower and two mirror-image flower types are found in the same plant (monomorphic enantiostyly; Barrett *et al.* 2000). The extent of anther dimorphism, bilateral symmetry and degree of enantiostyly varies within Section *Androceras*, sometimes within the same species

(Whalen 1979). In the taxa studied here, each flower possesses four shorter, centrally located stamens of approximately the same size and color (feeding anthers; Vallejo-Marín *et al.* 2014), and a single stamen of larger size and different morphology (pollinating anther), which can be of different color than the feeding anthers. The evolution of heteranthery is thought to result in the functional specialization of anther types into pollinator attraction and reward (feeding anthers) and fertilization (pollinating anther) (Muller 1883, Vallejo-Marín *et al.* 2009).

Here we selected three pairs of taxa in *Solanum* Section *Androceras*, which represent three independent transitions from large- to small-flowered taxa (Whalen 1978, Vallejo-Marín *et al.* 2014; Fig. 1). These transitions in flower morphology are thought to accompany shifts in mating system from pollinator-dependent outcrossing to increased selfing and reduced reliance on pollinators (Vallejo-Marín *et al.* 2014). The stamens for small-flowered taxa shown a variation not only in the amount of pollen produced (diminished) but also in the morphology of the pollinating anther, which considerably reduce its size and loss its sigmoid shape, resembling feeding anthers (Vallejo-Marín *et al.* 2014). Moreover, the apical pores of all anthers experience an enlargement, as well as the stigma surface, and a shift to a more flatter openings in their final form. Such characteristics, added to the reduction in distance among anthers and stigma, enables the slightest vibration produced by the wind or any other movement, to release pollen from the nearest anthers towards the stigma. Each series of *Solanum* Section *Androceras* was represented by a single taxa pair: Series *Violaceiflorum*: *S. citrullifolium* A. Braun (outcrosser) and *S. heterodoxum* Dunal (selfer); Series *Androceras*: *S. rostratum* Dunal (outcrosser) and *S. fructu-tecto* Cav. (selfer); Series *Pacificum*: *S. grayi* var. *grandiflorum* Whalen (outcrosser) and *S. grayi* var. *grayi* Whalen (selfer) (Fig. 1a; Vallejo-Marín *et al.* 2014).





**Figure 1. (a)** Phylogenetic relationships of the six *Solanum* section *Androceras* taxa used in the present study and their divergence time (95% highest posterior density or HPD) following Särkinen et al. (2013). Light green dots indicate small-flowered taxa, violet dots indicate large-flowered taxa. Below each taxa specific name the series name is indicated. Taxonomic classification follows Whalen (1979). **(b)** Frontal and lateral views of *S. citrullifolium* to exemplify the eight measured floral traits. Abbreviations used throughout this work: *cl* corolla length, *cw* corolla width, *pw* petal width, *a1w* feeding anther width, *a1l* feeding anther length, *a2w* pollinating anther width, *a2l* pollinating anther length, *sl* style length.

#### *Plant material and data collection*

Seeds were collected between 2007-2011 from natural populations in Mexico, except for *S. citrullifolium*, which was obtained from the Experimental Garden and GeneBank Solanaceae collection at Radboud University, Nijmegen (Table S1). Seeds from two populations per taxa were treated with a 1000 ppm aqueous solution of gibberellic acid (GA3; Sigma-Aldrich, Dorset, UK) for 24 h and sown in a seed trays with Modular seed growing medium compost (William Sinclair Horticulture PLC, Lincoln, UK) and kept in a controlled environment cabinet (Microclima 1750E; Snijders Scientific, Tilburg, the Netherlands) at 24C/16C and 18h/6h day/light cycles. (14 - 31 individuals per population) under greenhouse conditions. Two to three weeks after germination, seedlings were individually transplanted

to 1.5L pots with a mix of general purpose compost and perlite in a 3:1 ratio. Plants were fertilized approximately once a week with Tomorite fertilizer (Levington, United Kingdom). When plants started flowering, two open flowers per plant were randomly selected and collected to obtain morphometric floral measurements (N = 548 flowers; Table S1). Only one of the two populations of *S. fructu-tecto* flowered in the glasshouse (N = 39 plants), adding 11 populations among all taxa.

Floral measurements were obtained from digital images including a reference scale taken with a PowerShot A495 camera (Canon, Japan). Three images were taken from each flower: a frontal view photograph of the whole flower, a frontal view of each anther, and a lateral view of the gynoecium. The pollinating anther and gynoecium were preserved in 70% ethanol before taking the images. All pictures were processed transforming linear measurements from pixels to millimeters using ImageJ software (<https://imagej.nih.gov/ij/>). The following eight linear measures were obtained from each flower: corolla width (cw) and length (cl), petal width (pw), feeding anther width (a1w) and length (a1l), pollinating anther width (a2w) and length (a2l) and style length (sl) (Fig. 1b). Measurements from the same plant were averaged to obtain a single set of values per individual. Data were log-transformed to linearize the relationships among floral traits and for comparisons among traits and taxa (Benitez-Vieyra *et al.* 2019). These data were used to construct phenotypic variance-covariance matrices per population (**P**-matrix, Table S2).

### *Data analyses*

To determine the amount of variance explained by each source of variation (flower size, taxa, and populations), a random effect model for each trait was performed. Significant effects were tested with restricted maximum likelihood (REML; Zuur *et al.* 2009) and a selection model procedure using the *nlme* package (Pinheiro *et al.* 2019). All analyses were done in R v. 3.5.1 (R Core Team 2018).

### *Mean and covariance morphospace analyses*

To compare if there are dissimilarities in the floral matrices reflected in a divergence in morphospace occupation of populations from small and large flowered taxa, a Principal Component Analysis was performed using mean trait values of log-transformed data per population. The first two principal components explained more than 80% of the variation and were used to build the convex hulls of small-

and large-flowered taxa within the morphospace of all possible combinations of trait values (Chartier *et al.* 2014). The morphospace is a mathematical multidimensional space that provides an insightful visualization of evolutionary patterns among species (e.g., Benitez-Vieyra *et al.* 2019). Permutational Multivariate Analysis of Variance (PERMANOVA) (Anderson 2001) was conducted to test for differences in multivariate means between small- and large-flowered taxa using population mean values. PERMANOVA was performed using 1000 permutations without replacement to obtain an estimation of uncertainty of the multivariate combination of traits (Benitez-Vieyra *et al.* 2019). These analyses were performed with the *adonis* function of *vegan* package (Oksanen *et al.* 2017).

The morphospace approximation was also applied to examine whether small- and large-flowered populations and taxa diverged in their variance-covariance floral matrices following the rationale presented in Benitez-Vieyra *et al.* (2019). The matrix of pairwise Riemannian distances between population variance-covariance floral matrices was calculated using the function *MatrixDistance* of the *evolqg* package (Melo *et al.* 2016). This distance is the shortest distance between two matrices in the space of all possible variance-covariance matrices and invariant to linear transformations of the original variables (Mitteroecker & Bookstein 2009; Bookstein & Mitteroecker 2014). Riemannian distance is the square root of the summed squared logarithms of the relative eigenvalues between two matrices. Relative eigenvalues result from the eigendecomposition of **C** matrix, which specifies how to transform one matrix into the other. To visualize Riemannian distances, a Principal Coordinate Analysis (PCoA) was performed (Machado *et al.* 2018; Mitteroecker & Bookstein 2009; Bookstein & Mitteroecker 2014). In this analysis the 11 **P**-matrices, corresponding to the 11 populations, were represented each by a single point and their similarity was represented by the euclidean distances among them. As in the previous morphospace, convex hulls were computed and drawn to describe the morphospace portion occupied by populations from small- and large-flowered taxa. The first three principal coordinates were used as they accumulated 63% of the total variation. Again, significant differences between flower size groups were tested through a PERMANOVA.

