



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

**ESCUELA NACIONAL DE ESTUDIOS SUPERIORES UNIDAD MORELIA
ECOLOGÍA**

**“INFLUENCIA DE LA DOMESTICACIÓN DE LAS PLANTAS EN LAS INTERACCIONES
PLANTA-POLINIZADOR: CARACTERÍSTICAS FLORALES Y COMPORTAMIENTO DEL
VISITANTE FLORAL EN ESPECIES DE *Cucurbita* SILVESTRE Y CULTIVADA”**

TESIS

(POR ARTÍCULO CIENTÍFICO)

**INFLUENCE OF PLANT DOMESTICATION ON PLANT-POLLINATOR INTERACTIONS:
FLORAL ATTRIBUTES AND FLORAL VISITOR COMMUNITIES IN WILD AND
CULTIVATED SQUASH PLANTS (*Cucurbitaceae: Cucurbita*)**

QUE PARA OPTAR POR EL GRADO DE:

MAESTRA EN CIENCIAS BIOLÓGICAS

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MORELIA, MICHOACÁN, FEBRERO, 2022



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MORELIA, MICHOACÁN, 2022

COORDINACIÓN DEL POSGRADO EN CIENCIAS BIOLÓGICAS

ENTIDAD ESCUELA NACIONAL DE ESTUDIOS SUPERIORES UNIDAD MORELIA

OFICIO CPCB/081/2022

ASUNTO: Oficio de Jurado

M. en C. Ivonne Ramírez Wence
Directora General de Administración Escolar, UNAM
Presente

Me permito informar a usted que en la reunión ordinaria del Subcomité de Biología Evolutiva, Ecología, Manejo Integral de Ecosistemas y Sistemática del Posgrado en Ciencias Biológicas, celebrada el día **22 de noviembre de 2021** se aprobó el siguiente jurado para el examen de grado de **MAESTRA EN CIENCIAS BIOLÓGICAS** en el campo de conocimiento de **Ecología** de la alumna **GLASSER SONJA KRISTINA** con número de cuenta **519490860** por la modalidad de graduación de tesis por artículo científico titulado: **"Influence of plant domestication on plant-pollinator interactions: floral attributes and floral visitor communities in wild and cultivated squash plants (cucurbitaceae: cucurbita)"** que es producto del proyecto realizado en la maestría que lleva por título **"Influencia de la domesticación de las plantas en las interacciones planta-polinizador: características florales y comportamiento del visitante floral en especies de cucurbita silvestre y cultivada"**, ambos realizados bajo la dirección del **DR. MAURICIO RICARDO QUESADA AVENDAÑO**, quedando integrado de la siguiente manera:

Presidente: **DR. ALBERTO KEN OYAMA NAKAGAWA**
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Vocal: **DR. MILAN JANDA**
Vocal: **DRA. YVONNE HERRERÍAS DIEGO**
Secretario: **DR. RAFAEL LIRA SAADE**

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

ATENTAMENTE
"POR MI RAZA HABLARÁ EL ESPÍRITU"
Ciudad Universitaria, Cd. Mx., a 24 de enero de 2022

COORDINADOR DEL PROGRAMA



DR. ADOLFO GERARDO NAVARRO SIGÜENZA



AGRADECIMIENTOS INSTITUCIONALES

Primeramente, al Posgrado en Ciencias Biológicas, UNAM, por haberme aceptado en su programa y brindarme la oportunidad de seguir formándome académicamente.

Al CONACYT (Becario No. 928826) por otorgarme una beca que me permitió la completa dedicación a los estudios de posgrado.

A los proyectos que financiaron el presente trabajo:

Universidad Nacional Autónoma de México (PAPIIT # IV200418, IA207318, IA207618, IN2224920, IN219021)

CONACyT [Laboratorio Nacional de Análisis y Síntesis Ecológica, 2015-LN250996, 2016-LN271449, 2017-LN280505, 2018-LN293701, 2019-LN299033, 2020-LN314852, 2021-LN315810]

Secretaría de Agricultura y Desarrollo Rural-CONACyT [291333]

Programa Iberoamericano de Ciencia y Tecnología para el Desarrollo RED CYTED-SEPODI [417RT0527]

A mi Tutor Principal, Dr. Mauricio Ricardo Quesada Avendaño

A los miembros de mi Comité Tutor, Dr. Rafael Lira Saade y Dr. Antonio González Rodríguez.

AGRADECIMIENTOS A TÍTULO PERSONAL

Al LANASE por permitirme utilizar sus instalaciones.

A la Universidad Autónoma de Guerrero por prestarme espacio en su laboratorio LANASE.

A mis compañeras y compañeros del laboratorio de Ecología Evolutiva y Conservación de Bosques Tropicales de IIES-UNAM y Ecología y evolución de polinización y sistemas reproductivos de plantas de ENES-UNAM por compartir su conocimiento, regalarme su tiempo para formar ideas, preguntas y ofrecer su apoyo.

Al técnico del laboratorio, Biol. Gumersindo Sánchez Montoya.

A la técnica de la LANASE, M. en C. Violeta Patiño Conde

A Dr. Rogelio Cruz Reyes quien me compartió su tiempo, espacio y me apoyó en el desarrollo del proyecto en Tecpán de la Galeana.

A Dr. Ernesto Vicente Vega Peña y Dr. Francisco Mora Ardila por su apoyo y paciencia en la parte estadística.

A Ximena Contreras quien era mi compañera de campo y una colaboradora increíble.

A los agricultores quienes me compartieron sus parcelas, conocimiento y trabajo laborales, Napoleón Sahagún Gallegos, Carlos Godínez Cuevas, King Kong Cuevas, David Martínez Vega, y Baltazar Abarca Sayago.

A los estudiantes de la licenciatura quien me apoyaban de realizar el trabajo en campo, tomar datos, enseñarme cómo ser una mentora y cómo ser estudiante; Adonaji Shurave Cortés Pérez, Saul Espinoza, Jesús Torres Her, Elvia Pérez, Kenya Lizet Salgado Vásquez, Jazmín Villanueva

Acatitlán, Julia Giron Ulloa.

A Yolanda Vega por las ilustraciones originales para la figura de las partes florales de la flor de calabaza.

A César Liñares Ojeda quien me apoyaba en realizar el trabajo de campo, formar ideas y revisar varios trabajos escritos.

A Oliverio Delgado Carrillo por compartir su conocimiento del sistema de *Cucurbita* y su apoyo en campo. Gracias por ayudarme a tomar cada situación con calma y ser más cuidadosa y pensativa.

A mis compañeras de casa y mi familia sustituta, Lucia Rodríguez y Adela Rascón quien me apoyaba entre las sonrisas y las lágrimas. El hogar que construimos me aterrizó a lo largo de esta aventura que es el posgrado.

Por último, Martín Hesajim de Santiago-Hernández por ser un mentor increíble. Gracias por su paciencia eterna, entusiasmo y todas las conversaciones de teoría y estadística que desarrollaron este proyecto.

DEDICATORIA

Esta tesis está dedicada a la memoria de Baltazar Abarca Sayago. Gracias por abrir su casa, su terreno y su corazón. Gracias por su generosidad y cariño.

Se dedica esta tesis a su esposa, Virginia Espinoza Tabares y a su hijo, Saul Abarca Espinoza quienes siguen en su luz.

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RESUMEN

La domesticación de especies de plantas produce modificaciones fenotípicas e influye cambios en las interacciones bióticas. La mayoría de los estudios han analizado las relaciones ecológicas y evolutivas de interacciones antagonistas planta-herbívoro, teniendo en cuenta especies cultivadas y sus parientes silvestres. Sin embargo, se ha prestado poca atención al efecto de la domesticación sobre interacciones mutualistas entre plantas y polinizadores. En este estudio analizamos el efecto de la domesticación sobre las características florales y cómo estos cambios afectan la interacción entre los visitantes florales de calabazas domesticadas y sus parientes silvestres en su lugar de origen. Esperamos que la selección artificial produzca modificaciones en la morfología floral de las formas silvestres a las formas domesticadas. Estos cambios en el fenotipo floral afectaran la diversidad de visitantes florales, modificándose la tasa de visita y el comportamiento de los visitantes florales. Nuestro estudio se realizó en dos regiones en la costa del Pacífico de México. En esta región estudiamos tres especies de calabazas filogenéticamente relacionadas (*Cucurbita*: Cucurbitaceae); *Cucurbita argyrosperma* ssp. *sororia* (ancestro silvestre), *C. argyrosperma* spp. *argyrosperma* (domesticada) y *C. moschata* (domesticada). Identificamos tres poblaciones silvestres de *C. sororia* y cuatro parcelas cultivadas. Para cada especie cuantificamos la variación de los rasgos florales, incluidas las medidas morfológicas y la cantidad y calidad del néctar y el polen. Además, realizamos grabaciones en video de flores para describir las comunidades y el comportamiento de los visitantes florales.

Nuestros resultados indican que las características morfológicas de flores pistiladas y estaminadas en especies domesticadas fueron generalmente más grandes que las del pariente silvestre. Las características florales de *C. argyrosperma* fueron intermedias entre la especie más domesticada *C. moschata* y la menos domesticada *C. sororia*. Tanto en flores estaminadas como pistiladas en *C. argyrosperma* y *C. sororia* no existieron diferencias significativas en la longitud de las partes reproductivas. Los coeficientes de variación (CV) de los rasgos morfológicos de flores pistiladas y estaminadas de *C. moschata* fueron menores que en el resto de las especies analizadas. Caso contrario fueron los CV de los rasgos florales de *C. argyrosperma* que tuvieron los valores más altos entre todas las especies de *Cucurbita*. El índice de integración total para flores estaminadas y pistiladas en las especies domesticadas fue

más alto que en las especies silvestres. Para todas las especies, las flores pistiladas fueron más integradas que las flores estaminadas. Las especies de *Cucurbita* domesticadas tuvieron una mayor cantidad de néctar y polen y la proporción de macronutrientes de proteínas a lípidos fue mayor en las especies domesticadas, pero no se encontraron diferencias significativas en la concentración de azúcar del néctar. Registramos un total de 6635 visitas de 30 tipos de visitantes florales. Los visitantes más abundantes fueron las abejas del género *Eucera*, *Apis mellifera* y las tribus Meliponini y Augochlorini. La comunidad de visitantes florales fue similar para todas las especies, pero *Eucera* fue dominante en las flores de calabaza silvestre y tuvo la mayor probabilidad de visita en todas las especies de *Cucurbita*. La tasa de visitas fue más alta en ambas especies de calabazas domesticadas en comparación con las calabazas silvestres y forrajear por néctar fue el comportamiento más común. Para analizar la importancia de los visitantes florales construimos dos índices: 1) el índice de visita y contacto con partes reproductivas (VCI), y 2) el índice de importancia de polinizador (IPI). Las abejas de las tribus Meliponini, Augochlorini y *Apis mellifera* presentaron mayores valores de VCI en las flores domesticadas pistiladas. Mientras que las abejas del género *Eucera* presentaron mayores valores de VCI en las flores estaminadas, específicamente de *C. moschata*. Para el IPI, las abejas del género *Eucera* tuvieron los valores más altos en las tres especies de calabazas y principalmente en las flores de *C. sororia*.

Estos resultados sugieren que, aunque los rasgos florales pueden no haber sido seleccionados directamente, existen diferencias fenotípicas significativas entre las flores de las especies de calabaza domesticadas y su ancestro silvestre. Las comunidades de visitantes florales entre plantas de calabaza silvestres y domesticadas fueron parecidas pero el comportamiento de los visitantes florales fue distinto en función del nivel de domesticación de la planta y el sexo de la flor. Esto sugiere que los efectos indirectos de la domesticación de las plantas pueden resultar en la modificación de la relación planta-polinizadores.

ABSTRACT

Domestication of plant species results in phenotypic modifications and influences changes in biotic interactions. Most studies have analyzed ecological and evolutionary relationships of plant-herbivore interactions of cultivated plants and their wild relatives, but little attention has been given to how domestication may also influence the mutualistic interaction of plant-pollinators. This study investigates the potential changes to floral characteristics and interactions of floral visitors of domesticated squash with its wild relatives in its place of origin. We expect artificial selection modifies floral morphology from wild to domesticated forms, and these changes, in turn, may influence floral visitation rates and behavior as well as the diversity of floral visitors. Our study was conducted in three regions along the west coast in México where we studied three sister species of squash (Cucurbita: Cucurbitaceae), *Cucurbita argyrosperma* ssp. *sororia* (wild ancestor), *C. argyrosperma* spp. *argyrosperma* (domesticated) and *C. moschata* (domesticated). We identified three wild populations of *C. sororia* and four cultivated plots. For each squash species we quantified variation of floral traits including morphological measurements and quantity and quality of nectar and pollen. Additionally, we conducted video recordings of flowers and described floral visitor communities and behavior.

Our results indicated that floral morphological characteristics of pistillate and staminate flowers of domesticated species were generally larger from that of the wild relative, with *C. argyrosperma* having intermediate values between *C. moschata* and *C. sororia*. In both staminate and pistillate flowers *C. argyrosperma* and *C. sororia* did not differ in length of reproductive parts and for pistillate flowers there was no significant differences in the length of the corolla tube. The coefficient of variation (CV) for each morphological trait was generally lower for *C. moschata* in both pistillate and staminate flowers and highest *C. argyrosperma* in pistillate flowers. The total integration index for staminate and pistillate flowers in the domesticated species was higher than in the wild species. For all species, the pistillate flowers were more integrated than the staminate flowers. Domesticated cucurbit species had a greater amount of nectar and pollen and the macronutrient ratio of P:L was higher in domesticated species, but there was no difference in nectar sugar concentration. We recorded a total of 6635 visits from 30 floral visitor types. The most abundant visitors were bees from the genus *Eucera*,

Apis mellifera, and the tribes Meliponini and Augochlorini. The floral visitor community was similar for all cucurbit plants but *Eucera* was more dominant in wild squash flowers and had the highest probability of visitation in all cucurbit species. The visitation rate was higher in both domesticated squash species than the wild squash plants and nectar was the most common motive for visits. We synthesized the dependent variables of visitation, contact with reproductive parts and estimated pollen load into two indexes: the visitation and contact index (VCI) and the pollinator importance index (IPI). The bees from the tribe Meliponini, Augochlorini, and *Apis mellifera* presented high VCI values in pistillate flowers of domesticated cucurbits. Meanwhile the *Eucera* bees presented the highest VCI values in the staminate flowers, specifically in *C. moschata*. For the IPI, *Eucera* bees had the highest IPI values in all three cucurbit species, especially in *C. sororia*. These results suggest that although floral traits may not have been directly selected upon there are significant phenotypic differences between domesticated squash species and their wild ancestor. The community associated with the wild and domesticated squash plants were distinct and the behavior of the floral visitors differed, suggesting indirect effects of plant domestication can result in modification to plant-pollinator relationships.

Keywords: morphometric analysis; plant-pollinator interactions; *Eucera*; pollen macronutrients; floral rewards; squash; floral traits; domestication; *Cucurbita*; nectar; floral integration; native bees.

INTRODUCCIÓN

La domesticación de las plantas ocurre desde los primeros registros de su cultivo. Este proceso de selección artificial favorece que una planta silvestre adquiera rasgos fenotípicos deseables a los humanos (Milla et al. 2015), como un mejor sabor, homogeneización del fenotipo entre individuos, mayor contenido nutricional y producción del tejido reproductivo (Pickersgill 2007, Meyer et al. 2012, Turcotte et al. 2017). Este proceso evolutivo hace que la planta cultivada tenga divergencia genética de sus parientes silvestres (Milla et al. 2015). Los métodos de manejo también afectan los caracteres de la planta; por ejemplo, métodos de control de plagas, sistemas de irrigación, y la implementación de planes de fertilización entre otros (Weiner 2004, Turcotte et al. 2017). Los atributos de la planta adquiridos en respuesta a las condiciones agrícolas y el nivel de domesticación son diferentes a los que tendrían en un entorno natural (van Raamsdonk 1993, Rosenthal 1997).

Aunque la selección artificial se dirige a características específicas de la planta, también resulta en un conjunto de consecuencias indirectas (Hernández-Terán et al. 2017). Algunas de las más investigadas incluyen la reducción de la diversidad genética resultando en cambios al genoma, principalmente a las funciones de regulación, mutaciones de pérdida de función y poliploidización (Zhang et al. 2019, Meyer y Purugganan 2013). Además, hay otras modificaciones importantes, como cambios en la arquitectura de la raíz y cambios en las comunidades microbianas de la rizósfera (Pérez-Jaramillo et al. 2016), lo cual podría estar relacionado con diferencias en la plasticidad como respuesta a la disponibilidad de agua y nutrientes (Matesanz y Milla 2017). También se ha reportado que la domesticación influye en la disminución de la resistencia a herbívoros y patógenos, debido a la pérdida de metabolitos secundarios y defensas físicas (Whitehead et al. 2017, Macfayden y Bohan 2010, Meyer et al. 2012).

