UNIVERSIDAD NACIONAL AUTÓNOMA DE MÉXICO

POSGRADO EN CIENCIAS BIOLÓGICAS

INSTITUTO DE ECOLOGÍA

BIOLOGÍA EVOLUTIVA

Diversidad genética y adaptación local de

Aphelandra aurantiaca en la selva fragmentada de Los Tuxtlas

TESIS

QUE PARA OPTAR POR EL GRADO DE:

DOCTORA EN CIENCIAS

PRESENTA:

María del Pilar Suárez Montes

TUTOR PRINCIPAL DE TESIS: Dr. Juan Núñez Farfán (Instituto de Ecología, UNAM)

COMITÉ TUTOR: Dr. César Domínguez Pérez-Tejada (Instituto de Ecología, UNAM)

Dr. Victor Parra Tabla (Universidad Autónoma de Yucatán, UADY)

Ciudad de México Agosto 2017

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Directora General de Administración Escolar, UNAM
P r e s e n t e

Me permito informar a usted, que el Subcomité de Biología Evolutiva y Sistemática, en su sesión ordinaria del día 24 de abril de 2017, aprobó el siguiente jurado para el examen de grado de **DOCTORA EN CIENCIAS** de la alumna **SUÁREZ MONTES MARÍA DEL PILAR** con número de cuenta **402054623** con la tesis titulada: **"DIVERSIDAD GENÉTICA Y ADAPTACIÓN LOCAL DE *Aphelandra aurantiaca* EN LA SELVA FRAGMENTADA DE LOS TUXTLAS",** bajo la dirección del **DR. JUAN SERVANDO NÚÑEZ FARFÁN:**

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

A T E N T A M E N T E
"POR MI RAZA HABLARA EL ESPIRITU"
Cd. Universitaria, Cd. Mx., a 27 de junio de 2017

DRA. MARÍA DEL CORO ARIZMENDI ARRIAGA
COORDINADORA DEL PROGRAMA

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Agradecimientos

Al Posgrado en Ciencias Biológicas de la Universidad Nacional Autónoma de México (UNAM).

Al Consejo Nacional de Ciencia y Tecnología por la beca otorgada y al financiamiento del Proyecto de Investigación Papiit IN215111-3.para realizar esta tesis.

Al Dr. Juan Núñez Farfán por su apoyo y paciencia en el desarrollo de esta tesis. Gracias por guiarme en el estudio de la selva de Los Tuxtlas y en mi desarrollo profesional.

A los miembros del comité tutoral, Dr. Juan Núñez Farfán, Dr. César Domínguez Pérez-Tejada y Dr. Victor Parra Tabla por su valiosa orientación y disposición para desarrollar y mejorar este trabajo.

Agradecimientos personales

A los miembros del Jurado, Dr. Luis Eguiarte Fruns, Dr. Antonio González Rodríguez y Dr. Mauricio Quesada por sus valiosas sugerencias y comentarios para mejorar esta tesis y los manuscritos.

A la Dra. Rosamond Coates y los integrantes de la Estación de Biología de Los Tuxtlas por las facilidades para realizar la colecta y los experimentos en campo.

A la Dra. Rosalinda Tapia López por su ayuda en el desarrollo de los marcadores moleculares y por el apoyo logístico para realizar esta tesis.

A todos los integrantes del laboratorio de Genética Ecológica y Evolución por su valiosa y vital ayuda para realizar este trabajo.

A todos mis colegas y amigos del laboratorio, por su grata amistad y alegrías compartidas, Mariana, Lupita, Eunice, Vania, Erika Rosalba, Memo, Rosalinda, Laura Lorena, Iván Dario, Edson, Jorge, MariSol, Laura Judith, Iván Mijail, Lulu, Maried, Diana, Sabine, Adán y Citlalli. Agradezco a Adriana y Rafael por su ayuda en las tareas de campo y laboratorio. A Luis Ángel Ordoñez por su importante ayuda en el experimento, la toma y captura de datos.

A mi papá por su ejemplo y cariño.

A mi familia por su cariño siempre.

A mis amigos por su amistad.

Índice

Resumen

La fragmentación del hábitat es una de las mayores amenazas para la biodiversidad, ya que en muchas especies puede causar una reducción en el tamaño de las poblaciones y un aumento en el aislamiento entre ellas. Las poblaciones fragmentadas que son muy pequeñas y están aisladas son más susceptibles a la extinción local debido a la pérdida de variación genética ocasionada por procesos de endogamia, deriva génica y reducción del flujo génico. Hasta ahora, se han estudiado ampliamente los efectos de la fragmentación en la variación genética neutral. Sin embargo, aún son escasos los estudios sobre la variación genética cuantitativa y la adaptación de las especies en hábitat fragmentados. En este trabajo evaluamos los efectos de la fragmentación de la selva de Los Tuxtlas en la variación genética neutral y cuantitativa de *Aphelandra aurantiaca*. Utilizamos loci de microsatélites para comparar la variación genética neutral, la diferenciación poblacional, el sistema de apareamiento y la tasa de polinización cruzada en fragmentos de selva de diferentes tamaños. Comparamos diferentes escenarios del pasado demográfico para evaluar la estructura genética actual. A través de un experimento de trasplantes recíprocos evaluamos los efectos de la fragmentación en la variación genética cuantitativa y en la posible adaptación local. Trasplantamos familias de hermanos completos en el hábitat continuo y fragmentado. Monitoreamos la supervivencia, las medidas de vigor (área foliar, número de hojas, altura), el daño causado por herbívoros y las condiciones ambientales. Los resultados indican que a pesar del alto grado de fragmentación, *A. aurantiaca* posee alta diversidad genética neutral y baja diferenciación poblacional. Los tamaños efectivos poblacionales son relativamente grandes pero tienden a disminuir en fragmentos pequeños. Detectamos eventos de dispersión a distancia; la mayor parte de los migrantes derivan del fragmento grande y mediano, resaltando su importancia como reservorios genéticos. *A. aurantiaca* presenta un sistema de apareamiento mixto y no hay evidencia de un efecto negativo de la fragmentación en la tasa de entrecruzamiento. Dentro de las poblaciones, las nubes de polen están estructuradas y el movimiento de polen es limitado, indicando que la mayor parte del polen proviene de individuos cercanos. Las fluctuaciones en el pasado demográfico han afectado la estructura genética actual; detectamos un evento de expansión poblacional. Los efectos negativos de la fragmentación en la variación genética neutral parecen ser amortiguados por los tamaños efectivos grandes, eventos de dispersión a distancia y el sistema de apareamiento mixto. Los resultados del experimento de trasplantes recíprocos sugieren diferenciación en la variación genética cuantitativa debido a las diferencias ambientales entre el hábitat continuo y fragmentado. En general, las condiciones ambientales de los

fragmentos favorecen la supervivencia y el crecimiento de *A. aurantiaca*. Sin embargo, las plántulas locales en el hábitat continuo tienen mayor área foliar que las foráneas, sugiriendo un mejor desempeño y posible adaptación local a las condiciones lumínicas limitantes del sotobosque de la selva continua. Sugerimos que los tamaños efectivos grandes de la selva continua mantienen la variación en caracteres relacionados con la adecuación y con el potencial de adaptación local. El potencial de *A. aurantiaca* para responder ante los diferentes ambientes también puede estar relacionado con su historia de vida y el ciclo de regeneración de la selva.

Abstract

Habitat fragmentation is one of the biggest threats to biodiversity, since in many species it may reduces the size and increases the isolation of plant populations. Small isolated fragmented populations are more prone to extinction due to the loss of genetic variation through genetic drift, increased selfing, and reduced gene flow. The effects of fragmentation on the neutral genetic variation have been extensively studied; however, studies examining the effects of habitat fragmentation on quantitative genetic variation and adaptation to fragmented habitats are still scarce. Here, we examine the effects of Los Tuxtlas rainforest fragmentation on the neutral and quantitative genetic diversity of *Aphelandra aurantiaca*. We used microsatellite loci to compare neutral genetic variation, population differentiation, mating system and outcrossing rate of this species in forest fragments with different area. We compared different past demographic scenarios to assess its present-day genetic structure. We performed a reciprocal transplant experiment to assess fragmentation effects on quantitative genetic variation and potential local adaptation. Full-sib families of each of four populations were grown at the continuous and fragmented habitat, and were monitored through time to assess plant survival, vigour-related variables (plant height, number of leaves and leaf area), damage inflicted by herbivores and environmental variables. Results showed that despite prevailing habitat fragmentation, *A. aurantiaca* populations possess high neutral genetic variation and a shallow genetic structure. Effective population sizes were large, but with a trend to decrease in smaller fragments. Long distance gene dispersal events were detected; most migrants derive from large- and medium-sized fragments, highlighting their importance as genetic reservoirs. *A. aurantiaca* exhibits a mixed mating system and outcrossing rates have not been affected by habitat fragmentation. Within populations, a strong pollen pool structure was detected and low distance pollen movement, pointing that most plants received pollen from close neighbors. Past demographic fluctuations may have affected the present population genetic structure, revealing the signature of past population expansion. The predicted negative effects of habitat fragmentation on neutral genetic diversity and structure seem to have been buffered owing to its large effective populations, long distance dispersal events, and life history traits and mixed mating system. Results of reciprocal transplant experiment suggest quantitative genetic differentiation due to environment differences between continuous and fragmented habitat. In general, environmental conditions within fragmented habitat are favorable for *A. aurantiaca* survival and growth. However, local seedlings in the continuous forest exhibited higher leaf area than foreign plants from fragmented habitat,

suggesting a better performance and likely local adaptation to the limited light conditions of the continuous forest understory. We suggest that large effective population sizes within the continuous forest maintain variation in traits related to fitness and with the potential of local adaptation. The potential of *A. aurantiaca* to respond to different environments could be related with their life history and in relation to the forest regeneration cycle.