To determine if large-flowered populations occupied a wider portion of the mean and covariance morphospaces than small-flowered populations, a measure of disparity among populations within each category was estimated (Drake & Klingenberg 2010). Disparity was estimated as the area comprising the convex hulls generated by mean trait values, while the volume of the convex hulls were for the

covariance morphospace analysis. In both cases observed differences between small- and large-flowered taxa were contrasted against a distribution of 10,000 differences obtained by randomly reshuffling the labels “small-flowered” or “large-flowered” populations (e.g., Maubecin *et al.*, 2016)

#### *Comparison of matrices structure and size*

To analyze the size (total amount of variance) and shape (covariance internal structure) of floral matrices and detect a possible rearrangement in variance-covariance matrices reflecting structural change from large to small flowers, Random Skewers analysis was performed (Cheverud & Marroig 2007). The analysis compares two variance-covariance matrices through their evolutionary response to the same set of random selection vectors ( $\beta$ ). In the present study, each pair of matrices were subjected to the same set of 100,000 random selection vectors (Random Skewers) to obtain response to selection vectors following the multivariate breeder's equation (Lande 1979). The cosine of the angle (vector correlation) between response to selection vectors was used to test for differences in matrices structure. Zero value indicates complete differences whereas a value of one indicates similar orientation (structure). A Monte Carlo procedure using a distribution of 100,000 correlations between random vectors of  $k$  elements (where  $k$  is the number of traits) and unit length, was carried out to statistically evaluate similarity in orientation (Cheverud & Marroig 2007). Differences in matrices size were tested using the response vectors' length ratio. When two matrices have the same size, the magnitude of the response vectors will be equal and their ratio will be one. Analyses were performed after calculating an average correlation matrix for each taxa to compare small and large flowered taxa within pairs of independent transitions, pairs of taxa within the same category of flower size, and average flower size group matrices. Random Skewers were performed following Maubecin *et al.* (2016).

#### *Phenotypic Integration*

To identify if there were differences in floral integration during the transition to small flower taxa, for each population a correlation matrix was built computing Pearson correlation coefficients between pairs of morphometric floral traits. These matrices were then used to estimate the magnitude of integration of the whole flower, the corolla, and the sexual organs. To compare the magnitude of floral phenotypic integration between small and large flowered taxa, the level of integration in each

population was estimated using Wagner-Cheverud index (hereafter INT; Wagner 1984; Cheverud *et al.* 1989). This index is constructed after running a Principal Component Analysis (PCA) using the variance among the eigenvalues obtained for each matrix. Such variance is expressed in the index INT as a fraction of its maximal value for a given number of traits (Armbruster *et al.* 2009) and corrected for differences in sample size (Wagner 1984). INT can vary between 0% when the variance among eigenvalues is close to zero, to 100% when all the variance is concentrated on the first principal component. 95% confidence interval (CI) of INT values were estimated after bootstrapping. INT values and CI were estimated with *PHENIX* package (Torices & Muñoz-Pajares 2015). To assess differences in phenotypic integration between small- and large-flowered taxa, observed differences within each clade were contrasted against a randomized distribution of 1000 differences obtained by randomly reorganizing labels of small and large flowered taxa among individuals within populations (Maubecin *et al.* 2016).

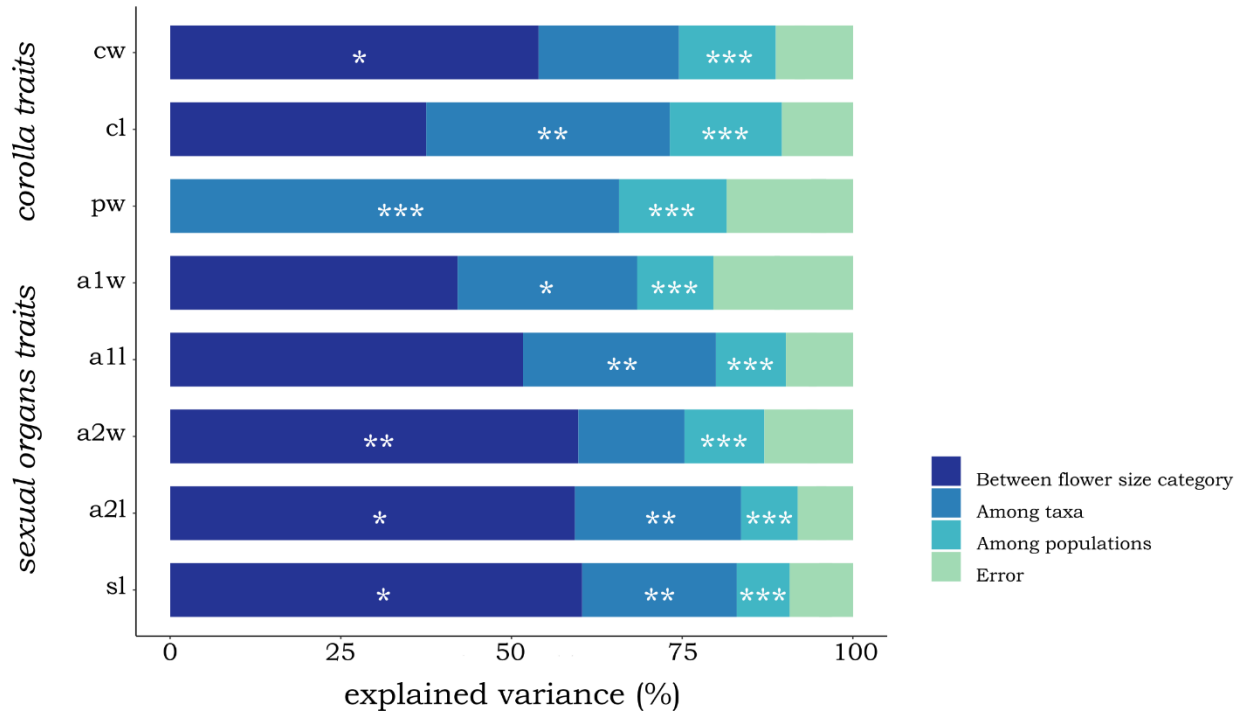
### *Modularity*

To test whether small flowered taxa have higher modularity between corolla and sexual traits than large flowered relatives outcrossing, CR coefficient (Covariance Ratio) was estimated (Adams 2016). First, two functional modules were *a priori* defined, one comprised of corolla traits (petal length, corolla width and length), and another one associated with sexual organs traits (anthers width and length, style length). **P**-matrices from each population were partitioned into sub-covariance matrices: the covariance matrix representing each module and the covariance matrix including correlations between modules. The index is a ratio of the covariation between modules relative to the covariation within modules (Adams 2016), and ranges from 0 to positive values. Low values of CR indicate low covariation between modules (i.e., high modularity) and high CR indicate stronger covariation between modules (i.e., low modularity). CR values between 0 and 1 characterize a significant modular structure while CR values ranging from 1 to any other positive result characterizes less modular structures.

### **Results**

Random effects model indicated that on average, flower size categorization explained most of the total variance, ranging between 50-60 %, following by taxa with 25-40 %, and finally by populations with 10-20 % (Fig. 2). A significant difference between small and large flowered taxa was detected for corolla width, pollinating anther traits and style length (Fig. 2), indicating that in the higher level the groups are

distinguished mostly by traits related with pollination accuracy. Among taxa differences were detected for all traits except for corolla width and pollinating anthers width. Among populations significant differences were detected in all floral traits, showing the phenotypic differentiation under the environmental influence (Fig. 2).



**Figure 2.** Percentage of total variance accounted by each source of variation after a random effects model. For each floral trait, sources of variation were flower size category, taxa (flower size category), and populations (taxa, flower size category). Traits are grouped according to their expected functional role: corolla traits (*cw* corolla width, *cl* corolla length, *pw* petal width) and sexual organs traits (*a1w* feeding anther width, *a1l* feeding anther length, *a2w* pollination anther width, *a2l* pollination anther length, *sl* style length). Significance levels \*0.05, \*\*0.01, \*\*\*0.001.