En términos de efectos de la domesticación sobre las interacciones bióticas, la mayoría de los estudios comparan los niveles de herbivoría entre plantas domesticadas y sus parientes silvestres (Rosenthal 1997, Macfadyen y Bohan 2010, Turcotte et al. 2014, Chen et al. 2015, Gaillard et al. 2017, Whitehead et al. 2017, Hernandez-Cumplido et al. 2018). Se ha propuesto que las plantas domesticadas disminuyen su resistencia a la herbivoría al incrementar la

asignación de recursos a los tejidos reproductivos (frutos y semillas) lo cual expone a las plantas a mayores niveles de daño y a una comunidad de plagas más diversa (Rosenthal 1997, Macfadyen y Bohan 2010, Chen et al. 2015, Gaillard et al. 2017, Whitehead et al. 2017, Hernandez-Cumplido et al. 2018). Así mismo, una mayor asignación de recursos al desarrollo y reproducción (Rosenthal 1997, Milla y Matesanz 2016, Gaillard et al. 2017, Züst y Agrawal 2017), puede afectar la asignación de éstos para atraer polinizadores. Sin embargo, pocos estudios han analizado los efectos de la domesticación de las plantas en las interacciones planta-polinizador (López-Uribe et al. 2016, Milla et al. 2017, Chen et al. 2017).

A la fecha son muy pocos los estudios que han analizado los cambios en los atributos florales entre las plantas silvestres y domesticadas (pero véase Kuriakose et al. 2009, Sapir 2009, Egan et al. 2018). Además, para algunas especies de plantas domesticadas, la selección artificial ha resultado en el aumento del tamaño de los frutos (Doebley et al. 2006, Fuller 2007, Tang et al. 2010) y a su vez en el tamaño de la flor, caracteres ligados por su relación alométrica (Primack 1987, Andersson 1996) o interacciones pleiotrópicas (Primack 1987). Un aumento en el tamaño de la flor o la asignación de recursos al éxito reproductivo puede tener un efecto en la interacción planta-polinizador.

Algunos estudios sugieren que las plantas domesticadas dependientes de polinización animal presentan un número mayor de flores (Kuriakose et al. 2009), mayor cantidad de néctar y mayor tamaño de los atributos florales (Kuriakose et al. 2009, Sapir 2009). Kuriakose et al. (2009) compararon el cardamomo silvestre y domesticado, *Elettaria cardamomum*, y encontraron un mayor número de flores, atributos florales más grandes y más néctar en los cultivos domesticados que en las plantas de poblaciones silvestres. En términos de interacciones planta-polinizador, las especies de abejas sociales dominaron la comunidad de visitantes florales de cardamomo cultivado, mientras que los principales visitantes florales en las poblaciones de cardamomo silvestre fueron abejas solitarias (Kuriakose et al. 2009). En otro estudio, las diferencias genéticas asociadas con los fenotipos de girasoles silvestres y domesticados, *Helianthus annuus*, afectaron indirectamente la interacción con los visitantes florales (Sapir 2009); en este caso, los girasoles del fenotipo domesticado presentaron una mayor área del disco floral, un tiempo de floración más temprano y una altura de tallo más

corta. A su vez, los girasoles del fenotipo domesticado tuvieron mayores tasas de visita e influyeron de manera más fuerte y positiva en el comportamiento de especies de abejas sociales de tamaño corporal mayor (Sapir 2009).

Los estudios que han analizado los cambios en las características florales a través de la domesticación de las plantas abarcan patrones generales, como el aumento del número de flores, tamaño y volumen de néctar, que pueden correlacionarse con diferencias en las interacciones planta-polinizador (Kuriakose et al. 2009, Sapir 2009, Pacheco-Huh 2020). El análisis de los rasgos florales morfométricos junto con observaciones de sus respectivos visitantes florales, se utiliza para conectar las respuestas evolutivas y las diferencias entre las poblaciones de plantas y las comunidades de polinizadores (Rosas-Guerrero et al 2011, Pérez-Barrales et al. 2007, Armbruster et al. 2004). La integración floral es un índice que describe la magnitud con la que varían y covarían los rasgos florales, revelando los efectos de la presión selectiva sobre el sistema reproductivo (Berg 1960, Armbruster et al. 2004). Por ejemplo, se han documentado que las poblaciones con flores con menos polinizadores efectivos tienden a tener una mayor integración de las partes de flores involucradas en la transferencia y depósito de polen que aquellas que interactúan con una comunidad generalizada de polinizadores (Berg 1960, Pérez-Barrales et al. 2007, Rosas-Guerrero et al. 2010). Hay muy pocos estudios que comparan la covarianza de las partes florales y los índices de integración de las plantas monoicas (flores femeninas y masculinas en la misma planta). A la fecha no hay ningún estudio que compare valores de integración floral entre flores de plantas silvestres y sus parientes domesticados. Uno de los objetivos de la domesticación es que la planta tenga mayor éxito reproductivo, así que nuestra hipótesis se enfoca en que la flor, el módulo reproductivo de la planta, tendría un mayor índice de integración en plantas domesticadas. Al calcular la magnitud de integración floral de las especies de plantas domesticadas y sus ancestros silvestres será una medida valiosa y novedosa para describir las consecuencias del proceso de domesticación.

Los rasgos morfológicos y la cantidad de recompensas florales no son los únicos atributos influenciados por la domesticación (Egan et al. 2018). Por ejemplo, en el caso de la mora azul silvestre y domesticada, *Vaccinium corymbosum*, el proceso de domesticación ha dado lugar a diferencias en la composición química tanto del néctar como del polen, lo que ha

resultado en polen con menos diversidad química (Egan et al. 2018). Otra forma de analizar cómo las diferencias en las recompensas florales afectan la salud y las preferencias de los visitantes florales, es analizar la concentración y proporción de los macronutrientes de polen (Vaudo et al. 2020). La concentración de proteínas y la relación con los lípidos (P/L) está determinada por múltiples factores, entre los cuales se incluye la relación filogenética entre las especies de plantas, el nivel de dependencia de la polinización animal (sobre todo las abejas) y la longitud del estilo de la flor (Roulston et al. 2000, Reudenauer et al. 2019). En caso de que la domesticación resulte en flores más grandes, se puede hipotetizar, por razones alométricas, que la concentración de proteínas y la proporción P/L también pueden aumentar, debido a una mayor inversión en rasgos asociados con la atracción de polinizadores como las recompensas florales (Roulston et al. 2000, Reudenauer et al. 2019). Para comprender mejor los efectos indirectos de la domesticación de plantas en la interacción planta-polinizador, es necesario hacer un análisis que compare combinaciones de rasgos florales, la morfología, cantidad y calidad de las recompensas florales y la composición y comportamiento de la comunidad de visitantes florales.

Mesoamérica se considera una de las regiones originarias de la agricultura y un centro de domesticación de plantas (Harlan 1992, Casas et al. 2007). Esta región se considera el origen de algunos cultivos de importancia económica global como el maíz, frijol, chile, entre otras especies. Un ejemplo de esta transformación de especies de sus formas silvestres a plantaciones agrícolas es la calabaza, *Cucurbita*, originaria de México, de suma importancia alimentaria y cultural a nivel local y mundial (Lira et al. 2016). La selección artificial de plantas del género *Cucurbita* comenzó hace aproximadamente 10,000 años, cuando el ser humano buscaba obtener variedades con semillas más grandes y menos amargas para su consumo (Lira et al. 2016). Desde entonces, 6 de las 22 especies de *Cucurbita* han sido cultivadas y ejemplifican los cambios de la domesticación (Castellanos et al 2018, Kates et al 2017, Lira et al. 2016). Cabe destacar que algunos de las modificaciones más destacadas incluyen notables cambios morfológicos y fisiológicos tales como: la incapacidad de la fruta para despegarse de la guía, y la disminución de las defensas externas por cambios en los tricomas de las hojas, tallos y fruto; además de la disminución o eliminación de metabolitos secundarios involucrados en la

defensa, compuestos conocidos como cucurbitacinas; el incremento del tamaño de los frutos y de las semillas que, en consecuencia, redujeron la cantidad de los mismos por planta; y por último, el cambio de crecimiento rastrero en forma de guía por un hábito de crecimiento en forma de roseta (Lira et al. 2016). Principalmente, las plantas de calabaza se cosechan por sus frutos tanto maduros como inmaduros, por sus semillas y flores (Lira 1995). Específicamente en México, las flores de calabaza fueron un componente alimenticio importante en la época prehispánica (Castelló y Piña-Luján 1987) y hasta el día de hoy, se encuentran comúnmente en muchos platos; las flores de calabaza son de los brotes comestibles más consumidos en el país, además tienen un mayor contenido de proteína en comparación con otras comestibles recolectadas en el territorio (Sotelo et al. 2007).

Las especies de abejas del género *Eucera* (subg. *Peponapis* y *Xenoglossa*) son especialistas en polen de *Cucurbita* (Hurd et al. 1971). Las abejas emergen de su nido durante la temporada de lluvias, sincronizándose con la temporada de floración de las calabazas y mostrando de esta forma una fenología emparejada con *Cucurbita* spp. (Hurd et al. 1971); adicionalmente, sus hábitos de forrajeo en horas del día, coinciden con la antesis de la planta y la presentación del néctar (Nepi et al. 2001, Delgado-Carrillo et al. 2018). Esta relación parece mutuamente beneficiosa tanto para la abeja especialista, como para el éxito reproductivo de las calabazas, lo cual resulta en su mayor eficacia para polinizar las calabazas *Cucurbita* spp. cultivadas en comparación con otros polinizadores (Canto-Aguilar y Parra-Tabla 2000, Cane et al. 2010, Delgado-Carrillo et al. 2018). Sin embargo, la polinización de otros visitantes florales también es importante especialmente en las temporadas secas, o cuando las calabazas son cultivadas fuera del rango de distribución de las abejas *Eucera* (Artz y Nault 2011, Pfister et al. 2017, Delgado-Carrillo et al. 2018).

En este estudio exploramos la relación entre el aumento de la asignación de recursos con la producción de frutos, así como la relación de la morfología floral y las propiedades de las recompensas florales (Primack 1987, Andersson 1996, Sasu et al. 2010, Egan et al. 2018). Consideramos que el proceso de domesticación ha dado lugar a modificaciones en las características florales, es decir, flores más grandes y en mayor cantidad, con diferentes calidades en sus recompensas. Proponemos que habrá menos variación en las características

florales de las plantas cultivadas y más integración floral. Además, dado que estos cambios se correlacionan con diferencias en la composición, frecuencia de visitas y comportamiento de la comunidad de visitantes florales, esperamos que las flores de las especies domesticadas sean visitadas con mayor frecuencia. Finalmente, nos interesa examinar el comportamiento de los visitantes florales en las flores, el contacto con las partes reproductivas, el horario, la duración y el motivo de la visita difieren entre las especies de calabaza cultivadas y silvestres.

Este estudio se realizó a lo largo de la costa oeste de México que se considera como el centro de origen de las calabazas del grupo *Argyrosperma* (Castellanos et al 2018, Gianna et al. 2011, Lira et al. 2016). Este grupo es un complejo de tres especies; un ancestro silvestre, *C. argyrosperma* ssp. *sororia* que se acepta como el ancestro directo de la especie cultivada *C. argyrosperma* ssp. *argyrosperma*, y ambos comparten un ancestro común con la especie de calabaza domesticada más derivada y ampliamente cultivada *C. moschata* (Lira et al 2016, Castellanos et al. 2018). Para comparar las especies de calabaza domesticadas y silvestres, realizamos mediciones morfométricas, cuantificamos la producción y concentración de azúcar en el néctar y grabamos videos de flores para observar al detalle su comportamiento. Este estudio pretende responder las siguientes preguntas: 1.) ¿Hay alguna diferencia entre los atributos florales, la covarianza de los caracteres morfológicos y el índice de integración floral entre las especies de *Cucurbita*? 2.) ¿Ha cambiado la cantidad y calidad de las recompensas florales entre plantas silvestres y domesticadas? 3.) ¿Están las especies silvestres y cultivadas asociadas con distintas comunidades de visitantes florales? 4.) ¿Existen diferencias en la tasa de visitas y el comportamiento de los visitantes florales?

MANUSCRITO DEL ARTÍCULO ENVIADO A LA REVISTA ANNALS OF BOTANY

Influence of plant domestication on plant-pollinator interactions: floral attributes and floralvisitor communities in wild and cultivated squash plants (Cucurbitaceae: *Cucurbita*)

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Running title: Plant domestication influences floral traits and visitation.

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ABSTRACT

Background and Aims: Domestication of plant species results in phenotypic modifications and influences changes in biotic interactions. Most studies have analysed ecological and evolutionary relationships of antagonistic plant-herbivore interactions of domesticated plants and their wild relatives, but little attention has been given to how domestication may also influence the mutualistic interaction of plant-pollinators. Floral attributes and interactions of floral visitors were compared between three sister taxa of the genera *Cucurbita*, two domesticated; *C. argyrosperma* and *C. moschata*, and one wild relative; *C. sororia* in the place of origin.

Methods: We conducted univariate and multivariate analysis to compare floral morphological traits and analysed floral reward (nectar and pollen) quantity and quality between wild and domesticated squash flowers. Staminate and pistillate flowers of all three species were video recorded and visitation and behaviour of floral visitors was registered and analysed.

Key Results: Floral morphological characteristics of domesticated species were generally larger, had more correlations between floral traits and higher magnitude of integration. Additionally, nectar accumulation and pollen quantity and pollen protein content and protein to lipid ratio of pollen was greater in domesticated cucurbits. Cucurbit pollen specialists, bees from the genus *Eucera*, had the highest probability of visit for all cucurbit species, most strongly dominating the wild cucurbit floral visitor community and had the highest probability of contact with stigma and anthers for all cucurbit species. Females *Eucera* spp. had the highest visitation to staminate flowers of domestic cucurbits while other floral visitors had highest visitation to pistillate flowers of domesticated cucurbits.

Conclusions: We provide evidence that domesticated plants which require animal pollination may invest more towards reproductive success and thereby increase attractiveness to pollinators. We also propose that wild ancestor plant populations should be conserved in the centre of origin to preserve original plant-pollinator interactions.

Keywords: morphometric analysis; plant-pollinator interactions; *Eucera*; pollen macronutrients; floral rewards; squash; floral traits; plant domestication; *Cucurbita*; nectar; floral integration; native bees.

INTRODUCTION

Plant domestication is a process in which artificial selection results in human-desired traits such as better taste and higher nutritional content, phenotypic homogenization, and adaptation to agricultural practices (van Raamsdonk, 1993; Pickersgill, 2007; Meyer *et al.*, 2012; Turcotte *et al.*, 2017). Although artificial selection targets specific plant characteristics, it also results in a suite of indirect consequences (Zohary, 2004; Milla *et al.*, 2015; Hernández-Terán *et al.*, 2017) such as a reduction of genetic diversity and genomic changes (i.e. regulatory functions) (Tang *et al.*, 2010; Meyer and Purugganan, 2013; Zhang *et al.*, 2019), differences in plasticity to abiotic factors such as water and nutrient uptake (Matesanz and Milla, 2017) and lower resistance to herbivores and pathogens due to loss of secondary metabolites and physical defences (Macfadyen and Bohan, 2010; Meyer *et al.*, 2012; Whitehead *et al.*, 2017). In terms of biotic interactions, most studies have compared herbivory in wild and domesticated species, hypothesizing that the reassignment of resources from defences to reproductive tissues exposes domesticated plants to higher levels of herbivory and a more diverse and generalist pest community (Rosenthal and Dirzo, 1997; Macfadyen and Bohan, 2010; Chen *et al.*, 2015; Chen, 2018; Gaillard *et al.*, 2017; Whitehead *et al.*, 2017; Shlichta *et al.*, 2018). Few studies have analysed the effects of plant domestication on floral traits and plant-pollinator interactions (López-Uribe *et al.*, 2016; Milla *et al.*, 2017; Chen *et al.*, 2018).

Studies which examine the effects of domestication on floral attributes and plant-pollinator interactions found that domesticated agricultural plants had larger and greater numbers of flowers (Kuriakose *et al.*, 2009; Sapir, 2009) and higher quantities of nectar

(Kuriakose et al. 2009) than their wild relatives. For example, in cardamom, *Elettaria cardamomum*, domesticated plants have a greater number of flowers, larger floral attributes and more nectar than in the plants of the wild populations (Kuriakose et al., 2009). In terms of plant-pollinator interactions, eusocial bee species dominated the cultivated cardamom floral visitor community whereas the principal floral visitors in wild cardamom populations were solitary bee species (Kuriakose et al., 2009). In another study with sunflowers, *Helianthus annuus*, domesticated phenotypes had larger floral disk area, earlier flowering time, and higher visitation rates by large bodied eusocial bee species than the wild phenotypes (Sapir, 2009).