Introducción general

Efectos de la fragmentación del hábitat

La selva tropical es el ecosistema más diverso sobre la Tierra, albergando más de la mitad de las especies identificadas, entre ellas el mayor número de plantas endémicas (Myers 1988; Lewis 2009; Seymour *et al.* 2014). Actualmente, gran parte de esta diversidad está en riesgo debido a que más del 50% de la selva original ha sido severamente degradada (FAO 2014; Seymour *et al.* 2014). Para preservar la biodiversidad es crítico entender los factores que la amenazan, así como sus causas y consecuencias (Haddad *et al.* 2015; Wilson *et al.* 2016). Una de las principales amenazas para la diversidad es la fragmentación del hábitat, un proceso que ocurre cuando un hábitat continuo es reducido en pequeños fragmentos aislados de vegetación. Se caracteriza por disminuir los tamaños poblacionales de las especies e incrementar el aislamiento entre ellas; sus efectos pueden modificar factores estrechamente relacionados entre sí, como las condiciones abióticas, los procesos ecológicos y genéticos de las especies (Young, Boyle & Brown 1996; Smulders *et al.* 2009).

La reducción del hábitat continuo a fragmentos aislados puede modificar el ambiente local, alterar negativamente las tasas vitales, la viabilidad y reproducción, disminuyendo el tamaño poblacional. Además, la falta de conectividad entre poblaciones puede modificar las interacciones bióticas, y en conjunto generar nuevas presiones de selección para especies propias de hábitats continuos (Laurance *et al.* 2002; Tscharntke & Brandl 2004; Matesanz, Gianoli & Valladares 2010). En específico, las condiciones abióticas de las selvas tropicales no perturbadas se caracterizan por los altos niveles de humedad, la baja incidencia de luz y viento, temperaturas estables y cobertura del dosel casi continua (Laurance 2004). Por el contrario, en los fragmentos de selva las condiciones ambientales cambian radicalmente, se reduce la humedad, aumenta la incidencia de luz, de vientos cálidos y secos, así como el incremento en las fluctuaciones de temperatura (Laurance 2004; Laurance *et al.* 2011; Ewers & Banks-Leite 2013). Las interacciones bióticas en los fragmentos también pueden ser alteradas, afectando el éxito reproductivo y la supervivencia de las plantas. Por ejemplo, la disminución en la abundancia de polinizadores y el decremento en la densidad de plantas pueden modificar los patrones de apareamiento, la polinización, la tasa de polinización cruzada y el éxito reproductivo de las plantas (Aguilar *et al.* 2006; Breed *et al.* 2012, 2013; Chávez-Pesqueira *et al.* 2015; Dáttilo *et al.* 2015; García-Guzmán, Trejo & Sánchez-Coronado

2016). Por lo tanto, para sobrevivir, las plantas que habitan en los fragmentos de selva, deberán responder ante las presiones selectivas de los cambios abióticos y bióticos.

La fragmentación del hábitat tiene efectos genéticos debido a la reducción del tamaño poblacional y al incremento en el aislamiento espacial entre poblaciones. Teóricamente estos cambios poblacionales pueden erosionar la variación genética e incrementar la diferenciación genética poblacional, debido a que se reduce el flujo génico y aumentan los efectos negativos de la deriva génica y la endogamia. Los efectos genéticos de la fragmentación tienen implicaciones importantes para la persistencia de especies; a corto plazo la pérdida de diversidad podría reducir la adecuación individual y la viabilidad de las poblaciones fragmentadas. A largo plazo, podría limitar la capacidad de las especies para responder a los cambios en las presiones de selección, incrementando el riesgo de extinción local de las especies (Young *et al.* 1996; Keller & Waller 2002; Fountain *et al.* 2016).

Respuesta de las plantas ante la fragmentación del hábitat

En general, los resultados de revisiones cuantitativas en plantas indican que la fragmentación del hábitat tiene efectos negativos en la diversidad genética y la tasa de polinización cruzada o entrecruzamiento (Leimu et al., 2006; Honnay & Jacquemyn, 2007a; Aguilar et al., 2008a; Eckert et al., 2010; Vranckx et al., 2011). Sin embargo, el tipo de respuesta de las especies ante las consecuencias genéticas de la fragmentación es diferencial y dependerá de los caracteres de historia de vida, longevidad, tiempo generacional, sistema de apareamiento, tipo de dispersión, así como de la estructura del paisaje en el que habiten (Leimu *et al.* 2006; Aguilar *et al.* 2008; Figueroa-Esquivel *et al.* 2010; Suárez-Montes, Fornoni & Núñez-Farfán 2011; Suárez-Montes, Chávez-Pesqueira & Núñez-Farfán 2016; Vranckx *et al.* 2012; Chávez-Pesqueira *et al.* 2014; Chung *et al.* 2014).

La respuesta de las especies ante los cambios ambientales es variable; algunas especies podrían extinguirse localmente, otras podrían escapar por medio de dispersión o bien enfrentar las nuevas condiciones a través de plasticidad fenotípica o vía cambios genéticos con adaptaciones locales (Saunders, Hobbs & Margules 1991; Leimu *et al.* 2010). Las especies pueden adaptarse a estos cambios a través de nuevas mutaciones o por medio de la selección de variación genética preexistente; para que se pueden adaptar, las poblaciones deben conservar variación genética

aditiva, esencial para una respuesta adaptativa a la selección (Lande & Shannon 1996). A diferencia de las nuevas mutaciones, se espera que la adaptación a partir de la variación genética preexistente sea más rápida, debido a la alta frecuencia de alelos benéficos disponibles que han sido mantenidos previamente por la selección (Barrett & Schluter 2008). Por ejemplo, en las selvas tropicales las especies adaptadas a altos niveles de luz y de disturbio (ej. pioneras en el proceso de regeneración) podrían ser favorecidas ante los cambios en el entorno físico ocasionados por la fragmentación del hábitat. Por el contrario, aquellas especies adaptadas a bajos niveles de luz podrían ser más susceptibles a los cambios ambientales de los fragmentos. Sin embargo, algunas especies tolerantes a la sombra también podrían responder positivamente a mayor disponibilidad de luz (Denslow 1987).

Adaptación local en hábitats fragmentados

Para entender el desempeño de las plantas en paisajes fragmentados, es necesario considerar de manera simultánea las respuestas adaptativas ante diferentes calidades de hábitat (Bowman *et al.* 2008). Las diferentes condiciones ambientales entre las poblaciones fragmentadas pueden ejercer presiones selectivas que maximicen la adecuación (*fitness*) individual y promuevan adaptación local, un proceso en el que los individuos locales exhiben mayor adecuación en su ambiente original en comparación con individuos de poblaciones introducidas. Por lo tanto, un prerrequisito para que exista adaptación local es la interacción genotipo × ambiente en la adecuación (Kawecki & Ebert 2004; Blanquart *et al.* 2013).

Los factores que promueven la adaptación local incluyen: la heterogeneidad ambiental espacial, bajo flujo génico (restricción de dispersión de polen y semillas o alta fidelidad al hábitat), selección intensa en contra de genotipos adaptados óptimamente a otros hábitats, selección moderada en contra de genotipos intermedios (recombinantes), baja variación temporal en las fuerzas de selección debido a que favorecen fenotipos generalistas, y costos o restricciones en la plasticidad adaptativa, ya que podría fijar un genotipo con fenotipos óptimos en todas las subpoblaciones (ver Kawecki & Ebert, 2004).

Los procesos de dispersión genética y adaptación local combinados con la estructura del paisaje, también pueden impactar la capacidad de respuesta ante cambios ambientales en los remanentes de hábitat. Sin embargo, el efecto general de la fragmentación del hábitat en la

adaptación local no es fácil de predecir, en parte debido al complicado balance entre los efectos positivos y negativos de la dispersión de genes. Por un lado, la adaptación local podría aumentar si la fragmentación del hábitat reduce el flujo génico entre los remanentes, pero también podría reducir o eliminar la adaptación local si los efectos de la deriva génica en los remanentes pequeños son más fuertes que la selección (Leimu *et al.* 2010). Además, la reducción de la variación genética podría limitar el potencial adaptativo de las especies para responder a los cambios ambientales (Becker *et al.* 2006; Leimu & Fischer 2008). Por otra parte, si la fragmentación del hábitat facilita la dispersión hacia poblaciones aisladas anteriormente, podría incrementar la tasa de alelos compartidos y reducir adaptaciones locales (Leimu & Fischer, 2008; Schiffers et al., 2013; Bourne et al., 2014). Se espera que especies con gran capacidad de dispersión atraviesen la matriz deforestada, aumenten su acceso a una mayor cantidad de hábitat y la dispersión de genes (Cheptou et al., 2016). En un escenario con hábitats heterogéneos, donde los individuos están adaptados localmente, la dispersión de genes puede tener consecuencias negativas en el fitness debido al incremento de mal-adaptaciones (Hereford, 2009; Schiffers et al., 2013; Bourne et al., 2014).

Las plantas son un excelente modelo para el estudio de la evolución adaptativa, ya que al ser sésiles deben enfrentar directamente las condiciones y cambios ambientales, siendo vital la adaptación a las condiciones locales. Proveen excelentes oportunidades para estudiar la variación en selección en escala temporal y espacial; su muestreo es relativamente sencillo a lo largo de gradientes ambientales y son relativamente fáciles de manipular para realizar experimentos de trasplantes recíprocos (Flood & Hancock 2017). Los transplantes recíprocos son una prueba experimental directa para medir la significancia adaptativa del cambio fenotípico. Tradicionalmente, la adaptación local se ha estudiado en plantas mediante el transplante de individuos entre ambientes, en los que se compara la adecuación de los genotipos locales contra genotipos foráneos bajo las mismas condiciones ambientales. Se considera que las plantas están localmente adaptadas cuando los individuos locales exhiben mayor adecuación que los individuos foráneos, o bien cuando las plantas tienen mayor adaptación en su propio hábitat que en otros hábitats (Kawecki & Ebert 2004; Blanquart *et al.* 2013). Los experimentos de transplantes recíprocos en poblaciones naturales, también permiten medir de manera realista los efectos genéticos y ambientales en el desempeño de las plantas. Además, en estos experimentos se consideran efectos bióticos que pueden ser importantes para el desarrollo de adaptaciones locales, como la competencia o la herbivoría (Bowman *et al.* 2008).