### Mean and covariance morphospace analyses

Morphospace occupied by populations mean floral trait values showed a clear divergence between small and large flowered taxa. The first two principal components explained 96.95 % of the variation (PC1: 86.63 % and PC2: 10.32 %). Variation on PC1 scores revealed clear differences in size between groups, whereas variation on PC2 mainly corresponds to differences in corolla width between taxa from

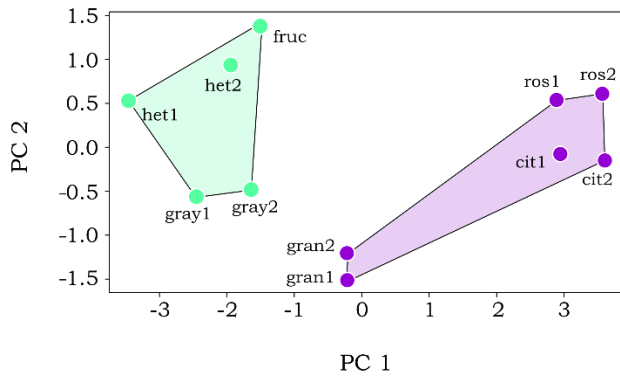
the series *Pacificum* and the rest of the taxa (Fig. 3a, Table 1). Results from PERMANOVA ( $F_{1,16} = 16,281$ ;  $p < 0.01$ ) revealed significant multivariate differences between small- and large-flowered taxa, as also shown by their non-overlapping convex hulls. Convex hulls of each group were not significantly different in their size indicating similar disparity ( $p = 0.34$ , Fig. 3a).

Trait	PC1	PC2
Corolla width	0.234	-0.853
Corolla length	0.3635	-0.303
Petal width	0.373	0.142
Feeding anther width	0.371	0.010
Feeding anther length	0.367	0.138
Pollinating anther width	0.346	0.333
Pollinating anther length	0.373	0.141
Style length	0.375	0.104

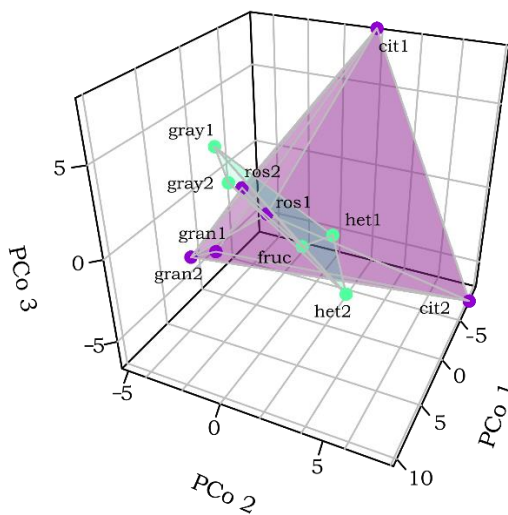
**Table 1.** Principal components analysis using populations mean floral trait values. The first two principal component are presented as they explained 96.95 % of the variation.

Principal coordinate analysis using the Riemannian distance matrix (representing pairwise distances between population matrices) indicated that the first three principal coordinate axes accounted for 63.17 % of the total variation. The first, second and third coordinate explained 28.46, 18.9 and 15.75 % of the total variation respectively. Contrary to PCA, convex hulls indicated a significant overlap between small and large flowered groups (Fig. 3b) as supported by PERMANOVA ( $F_{1,16} = 1.334$ ;  $p = 0.153$ ). Further, unlike PCA results here can be observed that small-flowered populations converged to a narrow portion of the morphospace of large-flowered populations as indicated by the significant differences in their magnitude of disparity ( $p < 0.0001$ , Fig. 3b).

a



b



**Figure 3. (a)** Morphospace representation using the first two principal components of a PCA performed with mean trait values of eight floral traits of eleven *Solanum* populations corresponding to three independent transition from large- to small-flowered taxa. Light green dots and convex hull shows the morphospace occupied by small-flowered populations. Violet dots and convex hull shows the morphospace occupied by large-flowered populations. Populations: cit1 and cit2 correspond to *S. citrullifolium*, ros1 and ros2 to *S. rostratum*, gran1 and gran2 to *S. grayi* var. *grandiflorum*, het1 and het2 to *S. heterodoxum*, fruc to *S. fructu-tecto*, and gray1 and gray2 to *S. grayi* var. *grayi*. **(b)** Variance-Covariance morphospace constructed using the Riemannian distance matrix among pairs of population matrices. Dots and convex hull's colors are similar as in the morphospace of mean trait values. Populations' references are the same as in Figure 2.

### Matrices size and structure

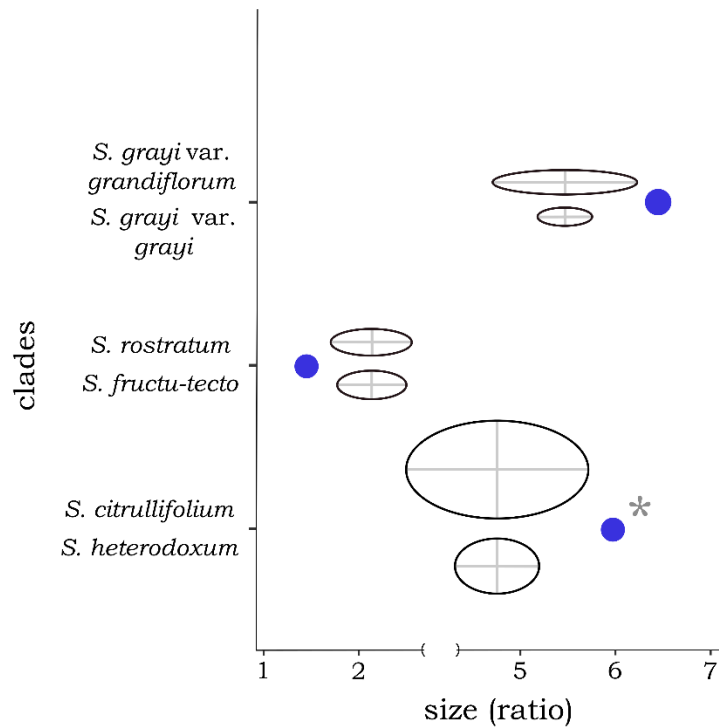
Convergence of small flowered taxa within the covariance morphospace indicate a reduction in the amount of variation in floral traits. Although this pattern can arise due to the association between mean and variance of metric traits, no clear pattern was detected (Fig. S1). Hence, differences in matrices size were not biased by differences in mean trait values. Random skewers analysis showed that small-flowered taxa expressed a reduced response to selection than their large sister taxa, excepting by the



clade represented by *S. rostratum* - *S. fructu-tecto* (Table 2, Fig. 4). Comparison of matrices structure through the correlation between response to selection vectors revealed that the transition represented by *S. citrullifolium* and *S. heterodoxum* of the series *Violaceiflorum* showed significant differences in their matrices structure (Table 2). This pair of taxa presented the lower correlation between their response to selection vectors ( $r = 0.62$ ). All other correlations were high and varied between 0.70 and 0.90 indicating no significant differences in matrices structure, hence, the only structural change reflecting a rearrangement in variance-covariance matrices was detected in the more divergent pair of taxons.

Comparisons by series	Size similarity (Mean response vectors' ratio)	Shape similarity (Mean response vectors' correlation)	p-value
<i>S. grayi</i> var. <i>grandiflorum</i> / <i>S. grayi</i> var. <i>grayi</i>	6.431032	0.9035817	> 0.05
<i>S. rostratum</i> / <i>S. fructu-tecto</i>	1.338158	0.9115384	> 0.05
<i>S. citrullifolium</i> / <i>S. heterodoxum</i>	5.987495	0.6183103	< <b>0.05</b>

**Table 2.** Matrices size and shape similarities between small- and large-flowered taxa within each clade. Only the taxa with the higher divergence time show significant differences in their matrix structures.