Several studies have shown that effective pollinators exert selection on morphological floral trait mean values positively affecting the fitness of plants; and that these traits show less variation (Armbruster et al., 1999; Herrera et al., 2002; Pérez-Barrales et al., 2007; Rosas-Guerrero et al., 2014; Ashworth et al., 2015). Additionally, when morphological floral traits associated with pollinator attraction or pollen collection and deposition concurrently increase plant fitness, a strong positive correlation is expected between these traits (Berg, 1960; Armbruster et al., 1999). In this case, the selection of these traits should favour the integration of floral traits (i.e., correlations among traits within functional units) (Berg, 1960; Stebbins, 1970; Armbruster et al., 1999; Murren, 2002; Perez-Barrales et al., 2007). The analysis of floral integration known as the strength at which floral traits vary and covary, considers the flower as one functional unit (Berg, 1960; Armbruster et al., 2004; Rosas-Guerrero et al., 2011). The specialization in pollination and plant breeding system determines the magnitude of floral trait integration. (Berg, 1960; Pérez-Barrales et al., 2007; Rosas-Guerrero et al., 2011). Evaluating floral trait variation, correlation between traits and comparing the integration of floral traits of

domesticated plant species with respect to their wild relatives would be a synthetic approach to understand selection via domestication. No studies have compared floral integration of domesticated plants to wild ancestor plant species. Homogenization of the domesticated plant species phenotype and selection for maximal reproductive success may result in less variation, higher correlation, and integration of floral parts.

Other floral traits, such as pollen and nectar could also be influenced by domestication (Kuriakose *et al.*, 2009; Sapir, 2009; Egan *et al.*, 2018). For example, as mentioned above, domesticated cardamom flowers produced more nectar than their wild relatives (Kuriakose *et al.*, 2009). Similarly, domesticated sunflowers with larger floral disk areas were hypothesized to present more nectar due to higher numbers of florets (Sapir, 2009). Domestication may also affect other floral rewards such as the pollen macronutrients and their relative concentrations. (Vaudo *et al.*, 2020). For example, high protein concentration and protein: lipid ratio (P:L) are positively associated with greater investment in pollinator attraction (Roulston *et al.*, 2000; Ruedenauer *et al.* 2019). If domestication results in larger morphological floral traits with greater rewards such as flowers with high nectar production, and high protein concentration and P:L, then domestication will indirectly increase pollinator attraction, thereby increasing plant reproductive success. Therefore, an analysis that integrates floral morphology, quantity, and quality of floral rewards as well as the floral visitor community composition and behaviour is needed to elucidate direct and indirect effects of plant domestication on the plant-pollinator interaction.

Squash and gourd plants, *Cucurbita* spp., originating from Mesoamerica, are an excellent system for exploring the effect of domestication on floral traits and plant-pollinator interactions. At least six independent domestication events occurred within the *Cucurbita* genera, the earliest of which dates back 10,000 years BP (Smith, 1997; Lira *et al.*, 2016). Squash plants are cultivated year-round, require animal pollination services, and are of high economic and cultural value (Lira, 1995). Wild cucurbit plants are found in natural and disturbed habitats throughout Mesoamerica (Montez-Hernández *et al.*, 2002; Balvino-Olvera *et al.*, 2017; Sánchez-de la Vega *et al.*, 2018). The pollen specialists of squash flowers are bees of the genus *Eucera* (Dorchin *et al.*, 2018). These bees have synchronized phenology and flight times to the natural growing season and daily floral anthesis of squash plants (Hurd *et al.*, 1971; Delgado-Carrillo *et al.*, 2018). This plant-pollinator interaction appears to be mutually beneficial in that the pollen specialists are the most efficient pollinators of the cultivated cucurbit species (Canto-Aguilar and Parra-Tabla, 2000; Cane *et al.* 2011; Delgado-Carrillo *et al.*, 2018). However, pollination by other floral visitors is significant, especially in the absence of *Eucera* spp. (Artz and Nault, 2011; Pfister *et al.*, 2017; Delgado-Carrillo *et al.*, 2018). The ecological and evolutionary history of wild and domesticated cucurbit plants and pollinator interactions is ideal for exploring the potential effects of plant domestication on a mutualistic interaction in its centre of origin.

In this study, we selected three phylogenetically related cucurbit species, one wild and two domesticated *Cucurbita argyrosperma* ssp. *sororia* (*C. sororia*, wild), *C. argyrosperma* ssp. *argyrosperma* (*C. argyrosperma*, domesticated) and *C. moschata* (domesticated), which comprise the monophyletic clade *Argyrosperma* (Castellanos-Morales *et al.*, 2018). Our study asked the following questions: 1) How do morphological floral traits differ between wild and

domesticated cucurbit species? 2) Has the quantity and quality of floral rewards changed between wild and domesticated plants? 3) Are wild and domesticated species associated with distinct floral visitors and pollinators? 4) Are there differences in the flower visitation and floral visitor behaviour between wild and cultivated cucurbit species? 5) Does the pollinator importance differ between wild and domesticated cucurbits? We argue that the process of domestication of plants that require animal pollination has resulted in modifications to floral characteristics, such as larger flowers and a higher quantity and quality of floral rewards than in their wild relatives. We propose that there will be less variation in floral characteristics in the cultivated squash flowers and more floral integration, meaning the flowers will have more uniform mean trait sizes and that these traits will be more correlated with each other. Due to differences in floral traits, we expect flowers of domesticated species will be visited at a higher frequency than the wild cucurbits. Additionally, *Eucera* spp. will be the most important floral visitor in all cucurbit species and non-pollen specialist bee species will be secondarily important in domesticated cucurbits. We also expect that the behaviours of floral visitors such as contact with reproductive floral structures, time of visit, motive and duration of visit differ between wild and domesticated cucurbit species.

MATERIALS AND METHODS

Study species

The west coast of Mexico is the centre of origin of the *Argyrosperma* clade of the *Cucurbita* genera (Giannini et al. 2011, Lira *et al.*, 2016; Balvino-Olvera *et al.*, 2017, Castellanos-Morales *et al.*, 2018). The *Argyrosperma* clade is a complex of three sister taxa; one wild

species, *C. argyrosperma* ssp. *sororia* (*C. sororia*, wild) and two domesticated species; *C. argyrosperma* ssp. *argyrosperma* (*C. argyrosperma*, domesticated), and *C. moschata* (domesticated) which is considered as the more derived of the domesticated cucurbit species (Lira *et al.*, 2016; Castellanos-Morales *et al.* 2018; Chomicki *et al.* 2019). *C. sororia* is commonly found growing alongside cultivated squash fields and in disturbed areas (Montez-Hernández *et al.*, 2002; Balvino-Olvera *et al.*, 2017; Sánchez-de la Vega *et al.*, 2018). The other domesticated cucurbits are cultivated year-round depending on the availability of water. Cucurbit species are annual monoecious plants, herbaceous vines, have unisexual flowers with one day anthesis and require a pollen vector (Lira, 1995; Nepi and Pacini, 2001). The cucurbit flowers are radially symmetrical flowers that are vertically oriented. Cucurbit flowers are gamopetalous with a bell-shaped corolla of varying from shades of yellow to pale yellow to orange (Lira, 1995). The staminate flowers display anthers fused into an antheriferous column (Lira, 1995; Nepi and Pacini, 2001); pistillate flowers exhibit an inferior ovary and the pistil complex has three fused bilobed styles with open nectary and is presented on a short peduncle relative to that of the staminate flower (Lira, 1995). The natural life cycle of these cucurbit species occurs in the wet season (June to October-November) and blooming begins one to three months after germination. The cultivated cucurbit plants are planted in both wet and dry seasons (Delgado-Carrillo *et al.*, 2018).

Study sites

We selected three regions in México: Jalisco (the municipality of La Huerta,), Guerrero (Técpan de Galeana) and Michoacán (Cuamio and Queretanillo). Sites with cultivated squash plants

showed local landrace varieties or plants grown from seeds acquired from the company Hortaflor (Nova-Gawepa©, Rancho Los Molinos). In Jalisco and Guerrero eight sites were observed and 45-50 plants studied from each species [for site descriptions see Supplementary Data Table S1]. In Jalisco, four sites were studied in the municipality of La Huerta, one planted field of each *C. argyrosperma* and *C. moschata* each with an adjacent wild population of *C. sororia*. These four sites were in bloom from Sept.-Oct. 2019. In Guerrero, three field plots were planted, two with all three species and one with *C. argyrosperma*. This first field site was planted with all three cucurbit species in Aug. 2019 and was adjacent to a wild population of *C. sororia*, all plants were blooming during Sept.-Oct. In 2020, this same field site was planted with *C. moschata* in July and was in bloom during September. The second field site was planted with all three species in Sept. 2019 and blooming began in late Oct.-Nov. and was adjacent to a wild population of *C. sororia*. The third field site was planted with *C. argyrosperma* in Mar. 2020 and blooming began in April. Collection of fresh pollen, for macronutrient analysis of protein and lipid concentrations, was conducted in 2020 in the states of Michoacán and Guerrero [Supplementary Data Table S1]. For all field plots watering was limited to initiate plant establishment and no chemical pesticides or herbicides were used during the growing season of the plants. Video recordings of flowers occurred for all sites in 2019.

Floral attributes

To determine whether floral morphology of wild and domestic cucurbits differ, we conducted floral measurements in Jalisco and Guerrero between 2019 and 2020. We measured 16 floral traits for the three cucurbit species: corolla diameter (CD), corolla tube length (TL),

corolla length (CL), corolla tube base diameter (TD1), corolla tube middle diameter (TD2), corolla tube opening diameter (TD3), nectary diameter of staminate flowers (NDm) and pistillate flowers (NDf), anther diameter (AD), stamen length (Stl), anther length (AL), stigma diameter (SD), stigma length (SL), stigma and style length (PL), ovary length (OL) and ovary diameter (OD) (Fig. 1 A-D). Before flower anthesis, we covered flower buds with a mesh bag to prevent insect visitation. Pistillate flowers were less frequent than staminate flowers (1:10 pistillate:staminate flowers approximately) and, when possible, we selected one pistillate and one staminate flower from each plant. Flowers collected from plants (ramets) of *C. sororia* in natural populations were separated by at least five meters to avoid sampling from the same individual (Balvino-Olvera *et al.*, 2017). Squash plants in field plots were assigned unique IDs to collect flowers from specific individuals. Flowers were cut from various plants in groups of five to eight. Floral anthesis of cucurbits begins before sunrise and lasts until midday, approximately 0500h-1200h. We conducted floral measurements for fully opened flowers from 0630h-1130h. Morphological characters were measured with a stainless-steel digital caliper (Traceable® Calipers) (± 0.01 mm of precision). We obtained nectar volume measurements every hour from 0700h-1200h, depending on floral senescence. Nectar was drawn from each flower using graduated 50 μ L glass capillary tubes. Nectar volume was calculated by measuring the length (mm) of nectar in a glass capillary tube with the digital calipers then multiplied by 50 μ L/73mm. Sugar concentration was recorded as BRIX percent from each individual flower using the "Pocket" Refractometer PAL-1 (± 0.2 %), ATAGO® (Bellevue, WA, USA).

One staminate flower from each plant was designated exclusively for pollen collection. Anthers were cut at the base and stored in glass vials left to dry in hermetic containers with

silica beads. The dry pollen was separated from the anthers and weighed with microgram precision using a OHAUS AX124 Adventurer Pro II Analytical/Precision Balance (Parsippany, NJ, USA). For macronutrient analysis, whole anthers were collected in non-visited flowers under field conditions. To avoid degradation of pollen nutrients, we placed the anthers in an ice chest or a liquid nitrogen tank to store pollen at -20°C until biochemical analysis. For each cucurbit species pollen was collected from two separate field sites: two localities in Michoacán, Cuamio (*C. argyrosperma* and *C. moschata*) and Queretanillo (*C. moschata* and *C. sororia*), and in one locality in Guerrero, Técpan de Galeana (*C. moschata* and *C. sororia*). For each site, we collected two milligrams of pollen from each one of the ten randomly selected flowers. Pollen collected was pooled for a 40 mg mixed sample. For each pollen sample, three replicates of 1.0 ± 0.1 mg were used for both protein analysis and lipid analysis. Absorbance readings of protein and lipid concentrations were performed at 595 and 525 nm, respectively, using spectrophotometry techniques (for details, see Vaudo *et al.*, 2020) Multiskan® GO, Thermo Fisher Scientific (Waltham, MA, USA). The mean for protein and lipid concentration (mean \pm SE $\mu\text{g}/\text{mg}$) was calculated from the three subsamples for all three species. The P:L ratio was calculated by dividing the average protein by average lipid concentration for each species (Vaudo *et al.*, 2020).

Floral visitor community and behaviour

To determine whether the floral visitor community and the behaviour of each floral visitor differed among cucurbit species, we filmed 142 flowers from 100 plants: in total *C. sororia* pistillate-22, staminate-46; *C. argyrosperma* pistillate-9, staminate-29; *C. moschata* pistillate-12, staminate-24. Each day, four to eight flowers were filmed using HERO5, GoPro

cameras (San Mateo, CA, USA) from different plants. We selected plants separated from each other by at least five meters. Only floral visitors that entered the corolla tube were counted. For each floral visitor, we recorded morphospecies, time of arrival, duration of the visit, motive for entering the flower, and contact with reproductive parts. The motive for entering the flower was separated into four categories: nectar, pollen, nectar + pollen and nothing. To calculate the visitation rate for each floral visitor group we divided the number of visits by the total duration of the video recording (decimal time) for each flower. For taxonomic identification of floral visitors, we collected specimens of each floral visitor observed in cucurbit flowers for 15-minute time intervals at three times; 0700h, 0900h and 1030h. We used taxonomical guides for insect identification (Michener *et al.*, 1994; Ayala and Griswold, 2012). We limited our analysis to the floral visitors that entered more than three flowers and/or had a motive for entering the flower other than just a consequential passive visit. Floral visitors were separated in two categories; primary floral visitors, six morphospecies groups that visited more than 30 flowers, and secondary floral visitors, a combination of all floral visitor morphospecies that visited less than 30 flowers [Supplementary Data Table S2].

STATISTICAL ANALYSES

Floral attributes

To determine whether floral attributes differ between cucurbit species, we compared the size of each floral trait using a generalized linear mixed model (lme4 function 'glmer' and stats function 'glm') followed by a post hoc Tukey test (emmeans function 'emmeans'). Floral trait measurements were \log_{10} transformed to obtain normality. Pollinator attraction traits

corolla (CD, TL, CL, TD1, TD2, TD3; Fig. 1A and B), and nectary diameter (NDm and NDf; Fig. 1C and D), were evaluated individually as response variables and the interaction between cucurbit species and floral sex as fixed factor. Because most pistillate flowers were sampled from the same plants as staminate flowers, we used the Plant ID as a random variable. Plant reproductive traits, androecium (AD, StL, AL; Fig. 1C) and gynoecium (SD, SL, PL, OL, OD; Fig. 1D), were evaluated individually as the response variables and the cucurbit species as the predictor variable. Significance level was found using type III Wald chi-square tests (car function 'Anova'). Additionally, to analyse the individual variation of each floral trait, we calculated the coefficient of variation ($CV = s / \bar{x}$). All floral attribute analysis were completed in the R program (version 3.6.2) (R Core Team, 2020).

In order to test for differences between the group of all floral morphological traits of cucurbit species, we performed a multidimensional scaling analysis (MDS) (stats function 'cmdscale', ggpubr function 'ggscatter'). To analyse the structure of correlation between floral traits, the staminate and pistillate flowers were analysed separately due to the differences in androecium and gynoecium features. We calculated Pearson's correlation coefficient (r) (psych function 'corr.test') and plotted significant correlations (p -value ≤ 0.05) in Cytoscape software (© Cytoscape Consortium version 3.8.2). To calculate floral integration indices, we used the variance of eigenvalues obtained from a multivariate analysis (Wagner, 1984; Torices and Muñoz-Pajares, 2015). We calculated four integration indexes: 1) total integration index using all morphological floral traits; 2) integration index of floral traits associated to attraction/restriction of floral visitors (CD, CL, TL, TD1, TD2, TD3; Fig. 1A and B); 3) integration index for traits associated to pollen placement of staminate flowers (NDm, AD, StL, AL; Fig. 1C); 4) floral traits

associated with female reproductive structures on pistillate flowers (NDf, SD, PL, SL, OL, OD; Fig. 1D). Because sample size differed between cucurbit species, we calculated a corrected integration index using the PHENIX package in R statistical software (Wagner, 1984; Armbruster *et al.*, 2004; Torices and Muñoz-Pajares, 2015).