Los estudios que exploran los efectos de la fragmentación del hábitat en la variación genética adaptativa son escasos (Willi et al., 2007; Eckert et al., 2010; Jacquemyn et al., 2012; Willi adaptación & Hoffmann, 2012; Bennington et al., 2012; Fraser et al., 2014; Ye et al., 2014; Chávez-Pesqueira & Núñez-Farfán, 2016; Cheptou et al., 2016), y en particular existen muy pocos estudios que evalúen la adaptación local (Hooftman, van Kleunen & Diemer 2003; Bowman *et al.* 2008; Lopez *et al.* 2009; Pickup *et al.* 2012). La mayor parte compara la diferenciación de caracteres cuantitativos (Q_{st}) con los marcadores genéticos neutrales (F_{st}) para obtener información de la importancia relativa de la selección, la deriva génica y los patrones de adaptación (Johansson, Primmer & Merilä 2007; Willi *et al.* 2007; Ellmer, Prentice & Andersson 2011; Dubois & Cheptou 2016). Algunos estudios han evaluado la variación neutral y adaptativa en "jardines comunes" (Johansson, Primmer & Merilä, 2007; González-Varo, Nora & Aparicio, 2012; Pickup et al., 2012; Zhao et al., 2013), pero pocos han evaluado el desempeño de las especies y las diferencias en la calidad del hábitat, debidas a la fragmentación del hábitat en poblaciones naturales (Hooftman, van Kleunen & Diemer, 2003; Bowman et al., 2008; Pickup et al., 2012) y a través de generaciones (Willi & Hoffmann 2012). Además, la mayor parte de los experimentos se han desarrollado en comunidades de plantas templadas, generalmente anuales y raramente en las selvas tropicales. Esto se debe principalmente a la inaccesibilidad de las selvas y a la longevidad de las plantas (Chen & Schemske 2015).

La falta de estudios sobre la variación genética adaptativa en paisajes fragmentados, puede deberse a la ausencia de un marco teórico y conceptual para predecir los efectos de la fragmentación en la composición adaptativa de las poblaciones, y las consecuencias que tiene sobre la respuesta de las especies a los cambios ambientales (Fraser *et al.* 2014). Recientemente, se han propuesto dos hipótesis principales (Willi & Hoffmann 2012): la “hipótesis direccional” sugiere que las características del hábitat cambian de manera consistente durante la fragmentación del hábitat, resultando en una relación direccional entre estas características, el tamaño poblacional, la extensión de la variación genética adaptativa y la diferenciación. Bajo esta hipótesis, las poblaciones más pequeñas tendrán menor variación adaptativa debido a los efectos combinados de la deriva génica, la endogamia y el limitado flujo génico (Fraser *et al.* 2014). Por otra parte, la “hipótesis variable” propone que las características del hábitat y las presiones de selección resultantes, serán altamente variables conforme disminuye el tamaño poblacional del fragmento (Willi *et al.* 2007; Willi & Hoffmann 2012; Fraser *et al.* 2014). Dado que los efectos evolutivos de la fragmentación dependen de las condiciones iniciales, algunos de los fragmentos

pequeños representarán la heterogeneidad del hábitat de las poblaciones grandes, mientras que otros serán más homogéneos, por lo que la variación adaptativa será variable.

Hasta ahora la evidencia sugiere que la fragmentación genera tanto cambios direccionales como cambios variables en las presiones selectivas, conforme se reducen los tamaños poblacionales. Esto sugiere que las respuestas a los cambios ambientales y la probabilidad de persistencia se vuelven más variables conforme la fragmentación y el tamaño poblacional se reduce (Fraser et al., 2014). Sin embargo, es necesario poner a prueba ambas hipótesis en múltiples escenarios.

Bases genéticas de la adaptación local

Las bases genéticas de la adaptación local pueden ser resultado de varios procesos que aún no se comprenden del todo. Pueden ser resultado de disyuntivas entre loci clave donde los alelos nativos tendrán mayor adecuación en relación a los alelos foráneos (pleiotropía antagonista). También puede deberse a múltiples loci independientes que interactúan para producir adaptación local; los alelos serán ventajosos en un ambiente pero pueden ser neutrales en el ambiente contrastante (neutralidad condicional) (Mitchell-Olds, Willis & Goldstein 2007; Hall, Lowry & Willis 2010; Anderson, Willis & Mitchell-Olds 2011). Ambas hipótesis no son mutuamente excluyentes, pueden ocurrir en la misma especie (Wadgymar *et al.* 2016), pero se espera que solo la pleiotropía antagonista conduzca a una fuerte selección balanceadora y mantenga la variación genética (Tiffin & Ross-Ibarra 2014). Asimismo, la inversión de cromosomas puede contribuir a la adaptación local y al aislamiento reproductivo; especialmente cuando las inversiones portan loci que despliegan pleiotropía antagonista (Lowry & Willis 2010; Anderson *et al.* 2011; Oneal *et al.* 2014; Lasne, Sgrò & Connallon 2017).

Los avances en genómica y en la tecnología de secuenciación de ADN están revolucionando nuestra comprensión de la variación genética en la naturaleza. Los estudios del genoma completo en organismos no modelo, junto con análisis de evolución adaptativa y experimentos en el campo, ayudan a diseñar estrategias para determinar las bases genéticas de la adaptación local (Anderson *et al.* 2011; Savolainen, Lascoux & Merilä 2013; O'Brien *et al.* 2017). Actualmente se ha incrementado el número de trabajos relacionados con las bases genéticas de la adaptación local (Savolainen *et al.* 2013; Tiffin & Ross-Ibarra 2014; Rellstab *et al.* 2017; Wellenreuther *et al.* 2017). Estos estudios han aplicado varias aproximaciones metodológicas,

como la búsqueda de "firmas de la selección" mediante pruebas de asociación, detección de outliers y genes candidatos (Pais, Whetten & Xiang 2016), en gradientes poblaciones (Fustier et al., 2017) o ambientes heterogéneos (Rellstab *et al.* 2017), junto con la validación funcional de los genes candidatos (Weigel & Nordborg 2005; Hoekstra *et al.* 2006). También, mediante réplicas de "firmas de la selección" en poblaciones independientes (Fournier-Level *et al.* 2011; Tiffin & Ross-Ibarra, 2014), o mediante la aplicación de loci de caracteres cuantitativos (QTL) con enfoques de mapeo en líneas de recombinación, a partir de poblaciones localmente adaptadas o divergentes (Agren *et al.* 2016; Ferris *et al.* 2016).

Además, se han desarrollado algunos modelos mediante simulaciones para comprender los procesos de adaptación local en paisajes heterogéneos (Forester *et al.* 2016) y la arquitectura genética de la adaptación en varios escenarios de migración (Yeaman & Whitlock 2011; Ferris *et al.* 2016; Tigano & Friesen 2016). Por último, se espera que en los próximos años aumente el interés en las bases genéticas de la adaptación local y que se utilicen ampliamente estas aproximaciones para comprenden la respuesta de las especies ante los cambios globales, en particular en paisajes fragmentados.

Objetivos y estructura de la tesis

El futuro de las especies que habitan la selva fragmentada aún no es del todo claro. Este trabajo aporta información sobre la respuesta de las plantas ante ambientes y paisajes alterados debido a la fragmentación del hábitat. Evaluamos los efectos de la fragmentación en la estructura genética y adaptación local de la herbácea *Aphelandra aurantiaca* en la selva de Los Tuxtlas, Veracruz, México. Integramos datos de variación genética neutral, cuantitativa y aspectos ecológicos poblacionales. Consideramos que este tipo de estudios ayudan a comprender los efectos de la fragmentación del hábitat sobre los procesos evolutivos, asimismo contribuyen con información relevante para promover la conservación de recursos genéticos y el manejo de selvas tropicales.

Los objetivos principales de este trabajo fueron dos. (a) Evaluar el efecto de la fragmentación de la selva en la variación y estructura genética neutral de *A. aurantiaca* en poblaciones fragmentadas de diferentes tamaños utilizando loci de microsatélites, así como evaluar la tasa de polinización cruzada (tasa de entrecruzamiento) y la dinámica del movimiento

de polen contemporáneo. (b) Evaluar el efecto de la fragmentación de la selva en la variación genética cuantitativa y la adaptación local de *A. aurantiaca* en dos hábitats contrastantes (continuo y fragmentado) mediante un experimento de transplantes recíprocos.

El Primer Capítulo de esta tesis trata sobre el desarrollo y estandarización de los marcadores neutrales utilizados (microsatélites) para la especie de estudio. El Segundo Capítulo se enfoca en la estructura genética de *A. aurantiaca* y los posibles efectos ocasionados por la fragmentación de la selva. El Tercer Capítulo aborda los efectos de la fragmentación del hábitat en la adaptación local de *A. aurantiaca*. En el Apéndice I se describen algunos aspectos del éxito reproductivo, depredación en inflorescencias, flores, frutos y semillas, así como en caracteres fenotípicos en poblaciones de la selva continua y fragmentada.

Sitio de estudio

La selva de Los Tuxtlas constituye el límite norte de la distribución de la selva tropical lluviosa en el continente americano (Dirzo & Miranda 1991). Ha sido severamente deforestada y fragmentada; el 90 % de su vegetación original ha desaparecido en los últimos 50 años (Dirzo & Garcia 1992; Guevara & Laborde 2012). Actualmente, el paisaje está compuesto por áreas de selva continua (zonas núcleo de la reserva de la biósfera) y fragmentos de ésta, en su mayoría pequeños (< 100 ha), rodeados por pastizales, vegetación secundaria, árboles aislados, cercas vivas y vegetación riparia (CONANP 2011; Salazar Arteaga 2015). A pesar de las implicaciones negativas para la conservación, el paisaje fragmentado de Los Tuxtlas nos permite realizar estudios ecológicos y evolutivos en poblaciones naturales. Asimismo, estos estudios pueden proporcionar información valiosa para proponer planes de manejo y conservación de la diversidad.