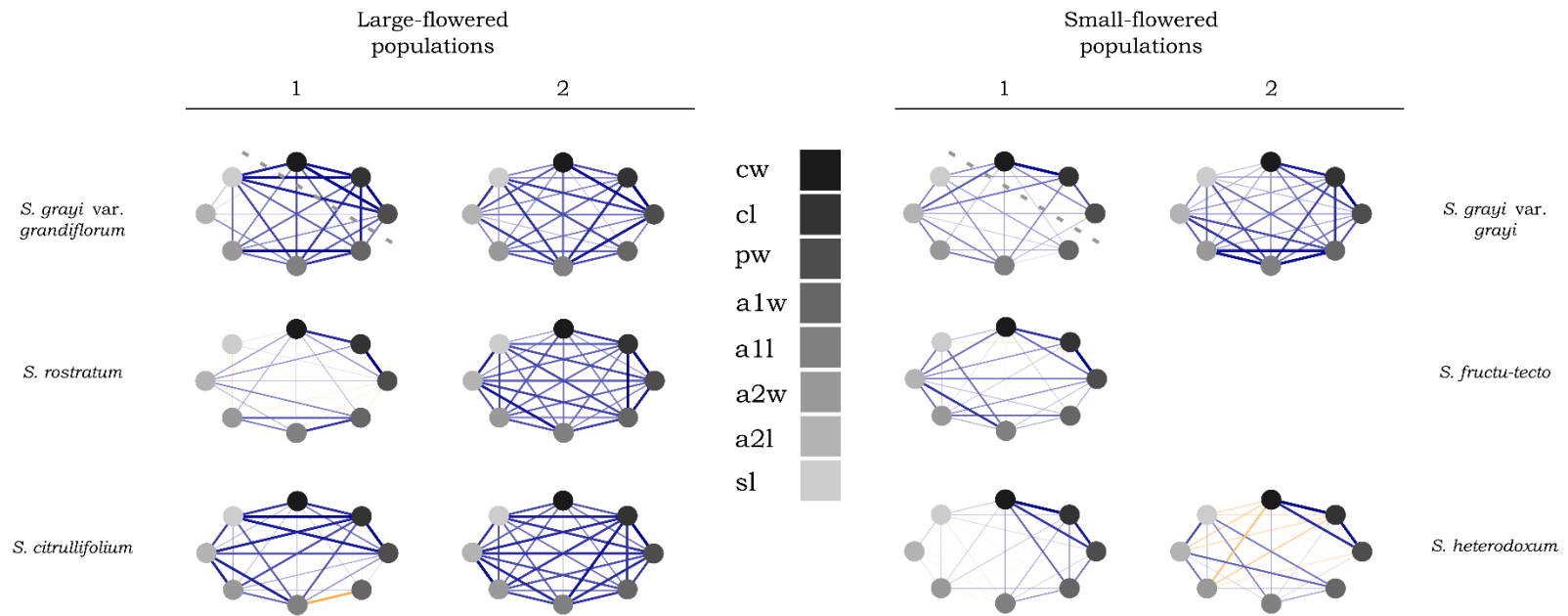


**Figure 4.** Blue dots indicate the length ratio of the response to selection vectors (large-/small- flowered taxa). The ratio was estimated for each evolutionary transition. The oldest transition (*S. citrullifolium* and *S. heterodoxum*) also presented significant differences in the matrices internal structure as indicated by the asterisk.

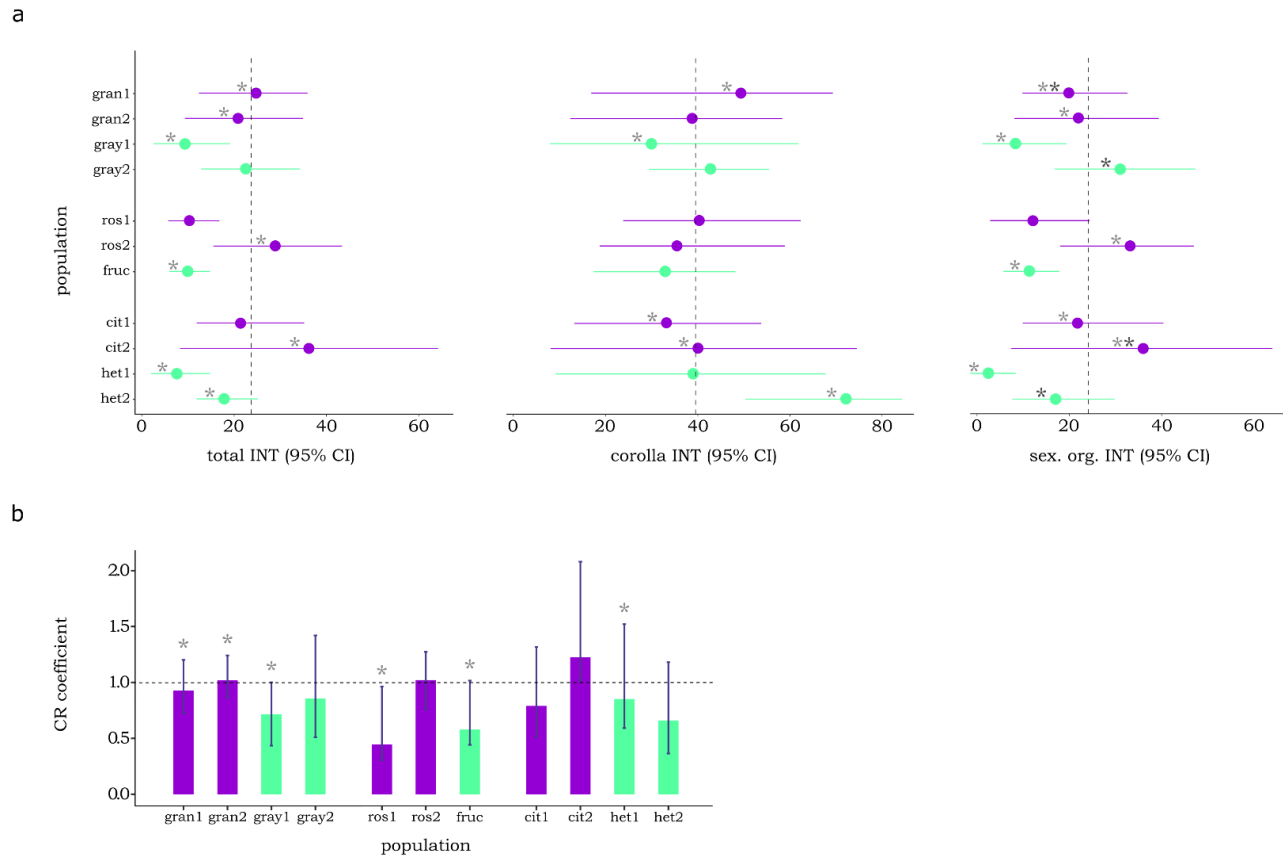
#### *Phenotypic integration and modularity*

Figure 5 shows the correlograms for each population. The two modules of the flower (corolla and sexual organs) are indicated in the first correlograms for small- and large- flowered taxa. Population level estimations of floral integration were all different from zero, ranging between 7.57% and 36.19% of the maximum possible level of integration. Within taxa, no differences between populations in floral integration were detected (Fig. 6a). Whenever a significant difference was detected between a small- and a large- flowered pair of populations within a clade, the former showed less integration than its large-flowered ancestor. This pattern was mainly driven by an apparent relaxation of correlations between sexual traits rather than those of the corolla. Modularity analyses using the Coefficient

Ratio calculation indicated that the same amount of large and small flowered populations are significantly modular in the phenotypic organization of floral traits. Although large flowered taxa tend to present higher values of CR indicating less modularity, most populations had values below or around 1 suggesting that the flower has a modular structure composed of two submodules, corolla and sexual organs (Fig. 6b).