To analyse whether volume and sugar concentration of nectar differs between cucurbits species, floral sex, and time, we performed a generalized linear mixed model (lme4 function 'glmer') followed by a post hoc Tukey test (emmeans function 'emmeans'). To normalize data, we converted nectar volumes using \log_{10} and sugar concentrations were converted to proportion. We used nectar volume and sugar concentration as response variables and the interaction between cucurbit species, floral sex and time transformed to decimal as predictive variables (emmeans function 'emmeans'). Because some pistillate and staminate flowers were sampled from the same plant, Plant ID was used as a random variable. For nectar volume, we used a normal distribution and for sugar concentration we used a binomial distribution.

To test for differences in pollen weight between cucurbit species, we performed a one-way ANOVA followed by a post hoc Tukey test (stats functions 'aov' and 'TukeyHSD'). Pollen dry weight was \log_{10} transformed to obtain normality. We used pollen dry weight as the response variable and cucurbit species as the predictive variable.

Floral visitor community and behaviour

To analyse the visitation and behaviour of floral visitors in staminate and pistillate flowers of cucurbit species, we conducted generalized linear models using the GLIMMIX procedure in SAS version 9.4 (SAS Institute Inc, 2014). All models included the interaction

between cucurbit species, flower sex and floral visitor type as the predictive variables. To test if visitation was affected by the predictive variables, we used a binomial model (logit link) and a gamma model (log link) (Bolker, 2021). The binomial model tested the probability that the flower was visited (yes or no), while the gamma distributed model tested differences in visitation rate (amount of visits/duration of video in decimal time). To test whether the predictor variables affect the probability of contact of floral visitors with floral reproductive parts (yes/no) we used a binomial distributed model. To test whether anthesis time affects visitation rate of each floral visitor, we used time of visit, assigned to one-hour time range blocks from 0600h-1200h, as the nesting factor for the predictor variables assuming a negative binomial distribution to control for zero inflated overdispersion of data. Finally, to test whether the visitation rate and the visit duration were influenced by the motive of visit, we used the interaction between cucurbit species, flower sex, floral visitor type and motive as the predictive variables. For all analysis, we used type III Wald chi-square tests and specified the ILINK option in the LS-MEANS statement to obtain the least square means back-transformed to the original scale.

To analyse the pollinator importance of each floral visitor, we created two indices, the visitation and contact index (VCI) and the pollinator importance index (IPI). VCI was calculated by multiplying the probability of visit, probability of contact with floral reproductive parts, and the rate of visitation of all floral visitors to each cucurbit species and for both flower sexes. Pollen deposition is the best proxy when predicting successful pollination if fruit and seed production data is not available (Ballantyne *et al.*, 2015, 2017; Santiago-Hernández *et al.*, 2019) therefore, we created the second index, IPI, by multiplying VCI values with the mean of pollen

grains carried on floral visitors' body reported by Delgado-Carrillo *et al.* (2018). Pollen means were available for *Eucera* female and male bees, *Apis mellifera*, and bees from the tribes Meloponini and Augochlorini. Although pollinator importance analysis uses the amount of pollen grains deposited on stigma (Ballantyne *et al.*, 2015, 2017), mean pollen grains on floral visitor bodies in combination with the VCI metrics also serve as an estimator of pollinator importance. Pollinator network visualizations for both VCI and IPI were constructed using Cytoscape software (© Cytoscape Consortium version 3.8.0) (Shannon *et al.*, 2003).

RESULTS

Floral attributes

A total of 201 individual plants were sampled for flowers: *C. sororia*; n=65, *C. argyrosperma*; n=65, *C. moschata*; n=71. The ratio of pistillate flowers to staminate flowers is approximately 1:10, therefore there was a greater number of staminate flowers sampled than pistillate flowers. Our result showed that most floral trait sizes associated with pollinator attraction (CD, TL, CL, TD1, TD2, TD3; Fig. 1A and B) differ significantly between cucurbit species and flower sex, but not for the interaction between cucurbit species and floral sex (Table 1). The diameter of the nectary (NDm, NDf; Fig. 1C and D) differed significantly between cucurbit species, flower sex and their interaction (Table 1). Most floral trait sizes associated to pollinator attraction and the diameter of the nectary were significantly larger in *C. moschata* than in *C. argyrosperma* and both were significantly larger than in *C. sororia* (Table 1), except for the diameter of the middle section and opening of the corolla tube (TD2 and TD3; Fig. 1b) which were not significantly different between *C. argyrosperma* and *C. moschata* (Table 1). Flower sex

had a significant effect on floral trait sizes (Table 1). Staminate flower traits were larger than in pistillate flowers except for the base and middle of the corolla tube (TD1, TD2) (Table 1). Reproductive floral trait sizes of staminate and pistillate flowers differed significantly between cucurbit species, where floral traits of *C. moschata* were significantly larger than *C. argyrosperma* and *C. sororia* (Table 2). Post hoc Tukey test showed that in staminate flowers anther length (AL) and stamen length (StL) was similar for *C. sororia* and *C. argyrosperma* and different to *C. moschata*. For pistillate flowers, stigma diameter (SD), stigma length (SL), and ovule length (OL) were not significantly different for *C. sororia* and *C. argyrosperma* and different from *C. moschata*.

The coefficient of variation (CV) for staminate flowers of *C. moschata* ranged from 3.31-7.71% and was lower in most morphological traits than for the other two cucurbit species (CD, CL, TD1, TD2, TD3, and NDm). The CV values for *C. argyrosperma* staminate flowers ranged from 4.4 - 8.97% and were highest in CD, CL, TD1, TD2 and AL (Table S2). The CV values for *C. sororia* staminate flowers ranged from 3.73 – 11.08% and had the lowest CV values for AL, StL and TL. The CV values for *C. moschata* pistillate flowers were lowest in most morphological traits; CD, CL, TL, TD1, TD2, TD3, NDf, PL, SD, SL, and had a range from 3.13 - 6.57%. The CV values for *C. argyrosperma* were the highest in all traits except for NDf and OD and ranged from 6.79 – 10.71%. *C. sororia* had intermediate CV values ranging from 4.14 – 7.86% for all traits except for OL and OD which presented lower CV values than the domestic cucurbits. For details of CVs for staminate and pistillate flowers for the three cucurbit species see Supplementary Data Table S3.

MDS analysis for both staminate and pistillate flowers separates *C. sororia* more clearly from *C. moschata* than from *C. argyrosperma* which overlaps between both *C. sororia* and *C. moschata* (Fig. 2A and B). Correlations between floral traits for staminate flowers showed that *C. sororia* and *C. argyrosperma* have similar correlation patterns while *C. moschata* has fewer but stronger correlations between floral parts (Fig. 3A-C). The trait correlations associated with the reproduction (StL, AL) and attraction/restriction (TL, CL) gradually strengthened from *C. sororia* to *C. argyrosperma* and were strongest in *C. moschata*. For pistillate flowers, *C. argyrosperma* had significant correlations between all floral parts, *C. moschata* had more correlated traits than *C. sororia*, but *C. sororia* had stronger trait correlations, specifically in traits associated with pollinator attraction (CD, TL, CL, TD1, TD2, TD3) and ovary dimensions (OL, OD) (Fig. 3D-F).

The total integration index showed that pistillate flowers were more integrated than staminate flowers (Table 3). For staminate flowers the total integration index was higher for *C. argyrosperma* followed by *C. sororia* and *C. moschata*, and for pistillate flowers was higher for *C. argyrosperma* followed by *C. moschata* and *C. sororia* (Table 3). For staminate flowers, the integration index for the attraction and restriction module was largest for *C. sororia* while the integration index for the reproductive module was larger in *C. argyrosperma* (Table 3). For pistillate flowers, the integration index for the attraction and restriction and reproduction modules were largest for *C. argyrosperma* (Table 3).

The volume and sugar concentration of nectar were not significantly affected by the interaction of cucurbit species, flower sex, and time of sampling [Supplementary data Table S4].

The Tukey test for nectar volume showed that pistillate flowers have significantly more nectar than staminate flowers for all cucurbit species [Fig. S1; Supplementary data Table S4]. For *C. moschata*, the volume of nectar for both pistillate and staminate flowers was significantly higher than flowers of *C. sororia* [Supplementary data Table S4].

Dry pollen weight (mg) in staminate flowers was significantly different between *C. sororia* staminate flowers from both *C. argyrosperma* and *C. moschata* (ANOVA, $F_{(2, 73)}=13.58$, $p < 0.001$, Table 4). Where, *C. moschata* and *C. argyrosperma* had significantly higher pollen weight than *C. sororia* (Table 4). The mean protein concentration was highest in *C. moschata*, followed by *C. argyrosperma* and then by *C. sororia* (Table 4). The mean lipid concentration was highest in *C. sororia* followed by *C. moschata* and then by *C. argyrosperma* (Table 4). The P:L ratio was highest in *C. moschata* followed by *C. argyrosperma* and then by *C. sororia* (Table 4).

Floral visitor community and behaviour

A total of 435 hours of video were reviewed from 145 flowers from 92 plants and 6501 visits were recorded from 30 morphospecies of floral visitors. Sixteen floral visitors were identified from the transect collections [Supplementary data Table S2]. Six taxonomic groups, primary floral visitors, made up 96% of all visits; *Eucera* female bees (30%), *Eucera* male bees (16%), from the Meliponini tribe (27%), *Megalopta tetewana* (10%) bees from the tribe Augochlorini (8%), *Apis mellifera* (5%), while the remaining 4% of floral visitors were grouped as secondary floral visitors and were made up of 11 other morphospecies. *Megalopta tetewana* is also a member of the Augochlorini tribe, but, because they are easily distinguishable from other

Augochlorini bees, crepuscular flight times, and high intensity of visitation, we decided to analyse them as a separate group.

The interaction between floral visitors, cucurbit species, and flower sex have a significant effect on the probability of visitation ($F_{(32, 955)}=1.82, p = 0.0037$), visitation rate ($F_{(31, 6444)} = 4.36, p < 0.0001$), and probability of contact of floral visitors with stigmas or anthers ($F_{(31, 6444)}= 8.16, p < 0.0001$). Female *Eucera* spp. has the highest probability of visit (86-97%) to all pistillate and staminate flowers of the cucurbit species, except for the group of secondary visitors to pistillate flowers of *C. argyrosperma* (100%) and the Augochlorini bees to staminate flowers of *C. argyrosperma* (92%). *Megalopta tetewana* did not visit the pistillate flowers of *C. sororia*, therefore had the lowest estimated probability of visit (0%). The bees from the Meliponini tribe and *M. tetewana* had the highest visitation rate, specifically to pistillate flowers of domestic cucurbits. Female *Eucera* spp. had the highest probability of contact with reproductive structures in flowers of all cucurbit species and floral sexes (91-100%). Secondly, male *Eucera* spp., *A. mellifera* and Augochlorini bees had the next highest probability of contact with reproductive structures.

The VCI values for staminate flowers of all cucurbit species were highest for both *Eucera* female and male bees (Fig. 4A). The VCI for all other visitors was larger in the pistillate flowers than in staminate flowers except for Augochlorini which was slightly higher in staminate flowers of *C. sororia* (Fig. 4A). The IPI values were highest for female *Eucera* spp. for all cucurbit species, especially in *C. sororia* (Fig. 4B). Augochlorini bees and *A. mellifera* had second highest values in the pistillate flowers of domesticated cucurbits.

The frequency of visits was affected by the time of day and the interaction between floral visitors, cucurbit species, and flower sex ($F_{(153, 842)} = 3.57, p < 0.0001$). The pistillate flowers of *C. sororia* had the shortest window of activity from primary flower visitors while the most extended window of activity for all visitors was in both staminate and pistillate *C. moschata* flowers (Fig. 5A and B). The peak of visitation in all flowers was in the time range of 0700-0800h (Fig. 5A and B). The interaction of motive of visit, cucurbit species, and flower sex had a significant effect on visitation ($F_{(102, 848)} = 5.01, p < 0.001$) (Fig. S2.A) and duration of visit ($F_{(101, 4874)} = 10.61, p < 0.001$) (Fig. S2.B). Collecting nectar was the most common motive for Meliponini bees and male *Eucera* bees in staminate flowers. Collecting pollen and nectar and pollen was the most common motive for female *Eucera* spp. and Augochlorini bees in the staminate flowers (Fig. 8). Nectar was the most common motive in pistillate flowers except for Meliponini bees in *C. sororia*, *M. tetewana* in *C. argyrosperma* and male *Eucera* spp. and the secondary floral visitor group in *C. moschata* (Fig. 8). The duration of the visits was the shortest for female and male *Eucera* spp. and *Apis mellifera* in both staminate and pistillate flowers of all cucurbit species. (Fig. 9).

DISCUSSION

Agricultural practices and phenotypic differentiation of domesticated plants from their wild ancestors has created novel niches for a suite of original and new biotic interactions (Chen *et al.*, 2015; Turcotte *et al.*, 2017). This study provides evidence that artificial selection has influenced changes in floral attributes that in turn are associated with the pollinator and floral visitor community. Few studies have explored the differences of floral morphology and the

flower visitor community of wild and domesticated plant species in their place of origin (Kuriakose *et al.*, 2009; Pacheco-Huh *et al.*, 2020). It is reasonable to expect that domesticated plants depend on pollinator communities originally found in natural habitats where the wild relatives of agricultural species originally occurred. Mutualistic interactions between cucurbit species and their pollinators are estimated to date back to 11 mya (Dorchin *et al.*, 2018), where natural selection resulted in a specialized relationship between flowers of wild cucurbits and *Eucera* bees. Meanwhile, artificial selection resulting in the domestication of cultivated cucurbit species began around 10,000 years ago (Smith, 1997; Lira *et al.*, 2016). Domestication in *Cucurbita* was driven by artificial selection on vegetative and reproductive traits. In terms of vegetative traits, plants changed from a vine growth habit to a reduction of internodal distance between leaves and/or a rosette like growth form, a decrease in defences found in terms of trichome density and secondary metabolites (cucurbitacin) in leaves, fruits, and seeds (Lira *et al.*, 2016). In terms of reproductive traits, domestication increased fruit and seed size, thereby reducing the number of fruits and seeds per plant (Lira *et al.*, 2016). However, little is known about the effects of domestication on floral traits and its consequences to maintain plant-pollinator mutualistic interactions. Our study is the first to show that domestication of *Cucurbita* species, belonging to the same phylogenetic group, increased in floral trait size, nutrient content, and nectar volume and the floral traits were more correlated and integrated with respect to the wild cucurbit species. In addition, these cucurbit species have similar floral visitor communities, but the probability and rate of visitation, behaviours, and relationship of the floral visitors with staminate and pistillate flowers differed between domesticated and wild cucurbit plants.

We show that domestication in cucurbit plants resulted in larger flowers, stronger floral trait correlations and higher floral integration. This pattern was most pronounced in the pistillate flowers of domesticated cucurbits. While *C. sororia* is the wild progenitor of *C. argyrosperma*; morphological attributes of both domesticated species, *C. argyrosperma* and *C. moschata*, were in general larger than in the wild cucurbit. The selective force experienced by domesticated cucurbits enlarged fruits, seeds and, indirectly, the floral parts. Some floral trait sizes were conserved between the wild progenitor and daughter domesticated species, *C. sororia* and *C. argyrosperma*, such as the length of the corolla and the dimensions of the reproductive parts, but all floral traits were larger for *C. moschata* (Table 1a-b). In experimental crosses between wild and domesticated cucurbit species, Merrick (1990) found symmetrical crosses between *C. argyrosperma* and *C. sororia* irrespective of which species acted as the pollen donor or receptor. These intraspecific crosses produced viable offspring. However, only asymmetric experimental crosses were possible between *C. moschata* and the other two species, because only viable offspring was produced when *C. moschata* was used as the pollen donor for both *C. sororia* and *C. argyrosperma*, but no fruits were produced on *C. moschata* when *C. argyrosperma* or *C. sororia* were the pollen donors (Merrick, 1990; Lira, 1995). One important step in plant domestication is reproductive isolation from wild populations (Meyer *et al.*, 2012). The larger length of the stigma and style of *C. moschata* may prevent wild species from crossing with the cultivated species but introgression of *C. moschata* with wild and other domesticated relatives is possible. The length of the pistil might act as a reproductive barrier (Tiffin *et al.*, 2001) that may limit the crosses of *C. argyrosperma* and *C. sororia* as pollen donors on *C. moschata*.