Especie de estudio

Aphelandra aurantiaca (Scheidw.) Lindl. (Acanthaceae) es una herbácea perenne que habita el sotobosque de la selva alta perennifolia. Se distribuye geográficamente desde el Sur de México hasta Bolivia. En México, es una de las especies del género con mayor distribución habitando

zonas con abundante lluvia como la selva (Daniel 1991). En Los Tuxtlas, las herbáceas son la forma de vida con mayor número de especies (30%) (Ibarra-Manríquez et al., 1997). *A. aurantiaca* es una de las hierbas dominante del sotobosque de Los Tuxtlas y crece en parches de regeneración de distintas edades, desde claros recién abiertos hasta sitios de bosque maduro (Calvo-Irabién, 1997 b). La floración de *A. aurantiaca* ocurre de septiembre a enero, es una especie auto-compatible, sus flores son hermafroditas protóginas y productoras de néctar. Las flores están dispuestas en una inflorescencia en forma de espiga, el color de sus flores cambia según su nivel de maduración, los botones florales son amarillos y las flores se tornan rojas al madurar. En los Tuxtlas, es polinizada por el colibrí *Phaethornis longirostris*, aunque también se han reportado visitas de mariposas y abejorros. El fruto es una cápsula ovoide con un número constante de cuatro semillas; la dispersión de las semillas es balística y es relativamente cercana a la planta progenitora (1.5 m) (Calvo-Irabién, 1989, 1997a,b).

A. aurantiaca es el objeto de estudio de esta tesis debido a que exhibe características importantes para evaluar los efectos de la fragmentación del hábitat en la adaptación local y en la estructura genética. En particular, el tiempo de vida de las plantas es un factor importante para detectar los efectos genéticos de la fragmentación. A diferencia de especies muy longevas, se espera que las especies de vida corta pierdan rápidamente diversidad genética debido a que están más expuestas a los efectos de la deriva génica(Ellstrand *et al.* 1993). En comparación con los árboles de la selva tropical (Lowe et al. 2005), la longevidad de *A. aurantiaca* es relativamente menor (< 20 años) (Calvo-Irabién, 1989), sugiriendo que algunas generaciones han pasado desde el inicio de la fragmentación en Los Tuxtlas. Esto permitiría evaluar la estructura genética posterior a la fragmentación y la posible pérdida de alelos. Sin embargo, también es importante considerar la rápida madurez reproductiva (un año, Calvo-Irabién, 1989) y el solape de las generaciones de *A. aurantiaca*.

Al ser una especie polinizada por animales, se esperaba que los efectos negativos de la fragmentación afectaran la dinámica de movimiento de polen, los patrones de apareamiento y disminuyeran la tasa de polinización cruzada. Debido a que habita uno de los estratos de la selva más vulnerable a los cambios micro-ambientales (Bruna 2002; Laurance 2004; Grimbacher, Catterall & Kitching 2006; Ewers & Banks-Leite 2013), se esperaba detectar cambios en los caracteres cuantitativos y en la respuesta adaptativa a la condiciones alteradas debidas a la

fragmentación del hábitat. Además, la limitada dispersión de semillas y el alto grado de aislamiento entre las poblaciones podrían promover la adaptación local.

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Capítulo I

Variación genética en microsatélites de la hierba tropical *Aphelandra aurantiaca*

Applications in Plant Sciences

Applications in Plant Sciences 2015 3(11): 1500058

Primer Note

Genetic variation at microsatellite loci in the tropical herb *Aphelandra aurantiaca* (Acanthaceae)[1]

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- *Premise of the study:* To assess the effect of forest fragmentation on genetic variation and population structure of *Aphelandra aurantiaca* (Acanthaceae), a tropical and ornamental herbaceous perennial plant, we developed the first microsatellite primers for the species.
- *Methods and Results:* Fourteen microsatellite markers were isolated and characterized from *A. aurantiaca* genomic libraries enriched for di-, tri-, and tetranucleotide repeat motifs. Polymorphism was evaluated in 107 individuals from four natural populations. Twelve out of 14 genetic markers were polymorphic. The number of alleles per locus ranged from two to 12, and the observed and expected heterozygosities ranged from 0.22 to 0.96 and from 0.20 to 0.87, respectively. Fixation indices ranged from −0.41 to 0.44.
- *Conclusions:* These newly developed microsatellite markers for *A. aurantiaca* will be useful for future population genetic studies, specifically to detect the possible loss of genetic diversity due to habitat fragmentation.

Key words: Acanthaceae; *Aphelandra aurantiaca*; gene flow; genetic structure; Los Tuxtlas tropical rainforest; outcrossing rate.

Aphelandra R. Br. is one of the largest genera of Acanthaceae, comprising ca. 175 species of perennial herbs, shrubs, and small trees restricted to the Neotropics (Wasshausen, 1975; Daniel, 1991). Species in this genus have colored flowering spikes (Wasshausen, 1975), and the genus is well known to horticulturists because some species are cultivated for ornamental purposes (Daniel, 1991). However, until now, no studies of molecular genetic diversity in this genus have been carried out. We focus on the understory herb *A. aurantiaca* (Scheidw.) Lindl., distributed from southern Mexico through Central and South America (Daniel, 1991). In Mexico, its distribution is restricted to regions with abundant rainfall such as Los Tuxtlas rainforest (Daniel, 1991), where it is one of the dominant understory species (Calvo-Irabién, 1997). The region of Los Tuxtlas, considered the northernmost limit of rainforests in the Americas, has been heavily impacted by deforestation and fragmentation (Dirzo and Miranda, 1991; Dirzo and García, 1992). Because fragmentation produces isolation between populations, it could impact their genetic structure (Chávez-Pesqueira et al., 2014), reducing genetic variation and gene flow, and increasing genetic divergence and inbreeding (Young et al., 1996). *Aphelandra aurantiaca* is a suitable model to study the genetic consequences of rainforest fragmentation due to the life history characteristics of the species. For example, it has a relatively short life span, which means that some generations have passed since the onset of fragmentation, and it depends on canopy cover, which is usually reduced in forest fragments. Furthermore, because *A. aurantiaca*'s attractive, nectar-producing flowers are pollinated by birds (Calvo-Irabién, 1997), its mating system can be affected by habitat fragmentation if this reduces species richness and abundance of pollinators (Aguilar et al., 2006). To date, little is known about its genetic structure, particularly in the context of rainforest fragmentation. Therefore, we aimed to develop variable genetic markers to elucidate the genetic diversity and structure of *A. aurantiaca*.

METHODS AND RESULTS

Using the DNeasy Plant Mini Kit (QIAGEN, Valencia, California, USA), we extracted genomic DNA from a single individual of *A. aurantiaca* for use in the isolation of microsatellite loci. A paired-end library was prepared by shearing 1 µg of genomic DNA following the standard protocol of the Illumina TruSeq DNA Library Kit (Illumina, San Diego, California, USA). Illumina sequencing was conducted on the HiSeq (Illumina) with 100-bp paired-end reads. Ten million of the resulting sequences were analyzed with the program PAL_FINDER_v0.02.03 (Castoe et al., 2012), extracting positive reads that contained di-, tri-, tetra-, penta-, and hexanucleotide microsatellites and sending to the program Primer3 (version 2.0.0; Rozen and Skaletsky, 1999) for primer design. To avoid duplicated loci, data were filtered and only primers that occurred one or two times were included; 24 loci out of 1722 that met this criterion were chosen. Primer pairs were tested for amplification and polymorphism using DNA obtained from five different individuals from the four Los Tuxtlas

[1]Manuscript received 13 May 2015; revision accepted 15 July 2015.

The authors thank the Los Tuxtlas Biological Research Station for logistics support; S. Lance and the University of Georgia Savannah River Ecology Laboratory for sequencing and developing primers; and L. Márquez-Valdemar, G. Andraca-Gómez, F. Baena-Díaz, and M. Chávez-Pesqueira for assistance in obtaining genetic data. The study was funded by a Universidad Nacional Autónoma de México (UNAM) Programa de Apoyo a Proyectos de Investigación e Innovación Tecnológica (PAPIIT) grant (IN 215111-3). This paper constitutes a partial fulfillment of the Graduate Program in Biological Sciences (UNAM) for P.S.-M., who acknowledges a scholarship and financial support by the Consejo Nacional de Ciencia y Tecnología (CONACyT) and UNAM.

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doi:10.3732/apps.1500058

populations sampled (Appendix 1), and amplified PCR products were then separated on 4% Metaphor agarose gels (Lonza, Rockland, Maine, USA). After excluding loci that did not amplify, we selected 14 potential polymorphic loci and marked these with fluorescent labels (Table 1). The PCR amplification was carried out in a 20-μL reaction containing 2 μL of 10× PCR buffer (KCl 500 mM, Tris-HCL pH 8.3, gelatin 100 μg/mL, 1% triton, bovine serum albumin [BSA] 1.5 mg/mL), 1 μL of $MgCl_2$ (30 mM), 2 μL of dNTPs (0.2 mM), 2 μL of DNA, 0.5 μL of each of the two primers (10 mM), 0.5 μL of *Taq* DNA polymerase (5 U/μL), and 12 μL of water (BIOTECMOL, Mexico City, Mexico), performed on a Thermo Scientific Hybaid Px2 thermal cycler (Thermo Scientific, Waltham, Massachusetts, USA) using the following conditions: 94°C for 10 min; followed by 35 cycles of 94°C for 1 min, at temperatures between 55–61°C for 1 min, and 72°C for 1 min; and a final extension step of 72°C for 7 min.