**Figure 5.** Correlation matrices for each population (correlograms), indicating the pattern and magnitude of correlations among traits. Blue lines are positive correlations, orange lines are negative correlations. Traits code is in a grey scale and abbreviations are detailed in Figure 1. Grey line divide corolla traits from sexual organs.



**Figure 6. (a)** Phenotypic integration using INT index (Wagner 1984) was estimated for the whole floral matrices, the corolla module and the sexual organs module. Light green dots and lines correspond to small-flowered populations with a CI 95%, while violet dots indicates large-flowered populations. For each transition, an asterisk indicates significant differences between one small- and one large-flowered populations ( $p$  value < 0.05). The second darker asterisk in some large-flowered populations indicates additional difference with both small-flowered populations. The dotted line indicates the hypothetical integration level of the large-flowered ancestor estimated as the average of the current populations. **(b)** CR coefficient for 11 populations of six *Solanum* taxa representing transitions from large- to small flowers. The dotted line indicate the threshold CR value indicating modularity: below this value modularity is expected while CR values above 1 indicate no modularity. Confident intervals (CI 95%) are indicate for each population. The asterisk indicates significant modularity.

## Discussion

Following a morphospace approximation, small- and large-flowered taxa showed clear differences between them in mean floral trait values. Within the covariance morphospace occupied by large flowered taxa, those with small flowers showed much more similarity in their floral matrices revealing a convergent pattern across three independent evolutionary transitions. In addition, small-flowered taxa showed a reduction in matrix size relative to large-flowered taxa supporting the expectation that inbreeding constrain phenotypic combinations and the potential for a response to selection. This pattern was remarkably stronger in the older clade, coupled with the detection of greater structural differences between matrices. Large-flowered taxa tended to have more integrated flowers than those with small flowers due to a higher level of integration of sexual organs.

Empirical evidence has shown significant changes in mean values of floral traits fitting the optimization hypothesis of Stebbins (1957) during the transition from outcrossing to selfing. A previous study with the same set of evolutionary transitions in the section *Androceras* also support Stebbin's expectations (Vallejo-Marín *et al.* 2014). Our results using mean trait values confirm that the overall reduction in flower size represent the major axis of multivariate variation, but also highlight additional adjustments on floral trait correlations. Convergence of small-flowered taxa on a restricted region of the covariance morphospace occupied by large-flowered taxa suggest a readjustment of the floral matrix during the evolutionary transition. In particular, phenotypic distances among the floral matrices of small-flowered taxa were shorter than among-large flowered taxa. Optimization of the floral phenotype towards the new adaptive peak resulting from the partial or complete independence of biotic pollination likely affect variances and covariance of floral traits. Recent studies also detected changes on the variance-covariance matrices within a single clade exposed to different ecological conditions or during evolutionary transitions of pollination syndromes (Turelli 1988, Murren 2002, Arnold *et al.* 2008, Calsbeek and Goodnight 2009, Benitez-Vieyra *et al.* 2019). For instance, the correlation between corolla traits (signal) and nectar sugar content (reward) differ between hummingbird- and bee-pollinated species of *Salvia* (Benitez-Vieyra *et al.* 2014). Thus, as long as changes recorded in small-flowered taxa represent functional adjustments, variances and covariances among floral traits should be considered as part of the selfing syndrome.

Unlike selfing taxa, those relying on cross-pollination for reproduction evolved under diverse selection pressures imposed by a wide spectrum of biotic vectors (Fenster *et al.* 2004). Within the

Neotropical genus *Salvia*, species pollinated by hummingbirds diverged from bee-pollinated species in their mean corolla traits and their correlation structure supporting a functional adjustment of the whole corolla matrix (Benitez-Vieyra *et al.* 2019). Similarly, populations visited by different pollinators also differ in their levels of floral integration (Pérez *et al.* 2007, Pérez-Barrales *et al.* 2014, González *et al.* 2015, Lázaro and Santamaría 2016). Given that outcrossing taxa of *Solanum* are visited by different species of bees and bumble-bees, our results suggest that the wider occupancy of the covariance morphospace by large-flowered taxa is likely due to their exposition to visitors of different size.

The evolution of selfing expose taxa to the consequences of homozygosity, inbreeding depression, genetic drift and an overall reduction in the amount of genotypic variation within populations (Charlesworth & Charlesworth 1995, Ashman and Majetic 2006, Charlesworth and Willis 2009, Wright *et al.* 2013). Theoretically, these effects translate to a reduction in the variance of individual quantitative traits and an increase in the covariation among traits due to linkage disequilibrium (Phillips *et al.* 2001, Charlesworth and Willis 2009; Maubecin *et al.* 2016). In the present study, analyses revealed a consistent reduction in matrix size of small compared to large flowered taxa. Hence constraining their potential evolutionary response to selection. This pattern fits the expectation that the transition from large to small flowers may accompanied the evolutionary dead end associated with selfing if reduction in the covariance morphospace increases the risk of extinction. At least in the Solanaceae family, selfing lineages had higher extinction rate than their outcrossing ancestors (Goldberg *et al.* 2010). However, the evolution towards a unique optimum phenotype during the transition to selfing may balance its negative genetic effect reducing the risk of extinction. On the other hand, no significant pattern of increment in covariances among small flowered taxa was detected and even a slight increase was observed among large flowered ones (i.e., higher phenotypic integration of sexual organs). Thus, while the transition to selfing apparently reduced the amount of phenotypic variation, the extent of linkage disequilibrium on quantitative floral traits was likely not as high as required to express a significant increase on covariances and floral integration. Further experiments should test this expectation since there is no published evidence to contrast our results. The balance between the genetic effects of mating and natural selection should be explored to better understand the sources of variation in phenotypic integration.

High floral integration has been historically associated with specialized pollination systems assuming that these facilitate an efficient pollen transfer among individual plants (i.e., outcrossing, Berg

1960; Armbruster 1991), but there is only indirect evidence of a correlation between floral integration and mating system (Pérez *et al.* 2007; Rosas-Guerrero *et al.* 2011; Fornoni *et al.* 2016). In the genus *Ipomoea*, incompatible taxa showed lower floral integration than self-compatible taxa suggesting that presumably outcrossing taxa have lower rather than higher floral integration (Rosas-Guerrero *et al.* 2011). Similarly, in the genus *Schizanthus*, autonomous selfing taxa have higher corolla integration than their pollinator-dependent relatives (Pérez *et al.* 2007). These two cases illustrate a possible general pattern found in a recent meta-analysis suggesting that the evolution of selfing is associated with an increase in the phenotypic integration of the floral phenotype (Fornoni *et al.* 2016). Nevertheless, within each of three independent clades examined in the present study the opposite pattern was detected for at least one pair of large- and small-flowered populations. When this occurred, small-flowered populations presented lower integration than their large-flowered partner. *Solanum* is characterized by having a specialized buzz-pollination system where anthers are directly manipulated by bees (Whalen 1978, De Luca and Vallejo-Marín 2013) probably favoring higher integration of sexual organs than in small-flowered taxa. In turn, this may explain why sexual organs rather than the corolla accounted for differences in floral integration. Our results suggest that the small-flowered taxa converged to a common matrix structure characterized by a reduced integration of sexual organs. This result is consistent with a previous study with the same set of taxa showing a breakdown of herkogamy among small flowered taxa (Vallejo-Marín *et al.* 2014). Changes in herkogamy may be enough to produce seeds by self-fertilization relaxing the selection pressure upon floral trait covariances. The contrast between our results and a previous review suggest that more information about the pollination ecology of taxa will help to better explain the variation in floral integration.