Domestication tends towards phenotypic homogenization facilitating management practices and desirable agricultural traits for human consumption (Berg, 1960; Doebley *et al.*, 2006). Domestication begins by selecting a subset of traits from a wild population that is used as the parental line and then crossed with each other for several generations to develop endogamic accessions with desirable traits (van Raamsdonk, 1993; Pickersgill, 2007; Hernández-Terán *et al.*, 2017; Milla *et al.*, 2018). We proposed that the domesticated cucurbits would have less variation in the floral morphological traits because the resulting endogamic accessions should be more genetically homozygous than the original parental lines (Doebley *et al.*, 2006; Purugganan and Fuller, 2009; Hernández-Terán *et al.*, 2017). This hypothesis was partially supported by our results, the most widely cultivated domesticated squash, *Cucurbita moschata*, presented the lowest coefficients of variation (CV) for most morphological traits of both staminate and pistillate flowers. However, floral traits of pistillate flowers of domesticated *C. argyrosperma* had the highest CV values. Higher CV floral trait values of *C. argyrosperma* could be a consequence of two factors; genetic exchange between local *C. argyrosperma* landrace varieties and adjacent wild *C. sororia* populations resulting in high genetic diversity and/or maintenance in high genetic diversity in traditional farming practices (Montes-Hernández and Eguiarte, 2002; Balvino-Olvera *et al.*, 2017; Sánchez-de la Vega *et al.*, 2018).

Although evaluating individual trait sizes and CV values illustrates phenotypic differences between species, a multivariate approach provides more robust evaluation of the relationships between floral traits and domestication across the three study species (Berg, 1960; Armbruster, 1991; Armbruster *et al.*, 1999; Herrera *et al.*, 2002). The multivariate analysis of the staminate and pistillate flowers showed differences between the three cucurbit species. Notably, for both

staminate and pistillate flowers, *C. argyrosperma* occupied an intermediate phenotypic space between the wild ancestor, *C. sororia*, and the more widely cultivated domestic sister taxa, *C. moschata*. This suggests that domestication of the two cucurbit species in the *Argyrosperma* group has led to gradual changes and enlargement of floral traits. The correlation networks of the staminate floral traits also follow this pattern of gradual phenotypic changes from wild to most derived domestic species. On the other hand, correlation networks of the pistillate flowers depicts a different pattern to those of the staminate flowers, *C. argyrosperma* exhibited a floral trait network with full correlation between all traits while *C. sororia* presented a stronger magnitude of correlated corolla traits, and *C. moschata* the weakest.

Our study is the first to compare floral integration between unisexual flowers of monoecious species. For all cucurbit species, the pistillate flowers had higher integration than the staminate flowers suggesting that pistillate flowers provide fewer variable cues to attract pollinators to maximize pollen deposition on stigmas. The modules for attraction/restriction for all cucurbit species in staminate and pistillate flowers have higher integration indices than the reproductive module. The fusion of floral parts, in the case of cucurbits the petals, results in higher integration of the attraction/restriction floral module. When comparing integration indices between cucurbit species we found that pistillate flowers of domesticated cucurbits were more integrated than those of the wild cucurbit species. Interestingly, *C. argyrosperma* pistillate flowers had the highest values for total flower integration and highest integration values for attraction/restriction and reproductive modules. Artificial selection of *C. argyrosperma* was primarily directed towards the production of large edible seeds (Jones, 1992; Lira *et al.*, 2016; Barrera-Redondo *et al.*, 2020) unlike *C. moschata* which is primarily cultivated

for its fruit flesh (Lira *et al.*, 2016). It is possible that for *C. argyrosperma* the selection for the higher rates of ovule fertilization to obtain a high number of fertilized seeds per fruit has resulted in a more integrated floral structure. As proposed by Meyer and Purugganan (2013), artificial selection primarily directed towards plant fruits should result in improved pollination success, which, we believe could be attributed to a higher magnitude of floral integration in domesticated cucurbit species. Future studies could compare other pollination-dependent fruit crops to test if this phenomenon holds true for other domesticated plant species and their extant wild ancestors.

Visual cues such as length and diameter of corolla can act as a signal to floral visitors of the quantity and quality of nectar and pollen resources (Elle and Carney, 2003; Galetto and Bernadello, 2004; Armbruster *et al.*, 2005; Theis *et al.*, 2014). We observed that the domesticated cucurbits have larger morphological floral attributes and accumulate more nectar and produce more pollen than the wild cucurbit species. Even though the sugar concentration did not differ between species it is possible that the sugar composition, the percentages of sucrose and hexose sugars, could be different according to the degree of domestication. Other cucurbit species present nectar with higher levels of sucrose than hexose, a pattern more pronounced in pistillate flowers and correlated to higher levels of attraction to bees (Nepi and Pacini, 2001; Ashworth and Galetto, 2002; Abrahamczyk *et al.*, 2018; Chatt *et al.*, 2018). Moreover, Theis *et al.* (2014) found a positive correlation between corolla length, sesquiterpenoids and nectar volumes of wild and domestic cucurbits. These correlated factors were also the best predictors of cucurbit pollen specialist species, *Eucera pruinosa*, attraction. Therefore, the quantity and perhaps the quality of floral rewards advertised by larger floral

traits could have positively influenced a higher intensity of visitation to flowers of domestic cucurbits.

Selection for higher reproductive success in domesticated cucurbits may have positively affected the pollen quality. Plants which require animal pollination experience two main selection forces which determine pollen quality, or pollen nutrition, the first being the production of vigorous microgametophytes for the successful fertilization of ovules and the second, to provide food for pollinators when pollen is considered as a reward (Quesada *et al.* 1991; Quesada and Stephenson 1993; Roulston *et al.*, 2000; Ruedenauer *et al.*, 2019). First, from the perspective of the plant, the length of the style directly affects pollen performance, therefore, the amount of pollen macronutrients resulting in increased protein concentrations and P:L will provide higher energy sources for better pollen tube growth for successful fertilization of ovules (Roulston *et al.*, 2000). Second, from the perspective of the floral visitor, macronutrients of pollen, principally protein concentrations and protein to lipid ratios (P:L) directly affect foraging preferences and nutritional quality for bee pollinators (Roulston *et al.*, 2000; Vanderplanck *et al.*, 2014; Vaudo *et al.*, 2016; Treanore *et al.*, 2019; Vaudo *et al.*, 2020). In support of our hypothesis, the domestic cucurbits, *C. moschata* (4.72:1) and *C. argyrosperma* (4.02:1) presented the highest P:L ratio followed by the wild cucurbit, *C. sororia* (2.92:1), which had lower protein concentrations and higher lipid concentrations than both domestic species. An alternative or complementary explanation for higher pollen protein content could be that artificial selection may have also acted directly on staminate flowers of cucurbit plants. Since pre-Hispanic times, in the region of Mexico, squash blossoms (staminate flowers) are the most consumed edible flowers and present higher protein content in floral tissues relative to other

edible flowers (Sotelo *et al.*, 2007). Floral tissues such as the corolla and floral rewards have been shown to share chemical composition due to genetic pleiotropic effects (Palmer-Young *et al.*, 2019). Potentially selection for flowers with higher nutritive properties could have resulted in higher protein content in pollen for human consumption.

Cucurbit domestication has increased pollinator attraction of the pollen specialist, *Eucera* spp. as well as other bee species. The *Eucera* spp. are oligolectic to cucurbit plants meaning that adult female bees use cucurbit pollen to provide for their brood cells (Hurd *et al.* 1971, Delgado-Carrillo *et al.* 2017, Brochu *et al.* 2020). Pollen protein, P:L ratio and quantity was highest in *C. moschata* staminate flowers. Although our study did not isolate the effects of quantity vs. quality, it appears that the pollen reward in *C. moschata* staminate flowers influences higher intensity of visitation from *Eucera* spp. than in the wild cucurbit plants. Like other studies, eusocial bees from the Apidae family were the least likely to collect cucurbit pollen than other floral visitors (Nepi and Pacini, 2001; Brochu *et al.*, 2020), and even less likely to collect pollen from the wild cucurbit species, *C. sororia* (*Apis mellifera* was only observed collecting pollen from the domestic cucurbits). Wild cucurbit pollen may be less attractive to these bee species because of lower protein concentration (Treanore *et al.*, 2019; Vaudo *et al.*, 2020) or, perhaps the pollen from wild cucurbit plants has more defences than found in the domestic species (Brochu *et al.*, 2020).

Plant-pollinator studies of the cucurbit system performed within its natural distribution find that the floral visitor community is dominated by *Eucera* spp. (Hurd *et al.*, 1971; Tepedino, 1981; Cane *et al.*, 2011; Parra-Tabla *et al.*, 2017; Delgado-Carrillo *et al.*, 2018; Lobo and Bravo,

2021; Contreras-Varela 2021). Our results corroborate this phenomenon and suggest that there is a bee community of floral visitors that likely play an important role as secondary pollinators for the domestic cucurbit species. In this study a pollinator efficiency experiment was not performed, but we observed floral visitor behaviour which we used as a proxy for pollinator importance, such as; visitation rate, contact with reproductive parts (Santiago-Hernández *et al.*, 2019) and pollen grains carried on the body. By synthesizing these metrics into two indices, the visitor and contact index (VCI) and the pollinator importance index (IPI), we were able to confirm that female *Eucera* spp. are the most important pollinators in cucurbit plants. We also propose that Augochlorini and *Apis mellifera* are likely to contribute secondarily to pollination of domesticated cucurbit species. Future studies should perform pollinator exclusion experiments to test the differences in pollinator effectiveness in wild and domesticated cucurbit species.

According to our observations, domestication of cucurbit species is associated with lengthening the floral anthesis resulting in more visitation opportunities from a broader floral visitor community. Although wild and domesticated cucurbit species are reported to have similar floral anthesis times (Montes-Hernández and Eguiarte, 2002), we observed that staminate flowers of the domesticated cucurbits, *C. moschata* and *C. argyrosperma*, had a more extended visitor activity window than the wild cucurbit flowers. Additionally, *C. moschata* presented the longest floral visitor activity window which we argue has increased the probability of visits from a bee community of floral visitors that may act as secondary pollinators. The *C. sororia* pistillate flower opened later in the morning and did not receive visits from the crepuscular bee species *Megalopta tetewana* that visited domesticated cucurbit

pistillate flowers in high intensities. Furthermore, *A. mellifera* presented higher visitation in the late morning to *C. moschata* flowers (Delgado-Carrillo *et al.*, 2018; Contreras-Varela 2021; Lobo 2021) when the wild cucurbit flowers were beginning to close (personal obs.). In agreement with previous studies (Hurd and Linsley, 1964; Hurd *et al.*, 1971; Nepi and Pacini, 1993; Montes-Hernández and Eguiarte, 2002), the floral anthesis of cucurbits is closely synchronized to the female cucurbit pollen specialist, *Eucera* spp, daily flight times. Like other solitary bee species, female *Eucera* spp. begin provisioning trips with pollen collection, in this case from 0600-0800h, and then end with nectar collection with most activity subsiding after around 0900h (Hurd *et al.* 1971). We propose that the shorter anthesis of the wild cucurbit flowers could limit the visitation and pollen theft from non-cucurbit pollen specialist bee species, like the crepuscular bees *M. tetewana* and later flying bee species like *A. mellifera*.

While domesticated cucurbit flowers were more attractive to floral visitors, wild cucurbit species should not be overlooked. Wild crop relatives are an important genetic reservoir for domestic plant species (Vaughan *et al.*, 2007) and in the case of cucurbit species, can maintain mutualistic interactions. Although wild cucurbit plants had lower visitation rates, we found that *Eucera* spp. was the most dominant in the floral visitor community. We propose that wild cucurbit populations most likely support *Eucera* spp. bee populations. For constant secure resources of the cucurbit pollen specialist, wild cucurbit populations should be conserved as pollinator sources to secure pollination services of domesticated cucurbits in neighbouring agricultural fields.

CONCLUSION

Looking back to wild ancestral plant species and their biotic interactions is essential for understanding the ecological and evolutionary effects of agricultural practices (Milla *et al.*, 2017). More studies like these need to be conducted in their centre of origin of the domesticated and wild plant species (Chen *et al.*, 2017). By comparing three sister taxa, two of which are domesticated, we found an increase in size of floral traits, distinct floral trait correlation patterns and a higher floral integration index of domesticated plants. Artificial selection for increased fruit size and/or more investment in reproductive success has resulted in larger flowers and higher quantities of nectar and pollen influencing higher visitation rates from floral visitors. Measuring floral attributes of wild plant populations and their domesticated counterparts would be an additional strategy to maintain particular traits in the natural germplasm and the genetic variation of natural populations. The conservation of wild ancestor plant populations needs to be prioritized to preserve key mutualistic interactions.

SUPPLEMENTARY INFORMATION

Supplementary data are available online at <https://academic.oup.com/aob> and consist of the following. Table S1: Description of sites, studies, and coordinates. Table S2: Floral visitor morpho species and groups. Table S3: Coefficients of variation of floral morphological traits. Table S4: Nectar sugar volume and concentration-LS means and ANOVA model results. Fig. S1: Estimated marginal means of nectar volume trends over time for staminate and pistillate flowers of cucurbit species. Fig. S2: a.) Relative proportions of LS means \pm SE of visitation rate and b.) LS

means±SE of duration of visit, for each motive; Nectar, Pollen, Nectar+Pollen, Nothing, for each floral visitor to staminate and pistillate flowers of cucurbit species.

ACKNOWLEDGEMENTS

We thank # anonymous reviewers for insightful comments that significantly improved this manuscript. We thank Adonaji Cortés Pérez, Saul Espinoza, Jesús Torres Her, Ximena Contreras, Elvia Pérez, Kenya Salgado, Jazmín Villanueva, Julia Giron, Estrella Páramo, Francisco Balvino, César Liñares Ojeda, Jacob Cristóbal Pérez, María de Jesús Aguilar for laboratory and field assistance, Violeta Patiño, Rogelio Cruz Reyes, Silvana Martén-Rodríguez, and Gumersindo Sánchez-Montoya for technical and logistical assistance, Napoleón Sahagún Gallegos, Carlos Godínez Cuevas, King Kong Cuevas, David Martínez Vega, and Baltazar Espinoza for lending us field plots and providing support for cultivating plants, Ernesto Vicente Vega and Francisco Mora for statistical analysis support, Anthony Vaudo for pollen macronutrient analysis consultation. We thank Yolanda Vega for the design and elaboration of Fig. 1. S.G., M.Q., A.G., R.L., M.H.S.H. planned and designed the research; S.G., and O.D. collected data; S.G. and M.H.S.H. performed statistical analyses; S.G., M.H.S.H., O.D., and MQ drafted the manuscript; and all authors contributed to the final version. This study was performed in fulfilment of the requirements of the Ecology M.Sc. degree of S.G. at the Graduate Program in Biological Sciences, UNAM.

FUNDING

This work was supported by grants from Dirección General Asuntos del Personal Académico, Universidad Nacional Autónoma de México [PAPIIT IV200418, IA207318, IA207618, IN2224920, IN219021]; Consejo Nacional de Ciencia y Tecnología (CONACyT) [Laboratorio Nacional de

Análisis y Síntesis Ecológica, 2015-LN250996, 2016-LN271449, 2017-LN280505, 2018-LN293701, 2019-LN299033, 2020-LN314852, 2021-LN315810]; Secretaría de Agricultura y Desarrollo Rural-CONACyT [291333] to M.Q. and Silvana Martén-Rodríguez; Programa Iberoamericano de Ciencia y Tecnología para el Desarrollo RED CYTED-SEPODI [417RT0527]; and graduate fellowship [928826] to S.G. financed by CONACyT.

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Table 1. Morphometric trait analysis comparing floral morphological attributes of the corolla between three cucurbit species and flower sex. Estimated marginal means \pm standard error. n= sample size.

Different letters indicate significant differences $p \leq 0.05$. * $p < 0.05$ ** $p < 0.001$ *** $p < 0.0001$

<i>Cucurbita</i> spp.	Flower sex	CD	CL	TL	TD1	TD2	TD3	ND
<i>C. sororia</i>	staminate (n=49)	79.40 $\pm 1.03^a$	80.57 $\pm 1.03^{ac}$	46.99 $\pm 1.03^a$	13.34 $\pm 1.03^a$	20.74 $\pm 1.03^a$	35.27 $\pm 1.04^a$	8.29 $\pm 1.03^a$
	pistillate (n=37)	74.89 $\pm 1.04^a$	73.19 $\pm 1.03^a$	40.56 $\pm 1.04^b$	17.11 $\pm 1.03^b$	24.88 $\pm 1.03^b$	34.91 $\pm 1.05^a$	12.52 $\pm 1.03^b$
<i>C. argyrosperma</i>	staminate (n=45)	108.22 $\pm 1.03^b$	99.61 $\pm 1.03^b$	61.83 $\pm 1.03^c$	18.90 $\pm 1.03^b$	34.70 $\pm 1.03^c$	53.14 $\pm 1.05^{bc}$	10.98 $\pm 1.03^c$
	pistillate (n=29)	95.28 $\pm 1.04^b$	85.73 $\pm 1.04^c$	53.22 $\pm 1.04^a$	25.22 $\pm 1.04^c$	36.18 $\pm 1.04^{cd}$	48.58 $\pm 1.05^b$	16.57 $\pm 1.03^d$
<i>C. moschata</i>	staminate (n=46)	129.00 $\pm 1.03^c$	134.24 $\pm 1.03^d$	81.59 $\pm 1.03^d$	22.88 $\pm 1.05^c$	34.30 $\pm 1.03^c$	62.95 $\pm 1.04^c$	12.97 $\pm 1.03^b$
	pistillate (n=31)	126.22 $\pm 1.04^c$	123.16 $\pm 1.04^d$	74.79 $\pm 1.04^d$	34.38 $\pm 1.04^e$	40.23 $\pm 1.04^d$	52.46 $\pm 1.05^b$	25.03 $\pm 1.03^e$
χ^2 values for model								
Model		CD	CL	TL	TD1	TD2	TD3	ND
<i>Cucurbita</i> spp.		187.5**	213.7** *	272.1**	316.1**	285.3**	123.5**	328.4**
Flower sex		6.6*	19.3**	22.1**	184.3**	29.1**	6.4*	428.1**
<i>Cucurbita</i> spp.:Flower sex		2.5	1.2	1.1	8.6*	6.0	4.7	23.2**

Table 2. Absolute morphological trait analysis for reproductive floral traits between three cucurbit species. Estimated marginal means \pm standard error. Numbers followed by different letters indicate significant differences. n= sample size.