To encompass the most genetic diversity of *A. aurantiaca* in the Los Tuxtlas rainforest, we collected leaf tissue of 107 individuals from four populations (Appendix 1). Genomic DNA was extracted following the cetyltrimethylammonium bromide (CTAB) MiniPrep protocol (Doyle and Doyle, 1987). We selected a subset of loci to function well together in four multiplex reactions (QIAGEN Multiplex PCR Kit) with labeled primers (Applied Biosystems, Foster City, California, USA) (Table 1). Each multiplex PCR mixture (10 μL) contained 2 μL of DNA template (20 ng), 0.2 μL of each fluorescent-labeled forward primer (0.2 μM), 0.2 μL of each reverse primer (0.2 μM), 5 μL of QIAGEN Reaction Mix (1×), and 2.6 μL of RNase/DNase-free water (the volume of water varied depending on the number of primers in each multiplex reaction) (QIAGEN). Multiplexed reactions were carried out on a Hybaid Px2 thermal cycler (Thermo Scientific) and a Veriti 96-Well Thermal Cycler (Applied Biosystems). PCRs were performed through touchdown reactions, starting with initial heat activation at 95°C for 10 min, followed by 31 cycles with denaturation of 94°C for 60 s, annealing for 60 s, and 60 s of extension at 72°C. Annealing cycling temperature began at 57°C and decreased 1°C every cycle for six cycles (to 51°C), followed by two stages of 12 cycles each (with annealing temperatures of 55°C and 54°C). To check amplification, 5 μL of the PCR products were subjected to electrophoresis in a 1.5% agarose gel with 1× TBE buffer and stained with ethidium bromide. The remaining PCR products (5 μL) were diluted in 10 μL of water. One or two microliters of these PCR products (20–50 ng) were run on ABI Prism 310 and ABI 3730xl (Applied Biosystems) automated capillary sequencers; allele sizes were scored manually using GeneScan 500 LIZ Size Standard (Applied Biosystems) in GeneMarker version 2.4.0 (SoftGenetics LLC, State College, Pennsylvania, USA).

Of the 14 primers tested, 12 were polymorphic and two were monomorphic with high-quality amplification (Table 2). For each polymorphic locus, we calculated the number of alleles (A), observed heterozygosity (H_o), and expected heterozygosity (H_e); tests of deviations from Hardy–Weinberg equilibrium (HWE) and linkage disequilibrium (LD) were performed using the software Arlequin version 3.5.1.3 (Excoffier and Lischer, 2010). Fixation indices (F_{IS}) were estimated by GenAlEx version 6.5 (Peakall and Smouse, 2006). The probability of null alleles was estimated using MICRO-CHECKER software (van Oosterhout et al., 2004). We detected higher probabilities of null alleles between two loci (5250 and 1071) as suggested by the general excess of homozygotes (Table 2). There was LD at 10 of 90 paired loci comparisons, and significant departure from HWE was inferred at six loci, although this figure varied depending on the studied population (Table 2). A ranged from two to 12 across the studied populations. H_o and H_e ranged from 0.22 to 0.96 and from 0.20 to 0.87, respectively, and F_{IS} ranged from −0.41 to 0.44.

CONCLUSIONS

We developed and characterized 12 polymorphic and two monomorphic novel microsatellite markers for the herb *A. aurantiaca*. The primers will be useful for assessing population genetic structure and mating system of *A. aurantiaca* in both

Table 1. Characteristics of 14 microsatellite loci developed in *Aphelandra aurantiaca.*

Locus	Primer sequences (5′–3′)	Allele size range (bp)	Fluorescent label	Repeat motif	GenBank accession no.
0432[a]	F: AGGCTGAAGAGATTTGCAGG R: AAGACAGGCTGATGCAGTCG	113–124	NED	$(AGCC)_{24}$	SRR1816885
1233[a]	F: GTTGCATTTGAGGCATGAGG R: TGTAATTGAACTAGGTCTTGTACTCGC	116–126	PET	$(AT)_{22}$	SRR1514097
4343[a]	F: TGTAAAGGAAAGTTGAAGAAATAAGGG R: TGATTCGTTGGAGACACATGC	150–172	6-FAM	$(ATT)_{27}$	SRR1817142
4914[a]	F: AGGAATTGTCCGGTCTTCCC R: CCGGCTGATTCTGCTTCC	130–152	VIC	$(AT)_{22}$	SRR1817143
5490[a]	F: GGTGTACGTAGCCCACAACG R: TGAAGAAGTTGTTCCAAGGTACG	174–184	NED	$(ATGC)_{24}$	SRR1816884
1810[b]	F: TGGCACTTATAGCCACATCCC R: GAACCAGTGTTGCGTGTCC	194–207	PET	$(AC)_{26}$	SRR1817168
4378[b]*	F: GAGAATATAGAGGCCACCGGG R: TCCGGTACATGCTCCAAAGG	216	VIC	$(TTC)_{18}$	SRR1817171
1721[c]*	F: TCCTCCTCTCTCATTACAAGTGG R: TGTTCTTTAGTTTGCACACGC	180	PET	$(TC)_{20}$	SRR1817170
4483[c]	F: GATGGAGGCAGTGGAGATAGC R: GCAGAATCTTCTGGAACCACC	206–229	NED	$(TC)_{30}$	SRR1817184
5250[c]	F: TTCCTTCTTGTTGTTATTCTTGGC R: GGAACAAAGAGTCATGATTGAAGC	208–293	6-FAM	$(TC)_{28}$	SRR1817169
1071[d]	F: TTGTATTTGAATTGAACCCTTTCG R: CGAATTGAAGTCCAATGTGGC	272–304	PET	$(AT)_{26}$	SRR1817193
1808[d]	F: TGCGTGTCTTTGTTGTACTATCTGG R: AATGCTCAAGGCATGCACC	294–318	NED	$(AGT)_{30}$	SRR1817198
4536[d]	F: AAGAATTGTAATCCTTGAAAGCCC R: GGAAATTTATATGGAATGCCGC	187–193	6-FAM	$(TGC)_{21}$	SRR1817191
5441[d]	F: CAAAGACCTGTAATAGATATAAGGAAGCC R: AACTTAATGGACCATGTCGGC	200–300	VIC	$(TC)_{30}$	SRR1817260

Note: Annealing temperature was the same for all primers (T_a = 57°C). For genotyping, we used: (a) one quintuplex reaction (loci 0432, 1233, 4343, 4914, and 5490), (b) one duplex reaction (loci 1810 and 4378), (c) one triplex reaction (loci 1721, 4483, and 5250), and (d) one quadruplex reaction (loci 1071, 1808, 4536, and 5441).

*Monomorphic locus.

TABLE 2. Genetic properties of the newly developed polymorphic microsatellite loci of *Aphelandra aurantiaca*.[a]

	F1 (n = 27)			Selva 1 (n = 21)			Selva 2 (n = 31)			Bambú (n = 28)			
Locus	A	H_o	H_e	A	H_o	H_e	A	H_o	H_e	A	H_o	H_e	F_{IS}[b]
0432	4	0.74	0.72	4	0.33	0.51	4	0.61	0.64	3	0.46	0.59	0.14
1233	7	0.66	0.63	5	0.66	0.68	5	0.61	0.65	9	0.60	0.73	0.04
4343	5	0.62	0.73	7	0.61	0.64	7	0.74	0.72	7	0.46	0.75	0.12
4914	4	0.59	0.51	4	0.52	0.55	4	0.48	0.60	7	0.57	0.65	0.05
5409	3	0.96	0.54*	4	0.42	0.47	3	0.51	0.54	3	0.46	0.59	–0.12
1810	7	0.92	0.62*	4	0.85	0.60	5	0.96	0.67	4	0.85	0.68	–0.41
4483	10	0.70	0.86*	7	0.76	0.74	9	0.58	0.73	7	0.42	0.59	0.14
5250	7	0.51	0.76*	6	0.38	0.48	7	0.63	0.74	7	0.39	0.76‡	0.28
1071	7	0.48	0.73	8	0.45	0.84‡	10	0.46	0.85*‡	6	0.31	0.73‡	0.44
1808	6	0.40	0.59	7	0.57	0.66	9	0.45	0.61	7	0.46	0.50	0.18
4536	4	0.22	0.27	5	0.35	0.54*	2	0.22	0.20	3	0.44	0.41	0.11
5441	7	0.81	0.80	11	0.73	0.87	11	0.87	0.82	12	0.67	0.83	0.05

Note: A = number of alleles; F_{IS} = fixation index; H_e = expected heterozygosity; H_o = observed heterozygosity.
[a] All values are based on 107 samples representing Los Tuxtlas rainforest located in southern Mexico. See Appendix 1 for locality and voucher information.
[b] Fixation index of each locus across populations.
*Loci that were not in Hardy–Weinberg equilibrium ($P < 0.001$).
‡Null alleles.

preserved and fragmented rainforest. Likewise, we expect these microsatellite loci could be useful for other *Aphelandra* species.

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APPENDIX 1. Geographic location and voucher information of populations of *Aphelandra aurantiaca* from Los Tuxtlas tropical rainforest. A voucher was collected only at the largest forest fragment (ca. 700 ha).

Species	No. of individuals	Voucher specimen[a]	Collection locality	Geographic coordinates
A. aurantiaca	27	—	Site F1	18°34.648′N, 95°4.105′W
A. aurantiaca	21	TUXsno3239	Site Selva 1	18°35.153′N, 95°4.609′W
A. aurantiaca	31	—	Site Selva 2	18°35.269′N, 95°6.023′W
A. aurantiaca	28	—	Site Bambú	18°36.607′N, 95°8.363′W

[a] Voucher specimen is deposited at the herbarium of the Instituto de Biología, Universidad Nacional Autónoma de México (MEXU); subcollection of Los Tuxtlas herbarium.

Capítulo II

Efectos de la fragmentación en la estructura genética, tasa de polinización cruzada y en el movimiento de polen contemporáneo

Life history and past demography maintain genetic structure, outcrossing rate, contemporary pollen gene flow of an understory herb in a highly fragmented rainforest

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ABSTRACT

Introduction. Theory predicts that habitat fragmentation, by reducing population size and increasing isolation among remnant populations, can alter their genetic diversity and structure. A cascade of effects is expected: genetic drift and inbreeding after a population bottleneck, changes in biotic interactions that may affect, as in the case of plants, pollen dynamics, mating system, reproductive success. The detection of the effects of contemporary habitat fragmentation on the genetic structure of populations are conditioned by the magnitude of change, given the few number of generations since the onset of fragmentation, especially for long-lived organisms. However, the present-day genetic structure of populations may bear the signature of past demography events. Here, we examine the effects of rainforest fragmentation on the genetic diversity, population structure, mating system (outcrossing rate), indirect gene flow and contemporary pollen dynamics in the understory herb *Aphelandra aurantiaca*. Also, we assessed its present-day genetic structure under different past demographic scenarios.