All sampled taxa showed significant modularity between the corolla and sexual organs, but no difference was detected between small- and large-flowered taxa. We provide three possible explanations for this result. First, since the corolla and sexual traits belong to different floral whorls modularity may reflect ontogenetic effects. Second, the transition to small flowers may not require an adjustment in the magnitude of modularity if partial or complete independence from pollinators can be achieved by isolated changes in sexual organs that did not affect the correlations between these and the corolla. Third, the evolution of small-flowered taxa within the genus *Solanum* may be too recent to detect significant differences in modularity within the flower. This explanation assumes that changes in

modularity are not a necessary condition for the evolutionary transition but may result from subsequent changes in the pattern of selection on the floral phenotype. However, the older clade represented by *S. citrullifolium* and *S. heterodoxum* showed significant differences in the matrix structure but not in modularity suggesting that the transition did not affect this emergent property of the flower.

Historically, the variation in floral integration has been assumed as the result of pollinator-mediated selection (Armbruster *et al.* 1999; Pérez *et al.* 2007; Pérez-Barrales *et al.* 2014; Rosas-Guerrero *et al.* 2011). However, although initially proposed, the connection between mating and floral integration was not directly explored. Our results support this connection and advocate for a deeper understanding of genetic and ecological effects of mating on the evolution of the multivariate floral phenotype. Overall, the present study described how two interacting forces affected the transition from large (outcrossing) to small (selfing) flowered taxa: (1) the genetic consequences of selfing affecting the expression of phenotypic variances, and (2) natural selection promoting floral convergence.

## **Acknowledgements**

This work is part of a master degree research in the Posgrado en Ciencias Biológicas, UNAM, Mexico. The authors thank the Animal-Plant Interaction Lab, whose comments and critiques helped us to structure and clarify our ideas of the manuscript. Rubén Pérez Ishiwara and Miguel Baltazar provided useful technical help. This research was supported by a scholarship grant to ARP from Consejo Nacional de Ciencia y Tecnología (CONACYT), by a project grant IN210617 PAPIIT-DGAPA-UNAM to JF, and by Scottish Plant Health License PH/38/2010–2018. We thank the University of Stirling for providing access to the research glasshouses and controlled environment facilities during plant growth, Lislíe Solís-Montero for seed collecting, the Vallejo-Marín Lab for assistance with plant maintenance, and Lucy Nevard for her help with seed counting. We thank Tiina Särkinen for help with calculating divergence times among *Solanum* taxa. ARP also thanks the Instituto de Ecología at the UNAM, the CONACYT and the University of Stirling, for technical support and facilities given to this project.

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## **Discusión general y Conclusiones**

Siguiendo una aproximación morfoespacial, los taxones de flores grandes y pequeñas manifestaron claras diferencias en los valores medios de sus rasgos florales. Dentro del morfoespacio de covarianzas ocupado por taxones de flores grandes, aquellos con flores pequeñas mostraron mucha mayor similaridad en sus matrices florales, revelando un patrón de convergencia a lo largo de las tres transiciones evolutivas independientes. Adicionalmente, los taxones de flores pequeñas mostraron una reducción en el tamaño de su matriz floral en relación a los taxones de flores grandes, apoyando la expectativa de que la endogamia restringe las combinaciones fenotípicas y el potencial de respuesta a la selección. Este patrón fue remarcablemente fuerte en el clado más antiguo, acoplado con la detección de mayores diferencias estructurales entre matrices. Los taxones de flores grandes tendieron a presentar

flores más integradas que aquellos con flores pequeñas debido a un mayor nivel de integración de sus órganos sexuales.

La evidencia empírica ha mostrado cambios significativos en los valores medios de los rasgos florales, ajustándose a la hipótesis de optimización de Stebbins (1957) de la transición de entrecruza a autofertilización. Un estudio previo con el mismo conjunto de transiciones evolutivas en la Sección *Androcera* también apoya las expectativas de Stebbins (Vallejo-Marín *et al.* 2014). Nuestros resultados, utilizando valores medios de los rasgos, confirman que la reducción general en el tamaño de la flor representa el eje mayor de variación multivariada, pero también destacan ajustes adicionales en las correlaciones de rasgos florales.

La convergencia de los taxones de flores pequeñas en una región restringida del morfoespacio de covarianzas ocupado por taxones de flores grandes sugiere un reajuste de la matriz floral durante la transición evolutiva. En particular, las distancias fenotípicas entre las matrices florales de taxones de flores pequeñas fueron menores que entre taxones de flores grandes. La optimización del fenotipo floral a través del nuevo pico adaptativo, resultante de la independencia parcial o completa de polinizadores bióticos, probablemente afecta las varianzas y covarianzas de los rasgos florales. Estudios recientes también detectaron cambios en las matrices de varianza-covarianza dentro de un clado expuesto a diferentes condiciones ecológicas o durante transiciones evolutivas entre síndromes de polinización (Turelli 1988, Murren 2002, Arnold *et al.* 2008, Calsbeek y Goodnight 2009, Benitez-Vieyra *et al.* 2019). Por ejemplo, la correlación entre los rasgos de la corola (señal) y el contenido de néctar (recompensa) difieren entre especies de *Salvia* polinizadas por colibríes o abejas (Benitez-Vieyra *et al.* 2014). Por lo tanto, siempre que los cambios registrados en taxones de flores pequeñas representen ajustes funcionales, las varianzas y covarianzas entre los rasgos florales deben considerarse parte del síndrome de autofertilización.

A diferencia de los taxones de autofertilización, aquellos dependientes de la polinización cruzada para su reproducción evolucionaron bajo diversas presiones de selección impuestas por un amplio espectro de vectores bióticos (Fenster *et al.* 2004). Dentro del género Neotropical *Salvia*, las especies polinizadas por colibríes divergieron de aquellas polinizadas por abejas en los valores medios de los rasgos de su corola y su estructura de correlaciones, apoyando un ajuste funcional en la matriz de toda la corola (Benitez-Vieyra *et al.* 2019). De manera similar, las poblaciones visitadas por diferentes

polinizadores también difirieron en sus niveles de integración floral (Pérez *et al.* 2007, Pérez-Barrales *et al.* 2014, González *et al.* 2015, Lázaro y Santamaría 2016). Dado que los taxones de entrecruza de *Solanum* son visitados por diferentes especies de abejas y abejorros, nuestros resultados sugieren que la amplia ocupación del morfoespacio de covarianzas por taxones de flores grandes es más probablemente debido a su exposición a visitantes de diferentes tamaños.

La evolución de la autofertilización expone a las especies a las consecuencias de la homocigosis, depresión por endogamia, deriva génica y a una reducción general en la cantidad de variación genotípica dentro de las poblaciones (Charlesworth y Charlesworth 1995, Ashman y Majetic 2006, Charlesworth y Willis 2009, Wright *et al.* 2013). Teóricamente, estos efectos se traducen en una reducción en la varianza de rasgos cuantitativos individuales y un incremento en la covariación entre rasgos debido al desequilibrio de ligamiento (Phillips *et al.* 2001, Charlesworth y Willis 2009; Maubecin *et al.* 2016). En el presente estudio, los análisis revelaron una consistente reducción en el tamaño de la matriz de los taxones de flores pequeñas, en comparación con los de flores grandes y por lo tanto, limitándose su potencial respuesta evolutiva a la selección. Este patrón se ajusta a la expectativa de que la transición de flores grandes a pequeñas es acompañada por un “camino evolutivo muerto”, asociado con la autofertilización, si la reducción en el morfoespacio de covarianzas incrementa el riesgo de extinción. Al menos en la familia Solanaceae, los linajes de autofertilización tienen mayor tasa de extinción que sus antecesores de entrecruza (Goldberg *et al.* 2010). Sin embargo, la evolución hacia un único óptimo fenotípico durante la transición hacia la autofertilización, puede balancear sus efectos genéticos negativos reduciendo el riesgo de extinción. Por otro lado, no fue detectado ningún patrón significativo de incremento en las covarianzas entre taxones de flores grandes, e incluso fue observado un ligero incremento (es decir, mayor integración fenotípica en sus órganos sexuales). Así, mientras la transición a la autofertilización aparentemente reduce la cantidad de variación fenotípica, el grado de desequilibrio de ligamiento en los rasgos florales cuantitativos probablemente no fue tan alto como se requería para expresar un aumento significativo en las covarianzas e integración floral. Experimentos adicionales deberían testear esta expectativa, ya que no existe evidencia publicada para contrastar nuestros resultados. Debe explorarse el balance entre los efectos genéticos del sistema de apareamiento y la selección natural para entender mejor las fuentes de variación en la integración fenotípica.