*p<0.05, **p<0.001, ***p<0.0001

Staminate flowers:					
<i>Cucurbita</i> spp.	AD	StL	AL		
<i>C. sororia</i> (n=49)	4.41 $\pm 1.02^a$	28.08 $\pm 1.02^a$	16.40 $\pm 1.03^a$		
<i>C. argyrosperma</i> (n=45)	5.55 $\pm 1.02^b$	31.59 $\pm 1.03^b$	17.03 $\pm 1.03^a$		
<i>C. moschata</i> (n=46)	5.97 $\pm 1.02^c$	42.61 $\pm 1.03^c$	27.93 $\pm 1.03^b$		
	χ^2 144.1***	150.3***	154.5***		
Pistillate flowers:					
<i>Cucurbita</i> spp.	SD	PL	SL	OL	OD
<i>C. sororia</i> (n=37)	11.53 $\pm 1.03^a$	21.16 $\pm 1.04^a$	9.71 $\pm 1.03^a$	23.94 $\pm 1.04^a$	16.22 $\pm 1.04^a$
<i>C. argyrosperma</i> (n=29)	12.90 $\pm 1.04^a$	23.90 $\pm 1.04^b$	10.50 $\pm 1.04^a$	24.31 $\pm 1.05^a$	23.65 $\pm 1.04^b$
<i>C. moschata</i> (n=31)	16.08 $\pm 1.04^b$	30.54 $\pm 1.03^c$	15.54 $\pm 1.03^b$	31.17 $\pm 1.05^b$	33.14 $\pm 1.04^c$
	χ^2 45.9***	55.4***	120.7***	22.5***	191.1***

Table 3. Integration indices of cucurbit staminate and pistillate flowers. n= sample size.

<i>Cucurbita spp.</i>	Total	Attraction/ Restriction	Reproductive
Staminate flowers:			
<i>C. sororia</i> (n=47)	1.378	1.471	0.361
<i>C. argyrosperma</i> (n=45)	1.56	1.309	0.502
<i>C. moschata</i> (n=46)	1.454	0.89	0.482
Pistillate flowers:			
<i>C. sororia</i> (n=36)	1.712	1.337	0.752
<i>C. argyrosperma</i> (n=26)	6.467	3.279	2.698
<i>C. moschata</i> (n=31)	2.213	1.241	0.985

Table 4. Macronutrient analysis; mean \pm SE values of pollen weight, protein, lipid, and protein/lipid ratio concentration. Different letters indicate significant differences. conc. = concentration, P:L = protein:lipid concentration, n= sample size. Concentration of macronutrients are described as micrograms per milligram ($\mu\text{g}/\text{mg}$) of pollen. *** $p < 0.0001$

Cucurbit species	Pollen weight (mg)	Protein conc. ($\mu\text{g}/\text{mg}$)	Lipid conc. ($\mu\text{g}/\text{mg}$)	P:L
<i>C. sororia</i> (n=35)	27.20 $\pm 1.07^a$	186.51 ± 1.88	66.40 ± 8.85	2.92 ± 0.42
<i>C. argyrosperma</i> (n=23)	38.07 $\pm 1.07^b$	211.09 ± 16.69	52.45 ± 2.88	4.02 ± 0.24
<i>C. moschata</i> (n=18)	46.35 $\pm 1.11^b$	241.79 ± 8.91	53.27 ± 6.17	4.72 ± 0.77
F value	13.58***			

Fig. 1. Illustration of floral characters for staminate and pistillate flowers of cucurbit flowers. a) corolla diameter (CD); b) corolla length (CL), corolla tube diameter at the base (TD1), in the middle (TD2) and at the opening (TD3). c) Staminate flowers base of nectary (NDm), anther diameter (AD), stamen length (StL), anther length (AL). d) Pistillate flowers base of nectary (NDf), stigma diameter (SD), style+stigma (PL), stigma length, (SL), ovary length and diameter (OL & OD). Original illustration: Yolanda Vega.

Fig. 2. MDS analysis results of all floral morphological attributes of a.) staminate and b.) pistillate flowers. Colours and shapes indicate different cucurbit species.

Fig. 3. Correlation networks of floral morphological traits for staminate and pistillate flowers. Lines represent significant Pearson (R^2) correlations. The solid lines represent the $R^2 > 50\%$ while the dotted lines are the $R^2 < 50\%$. The thickness of the lines increases according to R^2 values. On top staminate flowers and bottom pistillate flowers. Yellow circles represent floral traits associated with attraction and restriction of pollinators and orange circles represent floral traits associated with donation and reception of pollen.

Fig. 4. Floral visitor importance index networks: a) Visitation and contact index (VCI) network for staminate and pistillate flowers. Blue lines represent floral visitor importance to staminate flowers and coral lines represent floral visitor importance to pistillate flowers. b) Pollinator importance index (IPI) network for pistillate flowers. The thickness of the line for both networks represents the magnitude of importance of floral visitors and pollinators to cucurbit species.

Fig. 5. a) LS means \pm SE of the number of visits of each flower visitor for cucurbit species throughout anthesis. Top row staminate flowers and bottom row pistillate flowers for cucurbit species. Coloured lines represent different floral visitor groups. b) Separate representation of

visitation rates of Megalopta and Meliponini bees on *C. moschata* flower due to high visitation rates.

FIGURES

Fig. 1

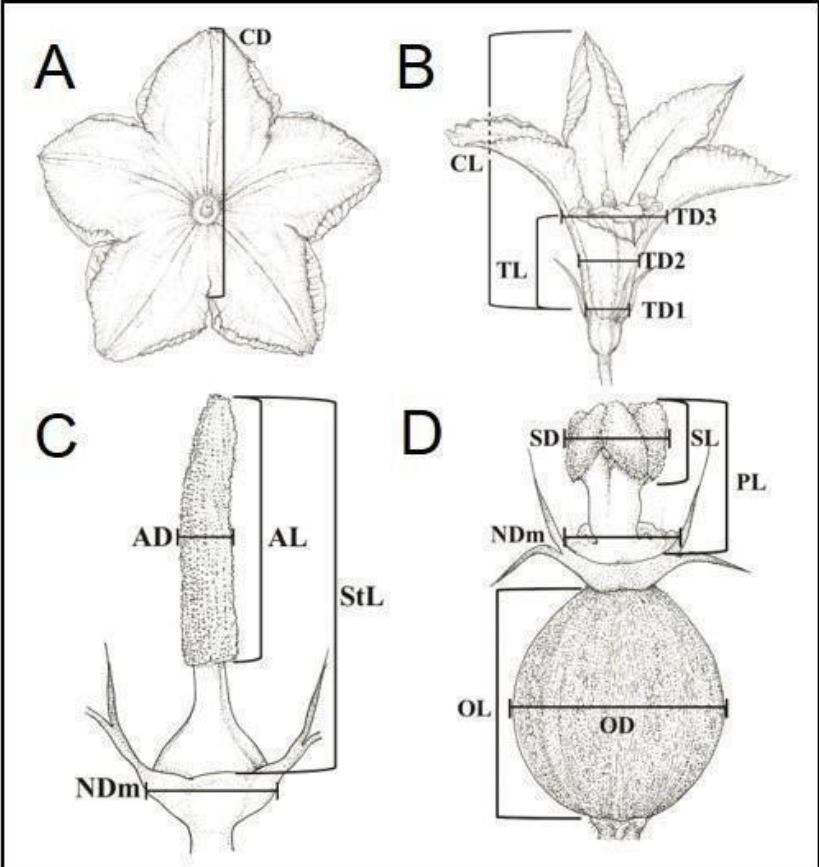


Fig. 2

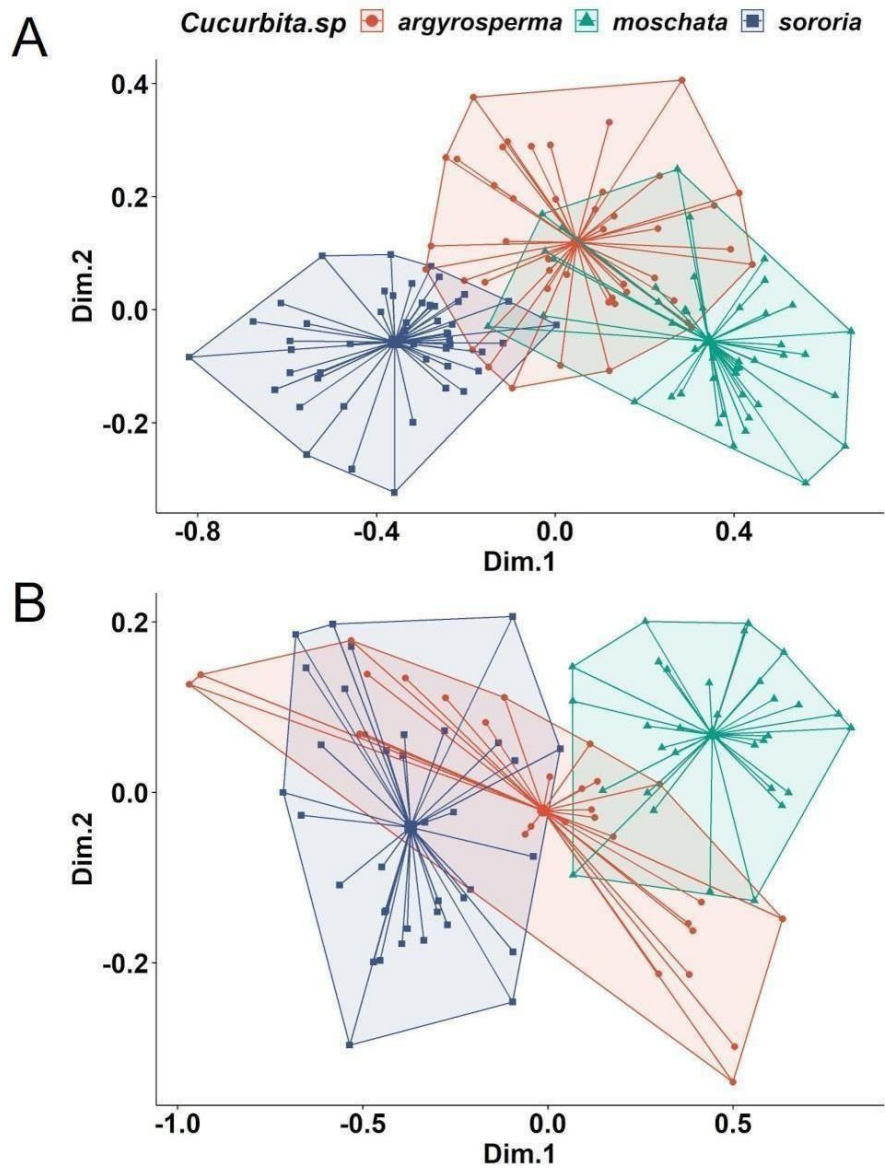


Fig. 3

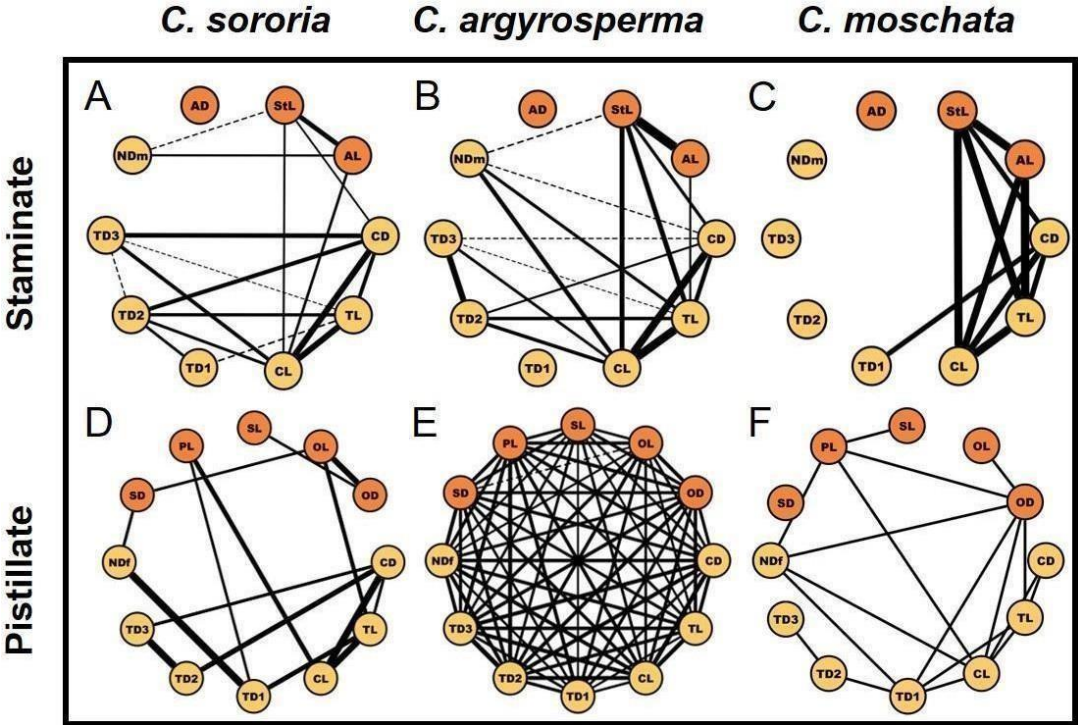
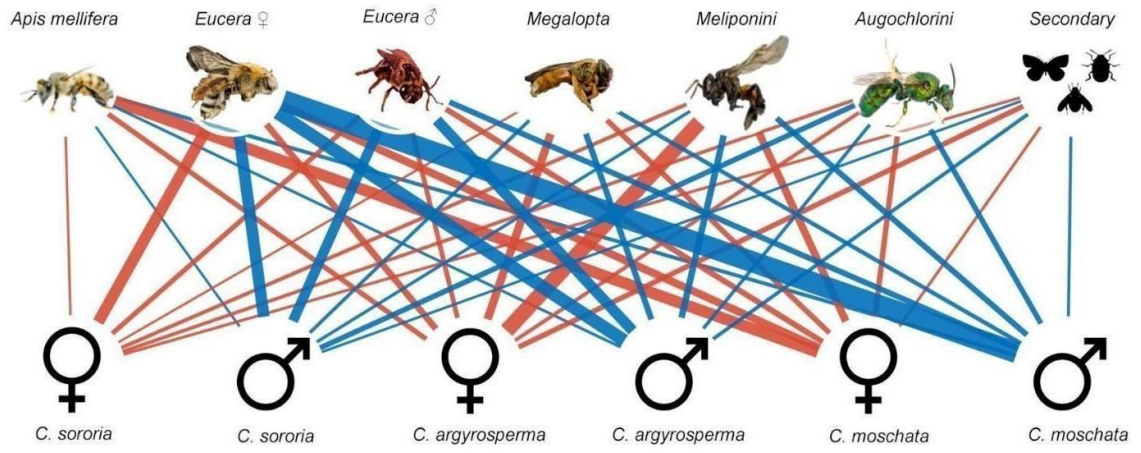