Methods. Twelve populations of *A. aurantiaca* were sampled in large (4), medium (3), and small (5) forest fragments in the lowland tropical rainforest at Los Tuxtlas region. Variation at 11 microsatellite loci was assessed in 28–30 reproductive plants per population. In two medium- and two large-size fragments we estimated the density of reproductive plants, and the mating system by analyzing the progeny of different mother plants per population.

Results. Despite prevailing habitat fragmentation, populations of *A. aurantiaca* possess high genetic variation ($H_e = 0.61$), weak genetic structure ($R_{st} = 0.037$), and slight inbreeding in small fragments. Effective population sizes (N_e) were large, but slightly lower in small fragments. Migrants derive mostly from large and medium size fragments. Gene dispersal is highly restricted but long distance gene dispersal events were detected. *Aphelandra aurantiaca* shows a mixed mating system ($t_m = 0.81$) and the outcrossing rate have not been affected by habitat fragmentation. A strong pollen pool structure was detected due to few effective pollen donors (N_{ep}) and low distance pollen movement, pointing that most plants received pollen from close neighbors. Past demographic fluctuations may have affected the present population genetic structure

Submitted 30 May 2016
Accepted 6 November 2016
Published 22 December 2016

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Academic editor
Todd Vision

Additional Information and Declarations can be found on page 18

DOI **10.7717/peerj.2764**

How to cite this article Suárez-Montes et al. (2016), Life history and past demography maintain genetic structure, outcrossing rate, contemporary pollen gene flow of an understory herb in a highly fragmented rainforest. **PeerJ 4:e2764; DOI 10.7717/peerj.2764**

as Bayesian coalescent analysis revealed the signature of past population expansion, possibly during warmer conditions after the last glacial maximum.
Discussion. Habitat fragmentation has not increased genetic differentiation or reduced genetic diversity of *A. aurantiaca* despite dozens of generations since the onset of fragmentation in the region of Los Tuxtlas. Instead, past population expansion is compatible with the lack of observed genetic structure. The predicted negative effects of rainforest fragmentation on genetic diversity and population structure of *A. aurantiaca* seem to have been buffered owing to its large effective populations and long-distance dispersal events. In particular, its mixed-mating system, mostly of outcrossing, suggests high efficiency of pollinators promoting connectivity and reducing inbreeding. However, some results point that the effects of fragmentation are underway, as two small fragments showed higher membership probabilities to their population of origin, suggesting genetic isolation. Our findings underscore the importance of fragment size to maintain genetic connectivity across the landscape.

Subjects Biodiversity, Conservation Biology, Evolutionary Studies, Plant Science
Keywords *Aphelandra aurantiaca*, Gene flow, Habitat fragmentation, Mating system, Outcrossing rate, Population expansion, Los Tuxtlas, Tropical rainforest

INTRODUCTION

Tropical rainforests sustain much of global biodiversity, including most endemic plant species of the world (*Myers, 1988*). Unfortunately, tropical rainforests have been reduced to half of their original area (*FAO, 2014*) and face intense pressures from agriculture and livestock expansion (*Seymour et al., 2014*). Forest fragmentation is, thus, one of the main threats to rainforest biodiversity due to its effects on physical environmental conditions, ecological interactions, and genetic processes (*Young, Boyle & Brown, 1996*; *Haddad, 2015*).

Theorized genetic consequences of habitat fragmentation have focused on effects brought about by reductions in population size and increasing spatial isolation between remnant populations (*Young, Boyle & Brown, 1996*; *Aguilar et al., 2008*). These changes may reduce genetic variability and increase population genetic structure. After sudden reductions of effective population size or recent bottlenecks, genetic drift and inbreeding will cause further loss of alleles—especially rare alleles—thus increasing homozygosity. On the other hand, reduced connectivity among populations, gene flow cannot prevent the loss of alleles leading to genetic structuring. Disruption of gene flow of plant populations inhabiting fragments may modify mating patterns, reducing outcrossing rates and reproductive success, and consequently increase inbreeding. In the long term, these effects may affect fitness of populations, their adaptability to novel environmental conditions, and increasing the risk of local extinction (*Young, Boyle & Brown, 1996*; *Aguilar et al., 2008*; *Breed et al., 2013*; *Finger et al., 2014*).

The impact of rainforest fragmentation on the genetic structure of plant populations is highly variable, depending on life history, life-span, and mating system (*Cuartas-Hernández & Núñez-Farfán, 2006*; *Figueroa-Esquivel et al., 2010*; *Suárez-Montes, Fornoni & Núñez-Farfán, 2011*; *Chávez-Pesqueira et al., 2014*). At the landscape scale, factors like

the spatial configuration of fragments can also explain the genetic change of populations (*Leimu et al., 2006*; *Vranckx et al., 2011*; *Aparicio et al., 2012*; *Chávez-Pesqueira et al., 2014*). Moreover, it is important to consider past demographic processes can impact the patterns of present-day of genetic diversity (*Hsieh et al., 2013*). Therefore, a comprehensive knowledge of life history, population genetic structure, effective population size, and demographic history changes is fundamental to develop conservation strategies aimed to maintain genetic variability and evolutionary potential of plant species across fragmented rain forests.

Herbs represent *ca.* 45% of vascular plant diversity and are the richest plant communities in lowland tropical rainforests (*Gentry Alwyn & Dodson, 1987*; *Parkes, Newell & Cheal, 2003*). Although tropical rainforest herbs may play an important role to maintain forest structure, functioning, and dynamics (*Richards, 1996*), the genetic effects of fragmentation on this life form have not been extensively studied. Tropical plants species represent only 20% of the total species analyzed in fragmentation studies; of these only 4% are herbs, whereas 88% are canopy trees (*Aguilar et al., 2008*; *Vranckx et al., 2011*). Specifically, understory herbs are ideal systems to detect genetic effects of habitat fragmentation on a shorter time-scale, owing to their dependence on canopy cover, and relative short life span in relation to long-lived canopy trees (*Lowe et al., 2005*). Moreover, their natural distribution is exposed to altered ecological and environmental conditions by forest fragmentation, which may modify outcrossing rates, contemporary pollen dynamics and mating patterns.

Very few detailed studies on herbaceous plants have measured contemporary pollen dispersal within and among fragmented populations (*Gonzales et al., 2006*; *Cuartas-Hernández, Núñez-Farfán & Smouse, 2010*; *Côrtes et al., 2013*). Results revealed restricted pollen movement of herbaceous plants with an unclear pattern of the effects of fragmentation. In some cases forest fragmentation has limited impact on pollen dynamics (*Cuartas-Hernández, Núñez-Farfán & Smouse, 2010*), while in others it increases pollen movement and decreases pollen structure, possible due to edge effects (*Gonzales et al., 2006*). Furthermore, since pollen dispersal could be associated to the density of reproductive plants, forest fragmentation can enhance/reduce gene dispersal depending on plant abundance (*Cuartas-Hernández, Núñez-Farfán & Smouse, 2010*; *Breed et al., 2012*; *Côrtes et al., 2013*).

Here, we assess the genetic structure, out-crossing rate, and contemporary pollen dynamics of populations of *Aphelandra aurantiaca* (Acanthaceae) in a highly fragmented tropical rainforest in southern Mexico. Also, using contemporary genetic data, we infer the demographic history to understand the current distribution of genetic diversity. *Aphelandra aurantiaca* is an important species of tropical rainforest understory, whose population dynamics is affected by the presence of sunflecks or forest light-gaps (*Calvo-Irabién, 1989*; *Calvo-Irabién, 1997*; *Calvo-Irabién & Islas-Luna, 1999*). Although species of Acanthaceae are among the most important flowering plants in the forest's understory, studies assessing their genetic diversity are still lacking. To our knowledge, this is the first study that assesses the effects of habitat fragmentation on the genetic structure and contemporary gene flow in a tropical herbaceous plant of the genus *Aphelandra*.

We assessed the potential effects of habitat fragmentation on the genetic structure of *A. aurantiaca* of populations inhabiting fragments of different area. Specifically, we tested the hypothesis that, unlike large or medium sized fragments, small ones will show (1) reduced genetic variation and effective population sizes, (2) higher population differentiation as a consequence of genetic isolation, (3) higher inbreeding, (4) lower out-crossing rate, and (5) higher differentiation among pollen pools, (6) reduced number of pollen donor parents, and (7) decreased effective pollination neighborhood due to a decrease in plant abundance. Complementary, we assessed the present day genetic structure of *A. aurantiaca* under different past demographic scenarios.

MATERIALS & METHODS

Study system

Aphelandra aurantiaca (Scheidw.) Lindl. is an understory herb of neotropical rainforests from southern Mexico to Bolivia. It is a self-compatible species that bears inflorescences with yellow floral buds that turn red when flowers open and produce nectar. In the rainforest of Los Tuxtlas in Mexico, this species is pollinated by the hummingbird *Phaethornis longirostris* (I Ramírez-Lucho, P Suárez-Montes & J Núñez-Farfán, pers. obs., 2013) altough it is also visited by butterflies and bumble-bees (*Calvo-Irabién, 1989*; *Islas Luna, 1995*). Seed dispersal is ballistic, ranging from 1 to 8.5 m (modal value of 1.5 m) from the maternal plant. This herb also exhibit vegetative reproduction by stolons. Its life span ranges from 13 to 18 years (*Calvo-Irabién, 1989*). Reduction in plant abundance of *A. aurantiaca* is related to the forest regeneration cycle where light is the most variable abiotic factor; the species inhabits both shaded forest understory and forest light-gaps (*Calvo-Irabién, 1989*; *Calvo-Irabién, 1997*).

Study site and data collection

The study was carried out at Los Tuxtlas Biosphere Reserve in southern Mexico, which constitutes the northernmost distributional limit of tropical rainforest in the Americas (*Dirzo & Miranda, 1991*). The region has lost more than 90% of its original forest cover in the past fifty years. Nowadays, the current landscape is composed of areas used for human settlement (1.27%), roads (0.78%), water bodies (1.92%), cattle ranching and crops (42.82%), riparian strips (4.29%), live fences (3.28%), isolated trees (1.03%), secondary vegetation of rainforest (0.53%), and fragments of rainforest (23.24%). Rainforest fragments are relatively small (<100 ha), surrounded by grassland and located in lowlands or restricted to the top of the mountains, in glens or areas of difficult access. At higher elevations (>600 m a.s.l.) cloud forest (4.61%) and secondary cloud forests (0.25%) is the predominant vegetation (*Dirzo & Garcia, 1992*; *CONANP, 2011*; see *Salazar Arteaga, 2015*) (Fig. 1).