La elevada integración floral ha sido históricamente asociada con sistemas de polinización

especializada, asumiendo que esto facilita una transferencia eficiente de polen entre plantas individuales (es decir, la entrecruza; Berg 1960; Armbruster 1991), pero existe sólo evidencia indirecta de una correlación entre la integración floral y el sistema de apareamiento (Pérez *et al.* 2007; Rosas-Guerrero *et al.* 2011; Fornoni *et al.* 2016). En el género *Ipomea*, las especies incompatibles mostraron menor integración floral que las especies autocompatibles, sugiriendo que presumiblemente las especies de entrecruza tienen una menor integración floral (Rosas-Guerrero *et al.* 2011). De manera similar, en el género *Schizanthus*, las especies de autofertilización autónoma presentan una mayor integración de la corola que sus parientes dependientes de polinizadores (Pérez *et al.* 2007). Estos dos casos ilustran un posible patrón general encontrado en un reciente meta-análisis, sugiriendo que la evolución de la autofertilización se encuentra asociada a un incremento en la integración fenotípica del fenotipo floral (Fornoni *et al.* 2016). Sin embargo, dentro de cada uno de los tres clados independientes examinados en el presente estudio, el patrón opuesto fue detectado para al menos un par de poblaciones de flores grandes y pequeñas. Cuando esto ocurrió, las poblaciones de flores pequeñas presentaron menor integración que su par de flores grandes. *Solanum* se caracteriza por un sistema de polinización especializado, en donde las anteras son directamente manipuladas por abejas (Whalen 1978, De Luca y Vallejo-Marín 2013), probablemente favoreciendo mayores niveles de integración de los órganos sexuales que en los taxones de flores pequeñas. A su vez, esto justificaría por qué los órganos sexuales antes que la corola explican las diferencias en integración floral. Nuestros resultados sugieren que los taxones de flores pequeñas convergen en una estructura matricial común, caracterizada por una integración floral reducida de los órganos sexuales. Este resultado es consistente con un estudio previo con el mismo conjunto de especies, que demuestra una ruptura de la hercogamia entre taxones de flores pequeñas (Vallejo-Marín *et al.* 2014). Los cambios en la hercogamia pueden ser suficientes para producir semillas por autofertilización, relajando la presión de selección sobre las covarianzas de los rasgos florales. El contraste entre nuestros resultados y una revisión previa sugiere que más información sobre la ecología de la polinización de estos taxones ayudará a una mejor explicación de la variación en la integración floral.

En todos los taxones muestreados se observó una modularidad significativa entre la corola y los órganos sexuales, pero no se detectaron diferencias significativas entre los taxones de flores grandes y de flores pequeñas. Proveemos tres posibles explicaciones para este resultado. Primero, dado que los



caracteres de la corola y sexuales pertenecen a diferentes verticilos florales, la modularidad puede reflejar efectos ontogenéticos. Segundo, si la independencia parcial o completa de los polinizadores puede ser lograda a través de cambios aislados en los órganos sexuales que no afectan las correlaciones entre éstos y la corola, la transición hacia flores pequeñas puede no requerir de ajustes en la magnitud de la modularidad. Tercero, la evolución de taxones de flores pequeñas en el género *Solanum* puede ser demasiado reciente para detectarse diferencias en la modularidad dentro de la flor. Esta explicación asume que los cambios en la modularidad no son necesariamente una condición para la transición evolutiva, sino que podrían resultar de cambios subsecuentes en los patrones de selección en el fenotipo floral. Sin embargo, el clado más antiguo, representado por *S. citrullifolium* y *S. heterodoxum*, manifestó diferencias significativas en la estructura matricial, aunque no en su modularidad, sugiriendo que la transición no afectó esta propiedad floral emergente.

Históricamente, la variación en la integración floral ha sido asumida como el resultado de la selección mediada por polinizador (Armbruster *et al.* 1999; Pérez *et al.* 2007; Pérez-Barrales *et al.* 2014; Rosas-Guerrero *et al.* 2011). Sin embargo, y aunque inicialmente fue propuesta, la conexión entre el sistema de apareamiento y la integración floral no fue explorada de forma directa. Nuestros resultados apoyan esta conexión y abogan por una comprensión más profunda de los efectos genéticos y ecológicos del sistema de apareamiento sobre la evolución del fenotipo floral multivariado. En general, el presente estudio describe cómo dos fuerzas interactuantes afectaron la transición entre taxones de flores grandes (entrecruza) a pequeñas (autofertilización): (1) las consecuencias genéticas de la autofertilización afectando la expresión de las varianzas fenotípicas, y (2) la selección natural promoviendo la convergencia floral.

## Supporting information

**Table S1.** Location of the eleven populations employed in this research.

Taxon	Population code	Reference	Accession number	Sample size	Latitude	Longitude
<i>S. grayi</i> var. <i>grandiflorum</i>	ZAP	gran1	07s197, 199	25	23.452	-106.462
<i>S. grayi</i> var. <i>grandiflorum</i>	VOL	gran2	08s68, 70, 74, 79	25	18.852	-100.131
<i>S. grayi</i> var. <i>grayi</i>	ILA	gray1	07s147, 149	19	23.452	-106.462
<i>S. grayi</i> var. <i>grayi</i>	RDC	gray2	07s85, 89, 91, 99	31	25.340	-107.951
<i>S. rostratum</i>	BER	ros1	10s40, 45, 49, 53	23	20.687	-100.021
<i>S. rostratum</i>	DOL	ros2	11s179, 193, 200, 213	24	21.165	-101.047
<i>S. fructu-tecto</i>	ATH	fruc1	10-AH-5, 7, 9, 10	39	20.066	-99.216
<i>S. citrullifolium</i>	199	cit1	894750199	26	NA	NA
<i>S. citrullifolium</i>	332	cit2	894750332	14	NA	NA
<i>S. heterodoxum</i>	FRZ	het1	10-FZ-24, 30, 32, 34	25	23.101	-102.802
<i>S. heterodoxum</i>	TEM	het2	11-PTEM-4, 2, 15, 14	25	19.685	-98.843

**Table S2.** Phenotypic variance-covariance matrices (**P**-matrix) constructed per population within taxa. References for traits (log transformed): *cl* corolla length, *cw* corolla width, *pw* petal width, *a1w* feeding anther width, *a1l* feeding anther length, *a2w* pollinating anther width, *a2l* pollinating anther length, *sl* style length.