Fig. 4

A



B

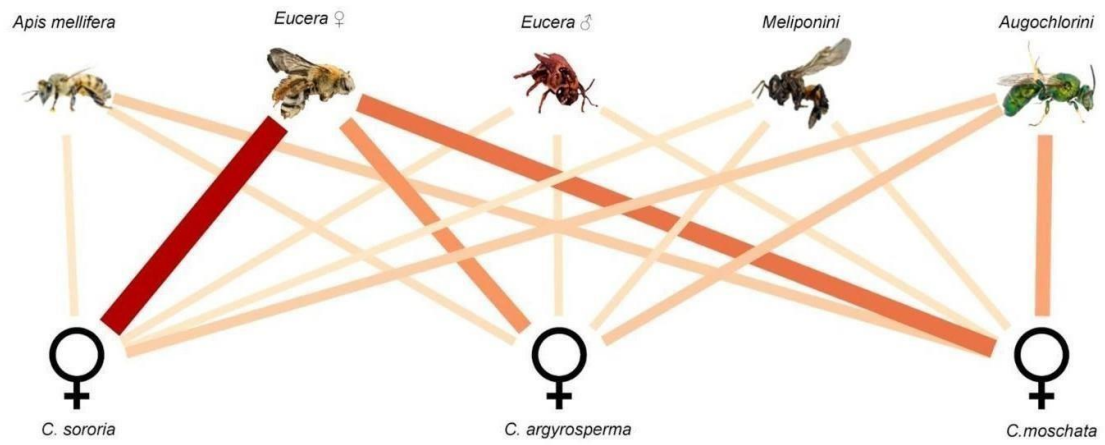


Fig. 5

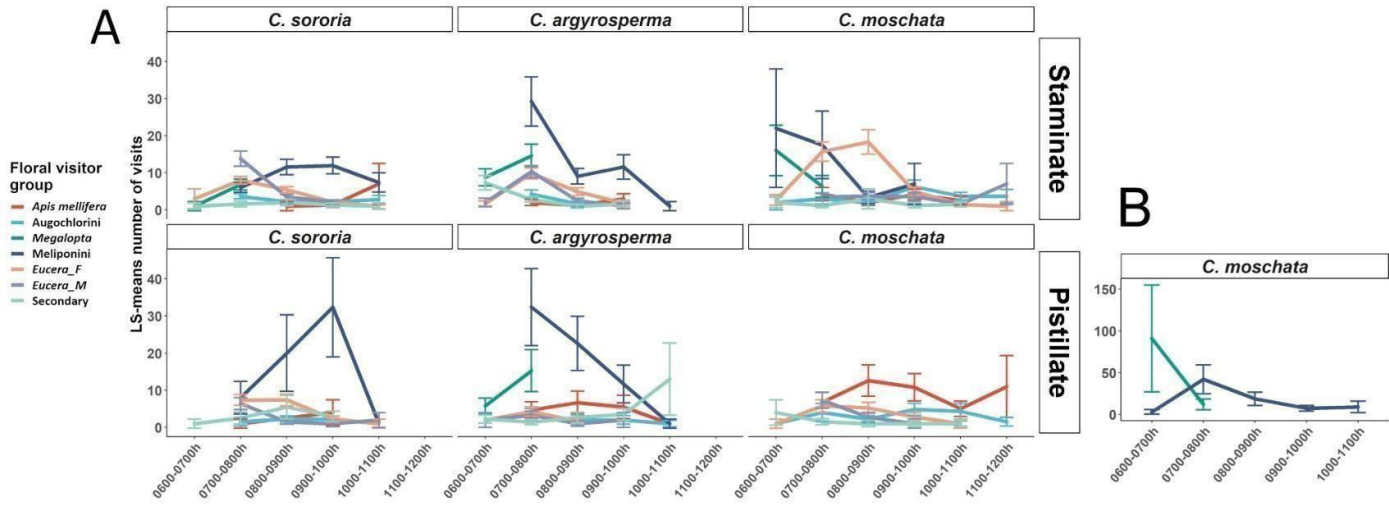


Table S1. Description of sites, studies and coordinates. Abbreviations; cucurbit species: Cs – *C. sororia*, Ca – *C. argyrosperma*, Cm – *C. moschata*; Study: FA - measurements of floral attributes, Vid - video recording of flowers. Type of site: wild pop - wild populations of Cs, plot - field plot. Region: Jal. - Jalisco, Gro. – Guerrero, Mich. – Michoacán.

Site	<i>Cucurbita</i> spp.	Year	Study	Site type	Latitude	Longitude (-)	Elev. (m)	Region
MC	Cs	2019	FA/Vid	wild pop	19°20'40.32"N	104°52'58.99"W	52	Jal.
MN	Cm	2019	FA/Vid	plot	19°21'45.82"N	104°53'53.62"W	52	Jal.
ZN	Ca	2019	FA/Vid	plot	19°22'29.79"N	104°57'43.27"W	15	Jal.
ZC	Cs	2019	FA/Vid	wild pop	19°22'07.51"N	104°57'39.53"W	55	Jal.
PS	Ca, Cm, Cs	0	FA/Vid	plot	17°16'56.51"N	100°34'1.20"W	740	Gro.
CS	Cs	2019	FA/Vid	wild pop	17°16'34.87"N	100°34'13.07"W	670	Gro.
PJ	Ca, Cm, Cs	2019	FA/Vid	plot	17°11'55.79"N	100°37'18.97"W	28	Gro.
PS2	Ca	2020	FA	plot	17°12'00.9"N	100°38'28.70"W	18	Gro.
C	Ca, Cm	2020	FA	plot	20°01'48.5"N	101°09'12.1"W	1840	Mich.
Q	Ca, Cs	2020	FA	plot/wild pop	19°29'9.94"N	100°53'44.52"W	1033	Mich.

Supplementary material 2

Megalopta tetewana is a member of the Augochlorini tribe, but, because they are easily distinguishable from other Augochlorini bees, crepuscular habits, and high visitation, we decided to analyze them as a separate group. *Eucera (Xenoglossa) gabbii* was included in the Secondary floral visitor group instead of the *Eucera* group because of their low visitation and distinct behavior from the other *Eucera* spp. Additionally, we separate female and male bees of *Eucera* because they have differences in floral visitation and pollination efficiency (Delgado-Carillo et al. 2018, Cane et al. 2011).

Table S2. Floral visitor morpho species and groups. Floral visitor morphospecies, group designations of floral visitors are in seven groups: *Eucera* (male and female), Meliponini, Augochlorini, *Megalopta*, *Apis mellifera*, Secondary. Group designations used for statistical analysis. Abbreviations, Region: Jalisco - Jal., Guerrero - Gro.

Floral visitor morphospecies	Group	Region
<i>Eucera (Peponapis) utahensis</i>	<i>Eucera</i>	Jal., Gro.
<i>Eucera (Xenoglossa) crassidentata</i>	<i>Eucera</i>	Jal.
<i>Eucera (Peponapis) azteca</i>	<i>Eucera</i>	Gro.
<i>Partamona</i> spp.	Meliponini	Jal., Gro.
<i>Trigona fulviventris</i>	Meliponini	Jal., Gro.
<i>Augochlora</i> spp.	Augochlorini	Jal., Gro.
<i>Caeneocorynura</i> spp.	Augochlorini	Jal., Gro.
<i>Megalopta tetewana</i>	<i>Megalopta</i>	Gro.
<i>Apis mellifera</i>	<i>Apis mellifera</i>	Jal., Gro.
<i>Acalymma vittatum</i>	Secondary	Gro.
<i>Agapostemon</i> spp	Secondary	Jal., Gro.
Sphecidae sp.	Secondary	Jal., Gro.
<i>Ceratina</i> sp.	Secondary	Jal., Gro.
Dermaptera	Secondary	Jal., Gro.
<i>Diaphania</i> spp.	Secondary	Gro.
<i>Euphoria</i> sp.	Secondary	Jal.
<i>Euglossa</i> sp.	Secondary	Jal., Gro.

Fig. S1. Estimated marginal mean trends (EMMs±SE) of nectar volume (μl) graphed using $\log_{10}[\text{nectar volume}]$ for a.) staminate and b.) pistillate flowers in cucurbit species. Nectar volume was influenced by cucurbit species and the covariate of time, but not their interaction. EMMs of nectar volume reported for five time points between 07:00-11:00h. Cucurbit species represented by colored lines.

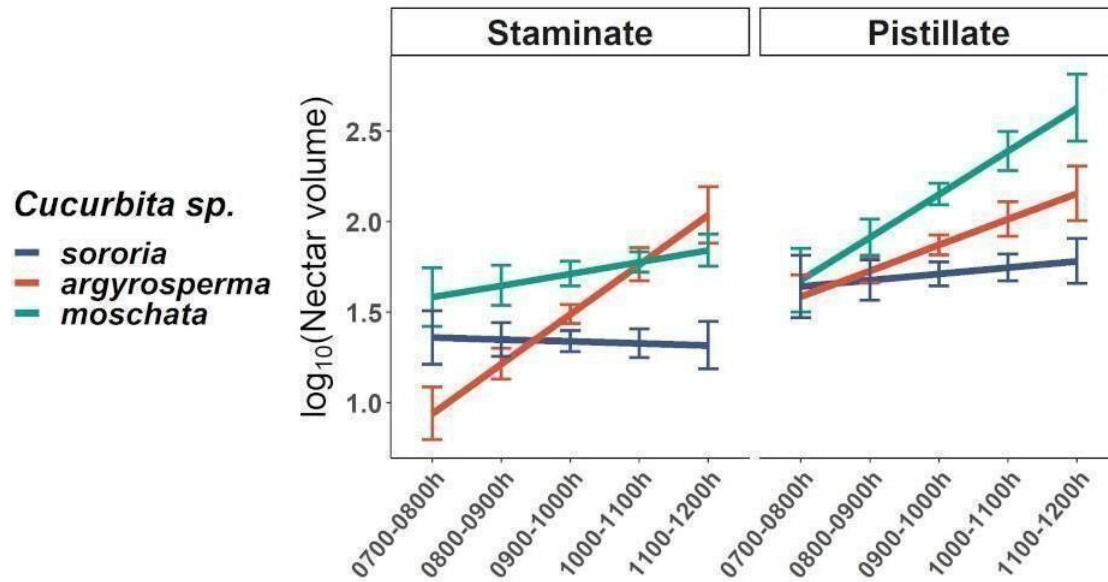
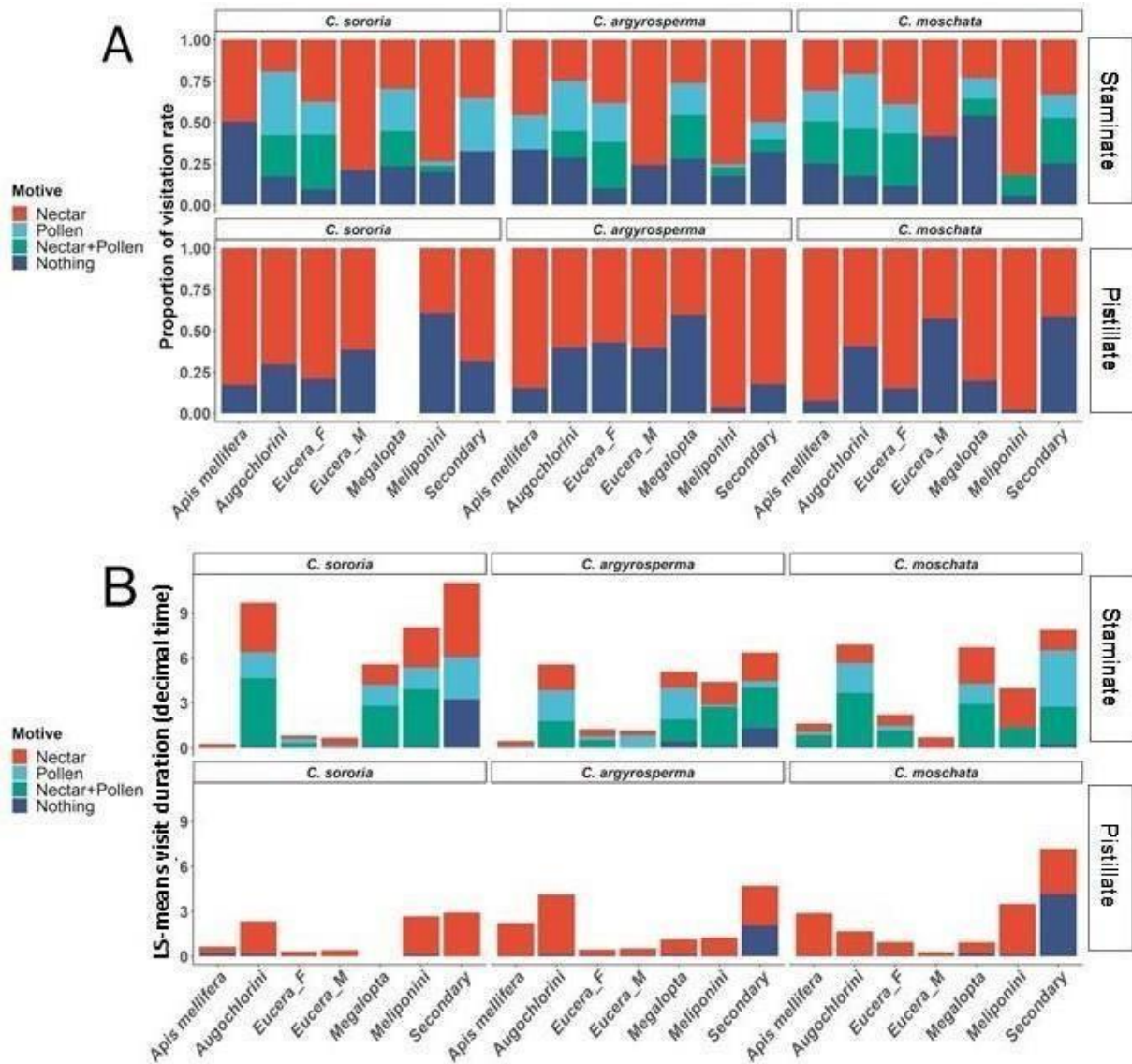


Fig. S.2 a.) Relative proportions of LS means±SE of visitation rate and b.) LS means±SE of duration of visit (decimal seconds) for each motive; Nectar, Pollen, Nectar+Pollen, Nothing, for each floral visitor to staminate (top row) and pistillate (bottom row) flower of each cucurbit species.



DISCUSIÓN GENERAL

Las prácticas agrícolas y la diferenciación fenotípica de las plantas domesticadas han influido en la creación de nuevos nichos y el establecimiento de otras interacciones bióticas que difieren de sus ancestros silvestres (Chen et al., 2015, Turcotte et al., 2017). Este estudio proporciona evidencia de cómo la selección artificial ha contribuido con cambios relevantes en los atributos florales, a su vez asociados con la comunidad de polinizadores y visitantes florales. Es preciso destacar que pocos estudios han explorado las diferencias de morfología floral y sus comunidades de visitantes, incluyendo especies de plantas domesticadas y sus homólogos silvestres en su lugar de origen (Kuriakose et al., 2009; Pacheco-Huh et al., 2020).

Es razonable esperar que las plantas domesticadas dependan de las comunidades de polinizadores que se encuentran en los hábitats naturales donde originalmente se encontraban sus parientes silvestres; sin embargo, aunque estas especies de *Cucurbita* tienen comunidades de visitantes florales similares, nosotros encontramos que la probabilidad, tasa de visitas, comportamiento, y relación de los mismos difiere entre plantas domesticadas y silvestres. Se estima que las interacciones mutuas entre las especies de *Cucurbita* y sus polinizadores se remontan a 11 millones de años (Dorchin et al., 2018), donde la selección natural dio como resultado una relación especializada entre las flores de las calabazas silvestres y las abejas *Eucera*. Mientras tanto, la selección artificial que originó a las especies de calabazas cultivadas, comenzó hace unos 10,000 años (Smith, 1997; Lira et al., 2016). La domesticación en *Cucurbita* fue impulsada por la selección artificial de rasgos vegetativos y reproductivos. En términos de rasgos reproductivos, la domesticación aumentó el tamaño de la fruta y la semilla, reduciendo el número de frutos por planta (Lira et al., 2016). Sin embargo, se sabe poco sobre los efectos de la domesticación en los rasgos florales y sus consecuencias para mantener interacciones mutualistas planta-polinizador. Nuestro estudio es el primero en mostrar que la domesticación de especies de *Cucurbita*, pertenecientes al mismo grupo filogenético, aumentó el tamaño de los rasgos floral, el contenido de nutrientes y el volumen de néctar. Además, las estructuras florales estuvieron más correlacionadas e integradas en especies de *Cucurbita* domesticadas que en sus parientes silvestres.

Nuestros resultados sugieren que la domesticación de las calabazas resultó en flores más

grandes, correlaciones de rasgos florales más fuertes y una mayor integración floral; éste patrón fue más pronunciado en las flores pistiladas de las calabazas domesticadas. Mientras que *C. sororia* es el progenitor silvestre de *C. argyrosperma*, los atributos morfológicos de ambas especies domesticadas *C. argyrosperma* y *C. moschata*, fueron en general mayores que en las calabazas silvestres. La fuerza selectiva experimentada por las *Cucurbita* domesticadas agranda frutos, semillas, e indirectamente las partes florales.