Twelve populations of *A. aurantiaca* were sampled throughout Los Tuxtlas rainforest (Table 1). Preserved areas covered by rainforest are mostly surrounded by a matrix of pasture lands used for cattle ranching. Because the study area is highly fragmented, the choice of sampling sites was based on size area, accessibility, and the possibility of getting large sample sizes for genetic analyses (~30 individuals). Forest fragment size best explains

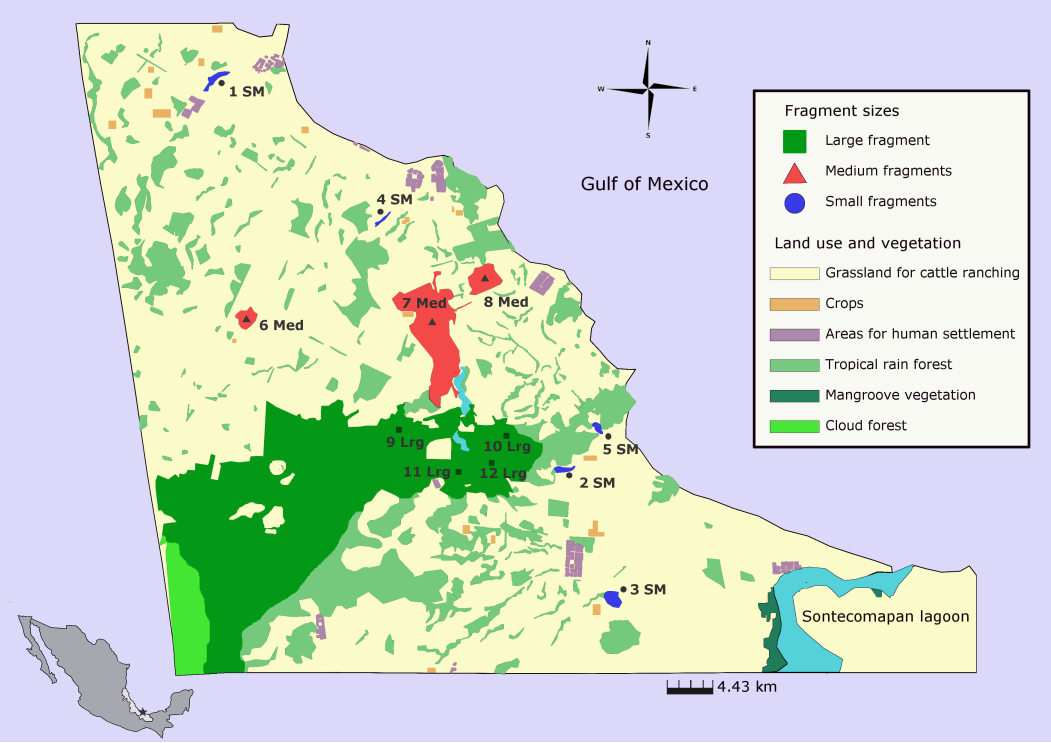

Figure 1 Populations of *Aphelandra aurantiaca* sampled for genetic analyses at Los Tuxtlas rainforest. Colors represent fragment size classes: blue (small), red (medium), green (large). Names of populations as in Table 1.

the differences in composition and plant structure (*Arroyo-Rodríguez & Mandujano, 2006*) therefore, we classified fragments as small (≤10 ha), medium (20–120 ha) and large and undisturbed fragments (>640 ha) (Fig. 1, Table S1). Unlike medium and large forest fragments, forest structure of small fragments is characterized by the absence of large primary trees in the canopy and lower abundance of palms and some herb species in the understory, but high abundance of shade intolerant secondary species (see *Arroyo-Rodríguez & Mandujano, 2006*). The large, undisturbed and continuous forest is in the core of Los Tuxtlas Biosphere Reserve; however it is partially separated by a deforested area produced by an illegal invasion (see Fig. 1). We established four sites within the large fragment as different populations based on their distance from deforested areas, and geographic distance between sampled sites (from 4. 39 to 14 km).

In each fragment size, we collected young leaf tissue from 29 to 38 adult plants. Individuals were selected as reproductive if they had an inflorescence/infructescence, or scars of these on the stem. We collected leaf tissue from individuals separated at least three meters apart. In total, we sampled individuals of *A. aurantiaca* in five small ($n = 153$), three medium ($n = 97$) and four large fragments ($n = 138$) (Table 1).

Microsatellites analysis and PCR amplification

We amplified 11 polymorphic microsatellites specifically developed for *A. aurantiaca* to determine its genetic structure (*Suárez-Montes, Tapia-López & Núñez-Farfán, 2015*)

Table 1 Genetic diversity of twelve populations of *Aphelandra aurantiaca* at the Los Tuxtlas tropical rainforest.

Size	Fragments	*ha*	*A (s.d.)*	*P(1) (s.d.)*	*Ho(s.d.)*	*He(s.d.)*	*Fis (C.I.)*	*Ne (C.I.)*
Small	1 SM	8	5.72 (2.10)	0.25(0.35)	0.57 (0.12)	0.66 (0.12)	0.134 (0.034, 0.201)	68.2
	2 SM	5	5.72 (1.79)	0.25(0.32)	0.63 (0.21)	0.63 (0.16)	−0.012 (−0.110, 0.047)	131.6
	3 SM	8	4.45 (1.96)	0.20(0.35)	0.38 (0.24)	0.54 (0.21)	0.290 (0.154, 0.361)	19.2
	4 SM	4	5.54 (2.54)	0.18(0.20)	0.59 (0.19)	0.61 (0.17)	0.038 (−0.051, 0.092)	70.1
	5 SM	5	5.09 (1.57)	0.14(0.20)	0.64 (0.21)	0.63 (0.14)	−0.012 (−0.105, 0.045)	30.6
Total small			9.0 (3.39)	0.74(0.48)	0.57 (0.16)	0.65 (0.11)	0.114 (0.071, 0.150)	119.2 (86.4–176.1)
Medium	6 Med	17	6.63 (2.94)	0.27(0.25)	0.50 (0.14)	0.64 (0.13)	0.217 (0.094, 0.312)	35.7
	7 Med	120	5.45(3.44)	0.23(0.33)	0.52 (0.24)	0.56 (0.17)	0.076 (−0.050, 0.176)	69.9
	8 Med	35	5.81 (3.86)	0.21(0.47)	0.66 (0.19)	0.63 (0.15)	−0.045 (−0.136, 0.014)	39.4
Total medium			8.72 (5.98)	0.65(0.65)	0.56 (0.16)	0.63 (0.15)	0.109 (0.045, 0.16)	146.7 (89.1–326.3)
Large	9 Lrg	640	6.27 (2.86)	0.22(0.25)	0.611 (0.18)	0.64 (0.16)	0.047 (−0.032, 0.101)	infinite
	10 Lrg	640	6.27 (2.32)	0.26(0.28)	0.55 (0.18)	0.59 (0.12)	0.068 (−0.031, 0.138)	510.1
	11 Lrg	640	4.81 (1.47)	0.14(0.25)	0.64 (0.19)	0.60 (0.15)	−0.066 (−0.176, 0.014)	24.7
	12 Lrg	640	5.09 (2.16)	0.16(0.15)	0.53 (0.22)	0.56 (0.19)	0.060 (−0.060, 0.144)	31.4
Total large			8.54 (4.32)	0.60(0.43)	0.59 (0.16)	0.622 (0.14)	0.051 (0.005, 0.089)	205.7 (123–480.6)

Notes.

ha, hectares by fragment; *A*, number of alleles per locus; *P(1)*, private allelic richness; *Ho*, observed heterozygosity; *He*, expected heterozygosity; *Fis*, inbreeding; *Ne*, effective population size; (*s.d.*), standard deviation; *C.I*, 95% confidence interval. Total values for each category of fragment size are provided.

(Table S2). DNA extraction, amplification, and laboratory setup are detailed in *Suárez-Montes, Tapia-López & Núñez-Farfán (2015)*. We genotyped all sampled individuals and scored alleles using the software GeneMarker V.2.4.0 (SoftGenetics, State College, PA, USA).

Genetic diversity analyses

We used MICRO-CHECKER software (*Van Oosterhout et al., 2004*) to detect null alleles. Deviations from Hardy–Weinberg equilibrium (HWE) and linkage disequilibrium were tested using GenePop 4.2 (*Rousset, 2008*) and FSTAT v.2.9.3.2 (*Goudet, 1995*), respectively. We estimated descriptive statistics of genetic diversity including allelic richness (*A*) and expected and observed heterozygosity (*He* and *Ho*) using Arlequin v.3.5.1.3 (*Excoffier, Laval & Schneider, 2005*). The private allelic richness (*P(1)*) was calculated by rarefaction with H_{P-RARE} (*Kalinowski, 2005*). Mean inbreeding coefficient (F_{IS}) was estimated using GENETIX v.4.05 (*Belkhir et al., 1996–2004*), based on 10,000 permutations. The hypothesis of isolation-by-distance was tested by a Mantel test based on 999 replicates using the ade4 package (*Dray & Dufour, 2007*) in R 3.1.3 (*R Development Core Team, 2015*).

Population size and demographic history analyses

We estimated effective population sizes (N_e) using a linkage disequilibrium method in NeEstimator V2 (*Do et al., 2014*). We employed two approaches to detect changes in population size. The first approach is based on the detection of heterozygosity excess or deficiency in a very recent period of time ($2N_e$–$4N_e$ generations) using the program Bottleneck 1.2.02 (*Piry, Luikart & Cornuet, 1999*). Populations recently bottlenecked would lose rare alleles faster than heterozygosity, resulting in an apparent heterozygosity excess in

comparison with a population at equilibrium (*Heq*) (*Cornuet & Luikart, 1997*; *Piry, Luikart & Cornuet, 1999*).