### 1. *S. grayi* var. *grandiflorum*

#### Population 1

<i>pw</i>	<i>cl</i>	<i>cw</i>	<i>a1w</i>	<i>a1l</i>	<i>a2w</i>	<i>a2l</i>	<i>sl</i>
0.03543823	0.02047483	0.01801246	0.00789507	0.00561904	0.00501685	0.0019051	0.00896139
	0.02263138	0.01449261	0.00900737	0.00476865	0.00481082	4.35E-05	0.0078673
		0.01608903	0.00504002	0.00335344	0.00299252	0.00168484	0.00596652
			0.00783492	0.00296239	0.00467551	-0.00061049	0.00294252
				0.00290197	0.00168928	0.00141309	0.00210838
					0.00466675	7.37E-05	0.00220096
						0.005318	0.00111629
							0.00484136

Population 2

<i>pw</i>	<i>cl</i>	<i>cw</i>	<i>a1w</i>	<i>a1l</i>	<i>a2w</i>	<i>a2l</i>	<i>sl</i>
0.01762899	0.00853823	0.01176239	0.00139485	0.00528007	0.00302145	0.00511202	0.00500289
	0.01487425	0.01135854	0.00069536	0.00660526	0.00391859	0.00342539	0.00426692
		0.01602646	0.00267904	0.00754683	0.00330757	0.00398246	0.00694477
			0.00310356	0.00257136	0.00185844	0.00096675	0.00283523
				0.00785941	0.00316917	0.00409549	0.00511337
					0.00501896	0.0020579	0.00182638
						0.0061763	0.00456477
							0.00933283

2. *S. grayi* var. *grayi*

Population 1

<i>pw</i>	<i>cl</i>	<i>cw</i>	<i>a1w</i>	<i>a1l</i>	<i>a2w</i>	<i>a2l</i>	<i>sl</i>
0.00825641	0.00397686	0.00282338	0.00067156	0.00128827	0.00070197	0.00235014	0.00101675
	0.00352054	0.0026236	-0.00013264	0.0009818	0.00012166	0.00127672	0.00057734
		0.00737477	0.00033395	0.00082648	-6.72E-05	0.00138595	-0.0001724
			0.00183	0.0002368	0.00047491	0.00036126	0.00039041
				0.00222312	0.00076008	0.00092774	0.00051535
					0.00158119	0.00083136	-3.60E-05
						0.00277575	0.0006276
							0.00103932

Population 2

<i>pw</i>	<i>cl</i>	<i>cw</i>	<i>a1w</i>	<i>a1l</i>	<i>a2w</i>	<i>a2l</i>	<i>sl</i>
0.01139594	0.00662016	0.00551732	0.00317994	0.00229028	0.00221575	0.00158973	0.00090915
	0.0082356	0.00674813	0.0040008	0.00379858	0.00308896	0.00176273	0.00097706
		0.00937419	0.00373922	0.00305525	0.00201553	0.00164676	0.00090388
			0.0052814	0.00398851	0.00410593	0.00244845	0.00134195
				0.00551276	0.0042396	0.00281624	0.00159095
					0.00609535	0.00233565	0.00142721
						0.00381293	0.0009978
							0.00166974

3. *S. rostratum*

Population 1

<i>pw</i>	<i>cl</i>	<i>cw</i>	<i>a1w</i>	<i>a1l</i>	<i>a2w</i>	<i>a2l</i>	<i>sl</i>
0.01275666	0.00790234	0.00377498	0.00234757	0.00084971	0.00220951	0.00197995	0.0005762
	0.01030951	0.00787357	-0.00061546	-0.00012138	-0.00055823	0.00184551	0.00029276
		0.00891367	-0.00154546	-0.0005617	-0.00090995	0.00105414	0.00058101

0.00811033	0.00269586	0.00365207	0.001943	-0.00039802
	0.00213239	0.00145512	0.0006064	-0.00010758
		0.0052328	-2.02E-06	-0.00066067
			0.00288571	-2.60E-05
				0.00238177

Population 2

<i>pw</i>	<i>cl</i>	<i>cw</i>	<i>a1w</i>	<i>a1l</i>	<i>a2w</i>	<i>a2l</i>	<i>sl</i>
0.01604547	0.00831403	0.0040288	0.00376548	0.00334302	0.00759908	0.0031674	0.00337927
	0.01072344	0.00811963	0.00597946	0.00301339	0.00558875	0.0036475	0.00453539
		0.01005499	0.00523192	0.0023774	0.00565439	0.00360236	0.00505328
			0.00513789	0.0027736	0.00364101	0.00307814	0.00370498
				0.003982	0.00402726	0.0032666	0.00263463
					0.01285359	0.00444279	0.00609376
						0.00499661	0.004389
							0.00762409

4. *S. fructu-tecto*

Population 1

<i>pw</i>	<i>cl</i>	<i>cw</i>	<i>a1w</i>	<i>a1l</i>	<i>a2w</i>	<i>a2l</i>	<i>sl</i>
0.01791141	0.00731564	0.00516273	0.00174932	0.00020446	0.00133178	0.00291487	0.00143051
	0.00889439	0.00617183	0.00071335	-0.00010241	0.00131299	0.00156326	-0.00081187
		0.00819855	0.00188986	0.00092746	0.00239041	0.00172696	-3.30E-05
			0.0071208	0.00104608	0.00304912	0.00089754	-0.00031611
				0.00295719	0.0010462	0.00164517	0.00116708
					0.00718331	0.00187817	0.00075226
						0.00273879	0.00108191
							0.00270376

5. *S. citrullifolium*

Population 1

<i>pw</i>	<i>cl</i>	<i>cw</i>	<i>a1w</i>	<i>a1l</i>	<i>a2w</i>	<i>a2l</i>	<i>sl</i>
0.08034469	0.02074917	0.00967742	0.01128856	0.00528108	0.00423382	0.00399855	0.0113087
	0.01360678	0.00740045	0.0028401	0.00545952	0.0046323	0.00485529	0.00588303
		0.00750702	0.00287763	0.00262551	0.00167847	0.00293454	0.00393405
			0.06694405	-0.01366179	0.00309568	-8.13E-05	-6.76E-05
				0.00889468	0.00325282	0.00395959	0.00305118
					0.00561652	0.00316905	0.00169664
						0.00402667	0.00244567
							0.00446629

Population 2

<i>pw</i>	<i>cl</i>	<i>cw</i>	<i>a1w</i>	<i>a1l</i>	<i>a2w</i>	<i>a2l</i>	<i>sl</i>
0.03057551	0.00753723	0.00472279	0.00764187	0.002833	0.00653415	0.00664692	0.00688746
	0.00505425	0.00358121	0.00524733	0.00370083	0.00366353	0.00373766	0.00347296
		0.00316361	0.00403773	0.00263581	0.002553	0.00284391	0.00247498
			0.01062999	0.00468728	0.00332193	0.0046245	0.00316009
				0.00394733	0.0031074	0.00331664	0.00214733
					0.00533727	0.00449607	0.00281628
						0.00485849	0.00308403
							0.00377285

6. *S. heterodoxum*

Population 1

<i>pw</i>	<i>cl</i>	<i>cw</i>	<i>a1w</i>	<i>a1l</i>	<i>a2w</i>	<i>a2l</i>	<i>sl</i>
0.00373316	0.00304664	0.00253012	0.00318774	0.00105301	0.00126835	-4.62E-05	0.00054387
	0.00448646	0.00315055	0.00193654	0.00084001	0.00149399	2.39E-05	0.00054533
		0.00493367	0.0032947	0.00055216	-0.0006176	-0.00010126	0.00046831
			0.01332958	0.00183583	0.00367698	-0.00028228	-0.00043203
				0.00272724	-0.00065478	0.0001071	0.0002272
					0.01644932	0.00059667	0.00081778
						0.00172778	0.00038208
							0.00248282

Population 2

<i>pw</i>	<i>cl</i>	<i>cw</i>	<i>a1w</i>	<i>a1l</i>	<i>a2w</i>	<i>a2l</i>	<i>sl</i>
0.02156213	0.01629253	0.0149597	-0.00187387	0.00339282	-0.01146586	-0.00338629	-0.0034925
	0.01409205	0.01375302	-0.00105261	0.00138824	-0.0066054	-0.0021454	-0.0024375
		0.01665566	0.00012913	0.00141253	-0.00435049	-0.00049376	-0.00127011
			0.01480228	0.0029212	0.00447028	0.00430532	0.00490357
				0.0046116	-0.00044858	0.00074131	0.00242495
					0.02074001	0.00473716	0.00321029
						0.0038008	0.00328461
							0.00617023

**Figure S1.** Variance in mean floral trait values for each population. The variance in small flowered taxa is lower than the variance in their relatives. Population references are gran1 and gran2 for *S. grayi* var. *grandiflorum*, gray1 and gray2 for *S. grayi* var. *grayi*, ros1 and ros2 for *S. rostratum*, fruc1 for *S. fructu-tecto*, cit1 and cit2 for *S. citrullifolium*, het1 and het2 for *S. heterodoxum*.