Al comparar los rasgos florales del ancestro silvestre *C. sororia*, y su pariente domesticado *C. argyrosperma*, se observaron algunos atributos conservados, como la longitud de la corola, y la dimensión de las estructuras reproductivas; sin embargo, todos estos rasgos florales fueron mayores para *C. moschata* (Tabla 1a-b). Un paso importante en la domesticación de plantas es el aislamiento reproductivo de las poblaciones silvestres (Meyer et al., 2012). De manera que se podría predecir que la longitud del pistilo actúa como una barrera reproductiva (Tiffin et al., 2001) que limita los cruces de *C. argyrosperma* y *C. sororia* como donantes de polen en *C. moschata*. En cruces experimentales entre especies de calabazas silvestres y domesticadas, Merrick (1990) encontró cruces simétricos entre *C. argyrosperma* y *C. sororia* independientemente de qué especie actuará como donador o receptor de polen; estos cruces intraespecíficos produjeron descendencia viable. Sin embargo, solo fueron posibles cruces experimentales asimétricos entre *C. moschata* y las otras dos especies, porque solo se produjo descendencia viable cuando se utilizó *C. moschata* como donador de polen para *C. sororia* y *C. argyrosperma*, pero no se produjeron frutos en *C. moschata* cuando *C. argyrosperma* o *C. sororia* eran los donadores de polen (Merrick, 1990; Lira, 1995). Posiblemente la longitud del pistilo, entre más factores, contribuye a que ocurra más hibridación en las poblaciones silvestres de calabazas.

La domesticación tiende a la homogeneización fenotípica facilitando prácticas de manejo y rasgos agrícolas deseables para el consumo humano (Berg, 1960; Doebley et al., 2006). La domesticación comienza seleccionando un subconjunto de rasgos de una población silvestre que se utiliza como línea parental, y luego se cruzan entre sí durante varias generaciones para desarrollar accesiones endogámicas con rasgos deseables (van Raamsdonk, 1993; Pickersgill,

2007; Hernández-Terán et al., 2017; Milla et al., 2018). Propusimos que las especies de *Cucurbita* spp. domesticadas tendrían menos variación en sus rasgos morfológicos florales, porque las accesiones endogámicas resultantes deberían ser genéticamente más homocigotas que las líneas parentales originales (Doebley et al., 2006; Purugganan y Fuller, 2009; Hernández-Terán et al., 2017). Esta hipótesis fue parcialmente apoyada por nuestros resultados, la calabaza domesticada más cultivada, *C. moschata*, presentó los coeficientes de variación (CV) más bajos para la mayoría de los rasgos morfológicos de flores tanto estaminadas como pistiladas. Sin embargo, los rasgos florales de las flores pistiladas de la otra especie domesticada *C. argyrosperma*, tuvo los valores de CV más altos. Especulamos que esta condición podría ser consecuencia de dos factores, el intercambio genético entre variedades locales de *C. argyrosperma* y poblaciones silvestres adyacentes de *C. sororia*, resultando en una alta diversidad genética, y / o el mantenimiento de variedad genética en las prácticas agrícolas tradicionales (Montes-Hernández y Eguiarte, 2002; Balvino-Olvera et al., 2017 ; Sánchez-de la Vega et al., 2018).

Aunque la evaluación del tamaño de los rasgos individuales y los valores de CV ilustra las diferencias de variación de fenotipo entre las especies, un enfoque multivariado proporciona una evaluación más sólida de las relaciones entre los rasgos florales y la domesticación en las tres especies de estudio (Berg, 1960; Armbruster, 1991; Armbruster et al., 1999; Herrera et al., 2002). A través del análisis multivariado encontramos diferencias tanto en las flores estaminadas y pistiladas para las tres especies de calabazas, en particular, *C. argyrosperma* ocupó un espacio fenotípico intermedio entre el ancestro silvestre, *C. sororia*, y la calabaza domesticada más ampliamente cultivada, *C. moschata*. Esto sugiere que la domesticación de las dos especies de *Cucurbita* del grupo *Argyrosperma* ha provocado cambios graduales y un incremento del tamaño de los rasgos florales. En el caso de las redes de correlación de atributos florales de flores estaminadas; se presentan correlaciones de menor magnitud a mayor magnitud desde las especies silvestres a las especies domesticadas. Por otra parte, las redes de correlación de las flores pistiladas muestran un patrón diferente al de las flores estaminadas; *C. argyrosperma* por ejemplo, mostró una red de rasgos florales con correlación total entre todos los rasgos, mientras que *C. sororia* presentó una magnitud más fuerte de rasgos de la corola

correlacionados, y *C. moschata* el más débil del correlaciones de rasgos de la corola.

Nuestro estudio es el primero en comparar la integración floral entre flores unisexuales de especies monoicas. Para todas las especies de *Cucurbita* analizadas, las flores pistiladas tuvieron una mayor integración que las estaminadas, lo que sugiere que las flores pistiladas proporcionan menos señales variables para atraer a los polinizadores, para maximizar el depósito de polen en los estigmas. Los módulos de atracción / restricción (atributos de la corola) para todas las especies de *Cucurbita* en flores estaminadas y pistiladas tienen índices de integración más altos en el módulo reproductivo (atributos del androceo y gineceo). La fusión de las partes florales, en el caso de las calabazas, los pétalos, da como resultado una mayor integración del módulo floral en términos de atracción / restricción. Al comparar los índices de integración entre las especies de *Cucurbita*, encontramos que las flores pistiladas de las calabazas domesticadas están más integradas que las de las especies silvestres. Curiosamente, las flores pistiladas de *C. argyrosperma* tuvieron los valores más altos para la integración total de flores y los valores más altos de integración para los módulos de atracción / restricción y reproducción. La selección artificial de *C. argyrosperma* se dirigió principalmente a la producción de semillas comestibles grandes (Jones, 1992; Lira et al., 2016; Barrera-Redondo et al., 2020) a diferencia de *C. moschata*, que se cultiva principalmente por la pulpa de su fruto (Lira et al., 2016). Es posible que para *C. argyrosperma* la selección de tasas más altas de fertilización de óvulos para obtener un número elevado de semillas fertilizadas por fruto, haya dado como resultado una estructura floral más integrada. Según lo propuesto por Meyer y Purugganan (2013), la selección artificial dirigida principalmente a frutos de plantas debería resultar en un mejor éxito de la polinización, que creemos podría atribuirse a una mayor magnitud de integración floral en especies de calabazas domesticadas. Sugerimos para estudios futuros se podrían comparar otros cultivos de frutas dependientes de la polinización animal, y así comprobar si este fenómeno es común para otras especies de plantas domesticadas y sus ancestros silvestres existentes.

Las señales visuales como la longitud y el diámetro de la corola pueden actuar como una señal para los visitantes florales de la cantidad y calidad de los recursos de néctar y polen (Elle y

Carney, 2003; Galetto y Bernadello, 2004; Armbruster et al., 2005; Theis et al., 2014). Observamos que las calabazas domesticadas tienen atributos florales de mayor tamaño, acumulan más néctar y producen más polen que las especies de *Cucurbita* silvestres; aunque la concentración de azúcares no difiere entre especies, es posible que su composición en cuanto a porcentajes de sacarosa y hexosa, puedan ser diferentes según el grado de domesticación. Se ha observado para otras especies de *Cucurbita* tienen niveles más altos de sacarosa que de hexosas en el néctar, patrón más pronunciado en flores pistiladas, y correlacionado con niveles más altos de atracción de abejas (Nepi y Pacini, 2001; Ashworth y Galetto, 2002; Abrahamczyk et al., 2018; Chatt et al., 2018). Theis et al. (2014) encontraron una correlación positiva entre la longitud de la corola, los sesquiterpenoides y los volúmenes de néctar de las calabazas silvestres y domesticadas; factores que también fueron los mejores predictores de la atracción de especies especializadas en polen de *Cucurbita*, *Eucera pruinosa*. Por lo tanto, la cantidad y quizás la calidad de las recompensas florales correlacionadas con rasgos florales más grandes podrían haber influido positivamente en una mayor intensidad de visitas a las flores de las calabazas domesticadas.

La selección para un mayor éxito reproductivo en las calabazas domesticadas pudo haber afectado positivamente la calidad del polen. Las plantas que requieren polinización animal experimentan dos fuerzas de selección principales que determinan la calidad de la línea germinal o el valor nutricional del polen; la primera es la producción de microgametofitos vigorosos para la fertilización exitosa de los óvulos, y la segunda, está enfocada en proporcionar alimento a los polinizadores cuando el polen se considera una recompensa (Quesada et al. 1991; Quesada y Stephenson 1993; Roulston et al., 2000; Ruedenauer et al., 2019). Desde la perspectiva de la planta, la longitud del estilo afecta directamente el rendimiento del polen, por lo tanto, la cantidad de macronutrientes del polen que resulta en un aumento de las concentraciones de proteína y P: L proporcionará fuentes de energía más altas para un mejor crecimiento del tubo polínico para una fertilización exitosa de los óvulos (Roulston y col., 2000). Por otra parte, desde la perspectiva de los visitantes florales, los macronutrientes del polen, principalmente las concentraciones de proteínas y las proporciones de proteínas a lípidos (P: L), afectan directamente las preferencias de alimentación y la calidad nutricional de las abejas

polinizadoras (Roulston et al., 2000; Vanderplanck et al., 2014; Vaudo et al., 2016; Treanore et al., 2019; Vaudo et al., 2020). En apoyo de nuestra hipótesis, las calabazas domesticadas, *C. moschata* (4.72: 1) y *C. argyrosperma* (4.02: 1) presentaron la relación P: L más alta, seguidas de la calabaza silvestre, *C. sororia* (2.92: 1), cuyas concentraciones de proteínas más bajas fueron compensadas por niveles de lípidos más altos que en ambas especies domesticadas. Una explicación alternativa para el mayor contenido de proteína en el polen podría ser que la selección artificial también pudo haber actuado directamente sobre las flores estaminadas de las especies domesticadas. Desde la época prehispánica, en la región de México, las flores de calabaza (estaminadas) son las flores comestibles más consumidas, y presentan mayor contenido de proteínas en sus tejidos en relación con las otras flores consumidas (Sotelo et al., 2007). Se ha demostrado que los tejidos florales como la corola y sus respectivas recompensas comparten la composición química debido a los efectos pleiotrópicos genéticos (Palmer-Young et al., 2019). La selección potencial de flores con mayores propiedades nutritivas podría haber resultado en un mayor contenido de proteínas en el polen para consumo humano.

La domesticación de las calabazas ha aumentado la atracción de polinizadores especialistas al polen de *Cucurbita*, *Eucera* spp. así como otras especies de abejas. Las abejas *Eucera* spp. son oligolécticas para las plantas de calabaza, lo que significa que las abejas hembra usan polen de *Cucurbita* para alimentar sus crías (Hurd et al. 1971, Delgado-Carrillo et al. 2017, Brochu et al. 2020). La proteína del polen, la proporción P/L y la cantidad fueron más altas en las flores estaminadas de *C. moschata*. Aunque nuestro estudio no aisló los efectos de la cantidad frente a la calidad, parece que la recompensa del polen en las flores estaminadas de *C. moschata* influye en una mayor intensidad de visitas de *Eucera* spp. en comparación con las calabazas silvestres. Al igual que otros estudios, las abejas eusociales de la familia Apidae fueron las menos propensas a recolectar polen de calabazas en contraste con otros visitantes florales (Nepi y Pacini, 2001; Brochu et al., 2020); además con menos probabilidades de recolección en especies de *Cucurbita* silvestres *C. sororia* (*Apis mellifera* solo se observó recolectando polen de las calabazas domesticadas). El polen de calabazas silvestres puede ser menos atractivo para estas especies de abejas debido a una menor concentración de proteínas (Treanore et al., 2019; Vaudo et al., 2020), o quizás el polen de plantas de cucurbitáceas

silvestres tiene más defensas que las que se encuentran en las especies domésticas (Brochu et al., 2020).

Los estudios del sistema planta-polinizador en *Cucurbita* que se han realizado dentro de su distribución natural, encuentran que la comunidad de visitantes florales está dominada por *Eucera* spp. (Hurd et al., 1971; Tepedino, 1981; Cane et al., 2011; Parra-Tabla et al., 2017; Delgado-Carrillo et al., 2018; Lobo y Bravo, 2021; Contreras-Varela 2021). Nuestros resultados corroboran este fenómeno y sugieren que existe una comunidad de visitantes florales que probablemente juegan un papel importante como polinizadores secundarios para las especies de cucurbitáceas domésticas. En este estudio no se realizó un experimento de eficiencia de polinizadores, pero observamos el comportamiento de los visitantes florales que usamos como una aproximación de la importancia de los polinizadores; analizamos la tasa de visitas, contacto con partes reproductoras (Santiago-Hernández et al., 2019) y granos de polen transportados en el cuerpo. Al resumir estas métricas en dos indicadores, el índice de visitantes y contactos (VCI) y el índice de importancia de polinizadores (IPI), pudimos confirmar que las hembras de *Eucera* spp. son los polinizadores más importantes en las plantas de calabazas. También proponemos que las abejas de la tribu *Augochlorini* y *Apis mellifera* probablemente contribuyen de manera secundaria a la polinización de especies de calabazas domesticadas. Se sugiere para estudios futuros realizar experimentos de exclusión de polinizadores para probar las diferencias de efectividad de polinización en especies de *Cucurbita* silvestres y domesticadas.

Nuestras observaciones indican que la domesticación de especies de calabazas se puede asociar con el alargamiento de la antesis floral, lo que genera más oportunidades de visita de una comunidad de visitantes florales más amplia. Sin embargo, otros estudios señalan que las especies de calabazas silvestres y domesticadas tienen tiempos de antesis florales similares (Montes-Hernández y Eguiarte, 2002), Nosotros observamos que las flores estaminadas de las calabazas domesticadas, *C. moschata* y *C. argyrosperma*, tuvieron una ventana de actividad de visitantes florales más extendida que las calabazas silvestres. Además, *C. moschata* presentó la ventana de actividad de visitantes florales más larga, por lo cual deducimos que se aumenta la probabilidad de visitas a una comunidad de abejas que actúan como polinizadores secundarios.

Observamos que la flor pistilada de *C. sororia* abre más tarde en la mañana, y no recibe visitas de la especie de abeja crepuscular *Megalopta tetewana*, la cual visitó flores pistiladas de calabazas domesticadas en intensidades altas. Por su parte, *A. mellifera* presentó mayor visitación a última hora de la mañana para *C. moschata* (Delgado-Carrillo et al., 2018; Contreras-Varela 2021; Lobo 2021), justo cuando las flores de calabazas silvestres comenzaban a cerrarse (observación personal). De acuerdo con estudios previos (Hurd y Linsley, 1964; Hurd et al., 1971; Nepi y Pacini, 1993; Montes-Hernández y Eguiarte, 2002), la antesis floral de las calabazas está estrechamente sincronizada con los tiempos de vuelo diarios de la hembra especialista *Eucera* spp. Como otras especies de abejas solitarias, la hembra *Eucera* spp. comienza los viajes de aprovisionamiento con la recolección de polen, en este caso de 0600 a 0800 h, y luego continúa con la recolección de néctar, disminuyendo la mayor parte de su actividad después de las 0900 h (Hurd et al. 1971). Proponemos que la antesis más corta de las flores de calabazas silvestres podría ser el factor limitante de las visitas, y hasta impedir el robo de polen de especies de abejas no especializadas, tales como, las abejas crepusculares *M. tetewana*.

Si bien las flores de calabazas domesticadas fueron más frecuentadas por los visitantes florales, las especies de calabaza silvestre cumplen una función importante en el mantenimiento de sus poblaciones. Proponemos que las poblaciones de calabazas silvestres probablemente sustenten a las poblaciones de *Eucera* spp., el cuál fue visitante dominante en general para *Cucurbita*; de esta manera esta abeja especialista de calabazas puede obtener recursos seguros y constantes. Es importante conservar las poblaciones de calabazas silvestres para asegurar los servicios de polinización de las calabazas domesticadas en los campos agrícolas vecinos. Además, la conservación de los parientes silvestres de plantas cultivadas son un importante reservorio genético para las especies de plantas domésticas (Vaughan et al., 2007) y mantienen interacciones mutualistas que benefician a las plantas cultivadas.

CONCLUSIÓN GENERAL

Analizar la evolución de plantas domesticadas y sus interacciones bióticas es esencial para comprender los efectos ecológicos y evolutivos de las prácticas agrícolas (Milla et al., 2017). Es necesario realizar más estudios como estos en su centro de origen de las especies de plantas

domesticadas y silvestres (Chen et al., 2017). Al comparar tres especies filogenéticamente relacionadas, dos de las cuales son domesticadas, encontramos un aumento en el tamaño de los rasgos florales, patrones de correlación de rasgos florales distintos y un índice de integración floral más alto de las plantas domesticadas. La selección artificial para aumentar el tamaño de la fruta y / o una mayor inversión en el éxito reproductivo ha dado como resultado flores más grandes y mayores cantidades de néctar y polen que influyen en las tasas de visita más altas de los visitantes florales. Es necesario priorizar la conservación de las poblaciones de plantas ancestrales silvestres para preservar las interacciones mutualistas clave.

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