The second approach employs coalescent simulations in an approximate Bayesian computation (ABC) framework to infer past demographic history, as implemented in the software DIYABC 2.0 (*Cornuet et al., 2008*). ABC chooses a demographic scenario that best fits the observed data by running simulations constrained by the specifications of the model (e.g., a demographic bottleneck). It approaches the posterior probability distributions of parameters by selecting the simulated datasets with the smallest Euclidian distances to the observed data, as measured by summary statistics (*Cornuet et al., 2008*; *Cornuet et al., 2014*). We compared four demographic scenarios: two with constant N_e, one with a decline, and one with an expansion. We ran one million simulations for each scenario. The parameter settings and priors are shown in Table S3. The change time in N_e was set at 10–10,000 before present, assuming generation time of one year for the species (*Calvo-Irabién, 1989*). We used a generalized stepwise mutation model with a mutation rate of 10^{-4}–10^{-3}. The summary statistics were: mean number of alleles, mean heterozygosity, and mean allelic size variance. We assessed the fit of the models to the data by principal components analysis (PCA), implemented in DIYABC. We estimated the posterior probabilities using a logistic regression approach on the first 1% simulations closest to the observed dataset. To check the confidence of model choice we estimated type I and type II error rates by simulating 500 pseudo-observed data sets (*Cornuet et al., 2008*; *Cornuet et al., 2014*).

Genetic structure and clustering analyses

We estimated *Rst* and a hierarchical analysis of molecular variance (AMOVA) using Arlequin v.3.5.1.3 program (*Excoffier, Laval & Schneider, 2005*). Population structure was explored with both model based (Structure) (*Pritchard, 2010*) and distance based approaches (DAPC, Pop Graph and NetStruct) (*Jombart, Devillard & Balloux, 2010*; *Greenbaum, Templeton & Bar-David, 2016*).

The Bayesian clustering algorithm implemented in Structure V2.3 (*Pritchard, 2010*) estimates the probability of genotypes being distributed into K number of clusters. Simulations were run using correlated allele frequencies, under admixture ancestry models, conducting a burn-in of 10^6, MCMC iterations of 10^6, and K varying from 1 to 12. The total number of clusters (K) was inferred with the Evanno ΔK method (*Evanno, Regnaut & Goudet, 2005*) using STRUCTURE HARVESTER (*Earl & vonholdt, 2012*) and CLUMPAK pipeline (Cluster Markov Packager across K) (*Kopelman et al., 2015*) to visualize bar plots.

NetStruct 1.2 program (*Greenbaum, Templeton & Bar-David, 2016*) infers genetic structure using network theory. The equivalent of a genetic population structure is the community partition of a network constructed with individuals as nodes and edges (paired connections of nodes), defined by using a similarity measure. Clustering is done by locating groups of nodes (community) that are strongly connected within the group but weakly connected to nodes outside the group. The strength association (SA) measures how strongly the individuals are related to the community at which they were assigned to. The strength association distribution (SAD) analysis examines the distribution of SA values

of different communities and provides information about relative gene flow. A narrow SAD indicates low gene flow, while left-skewed SAD suggests constant moderate gene flow; recent migrants will display low SA values, increasing the variance and left–skewness of the distribution. Finally, NetStruct evaluates the statistical significance of community partitions using permutation tests (*Greenbaum, Templeton & Bar-David, 2016*). We used the Fast Greedy algorithm with a medium threshold of 0.24 and 999 permutations for modularity significance test. We characterized the SAD of communities by the Coefficient of Variation (CV), as a measure of dispersion.

Membership probability analysis

To evaluate the membership probability of individuals to their population of origin we used the discriminant analysis of principal components (DAPC), a multivariate analysis implemented in the *adegenet* package (*Jombart, 2008*; *Jombart, Devillard & Balloux, 2010*) in R 3.1.3 (*R Development Core Team, 2015*). Based on the retained discriminant functions, the analysis derives membership probabilities for each individual of original source populations (*Jombart & Collins, 2015*). We followed *adegenet* directions for alpha scores optimization; we performed the analysis with 50 PCs retained. We also evaluated admixed individuals with no more than 0.5 probability of membership to any population.

Spatial structure and genetic barriers analyses

To visualize the spatial genetic structure and the connectivity across the landscape we used Population Graphs implemented in *gstudio* (*Dyer & Nason, 2004*) and *popgraph* packages (*Dyer, 2009*; *Dyer, 2014*) in R 2.15.3 (*R Development Core Team, 2013*). Population Graphs is a graph-theoretical approach where the total genetic variation is decomposed into a geometric interpretation of components within and among the strata, and then modified using conditional covariance to the minimal topological configuration. The genetic structure within population variance is represented as nodes that are connected by edges whose magnitude is proportional to their interpopulation variance (*Dyer, 2015*). Nodes and edges were then mapped on their spatial coordinates. We tested isolation across graph distance (IBGD) through physical and graph distances using *Graph* software of GeneticStudio software (*Dyer, 2009*; *Dyer, 2014*). To identify long distance dispersal events or restricted gene flow we compared pairwise physical distances with their corresponding pairwise edge lengths using *Graph* software (*Dyer, 2009*). Edges whose length is significantly longer than expected indicate long distance dispersal events while edges with length shorter than expected indicate limited dispersal across the landscape (*Dyer, 2015*).

Recent migration analyses

Recent bidirectional migration rates (in the last 2–5 generations) were estimated for paired populations using the program BayesAss v 3.0 (*Wilson & Rannala, 2003*). Because we expected reduced connectivity for smaller and more isolated fragments, we also estimated migration rates between different fragment sizes. BayesAss does not depend on Hardy–Weinberg equilibrium and estimates m as the fraction of individuals in population i that are migrants derived from population j (per generation) (*Wilson & Rannala, 2003*). To check for consistency, we performed 10 runs with a different random seed number, and

calculated their respective Bayesian deviance (*Meirmans, 2014*). The run with the lowest deviance value was used to select the best-fitting model. Each run consisted of three million iterations for the chain with an initial burn-in period of one million iterations, and interval between samples for 2,000 chains of MCMC.

To detect possible first-generation migrants and their source population we used GENECLASS2 (*Piry et al., 2004*). We used the likelihood-base estimator *L_home* using *Paetkau et al.*'s, *2004* algorithm. *L_home*, is the likelihood of the individual genotype within the population where it was sampled. GENECLASS2 assumes Hardy–Weinberg equilibrium and species, sexual reproduction. The probability of each individual to be encountered in a given population was estimated using 100,000 simulated individuals with a threshold value of 0.01.

Mating system and contemporary gene flow

We established a 50 × 20 m plots (200 m^2) within four fragments, two medium size fragments and two populations from large fragments. We did not include small fragments due to the lack of enough samples of maternal families (mother and progeny). Plant density was characterized in each population by counting the number of flowering individuals. In each plot, we collected leaf tissue and mature infructescences of maternal plants ($n = 33$). We germinated seeds and collected the leaf tissue from the emergent seedlings for DNA extraction. The number of maternal families varied between populations and each family between 2 and 11 individual plants (Table 2). To estimate outcrossing rate (t) and pollen pool structure (Φ_{FT}) we used six highly polymorphic unlinked microsatellite loci (0432, 5409, 1233, 4536, 4483, and 1808) (Table S2) (*Suárez-Montes, Tapia-López & Núñez-Farfán, 2015*) and analyzed all members of each maternal family (6–10 families per population).

We estimated parental inbreeding coefficient (F), multilocus outcrossing rate (t_m), single-locus outcrossing rate (t_s), and biparental inbreeding due to mating among relatives ($t_m - t_s$) using MLTR (*Ritland, 2002*). Standard errors were derived from 1,000 bootstraps. To estimate the confidence interval (CI at 95%) we used the estimated MLTR mean ± 1.96 × 1 sd. We also estimated the CI for F, t_m, and t_s by the bootstraping in MLTR (*Ritland, 2002*). Progeny inbreeding was calculated with GENETIX v4.05, based on 10,000 permutations (*Belkhir et al., 1996–2004*).

We estimated the differentiation of pollen pools (Φ_{FT}) sampled by different maternal families using the TwoGener method (*Austerlitz & Smouse, 2002*) as implemented in GenALEx 6.502 (*Peakall & Smouse, 2012*). This model assumes uniform individual distribution and accurate density estimates. If the analysis reveals high Φ_{FT} then pollen dispersal is restricted, suggesting that different mothers are sampling pollen from, at least partially, non-overlapping sets of fathers. To avoid overestimation of Φ_{FT} values due to parental inbreeding (F_p) and selfing (s), we used the formula

$\Phi'_{FT} = \frac{\Phi_{FT}}{1+F_p}$, and given s as $1 - t_m$ where t_m is the multilocus outcrossing rate, Φ'_{FT} transforms to $\Phi'' = \frac{2\Phi'_{FT} - S^2}{2(1-S)^2}$ for the selfing rate. Finally the pollen dispersal distance (δ), the effective number of pollen donors ($N_{ep} = \frac{1}{2}\Phi''_{FT}$), and the effective pollination neighborhood area, ($A_{ep} = \frac{N_{ep}}{d}$), where d is the density of reproductive plants (*Austerlitz & Smouse, 2001a*; *Austerlitz & Smouse, 2001b*).

Table 2 Mating system and pollen structure parameters of *Aphelandra aurantiaca* from Los Tuxtlas.

Fragment size:	Medium		Large	
Population:	**6 Med**	**7 Med**	**9 Lrg**	**11 Lrg**
Density/m^2	1.6	0.20	0.18	0.18
n-mothers	11	6	10	6
n-progeny	91	56	75	32
Parental inbreeding: F (sd)	0.13 (0.11)	−0.20 (0.01)	0.10 (0.11)	0.09 (0.09) (0.03, 0.12)
B.C.I	(0.06, 0.19)	(−0.22, −0.18)	(0.04, 0.13)	(0.03, 0.12)
Progeny inbreeding: F	0.13	0.03	0.03	0.04
B.C.I	(0.05, 0.2)	(−0.04, 0.1)	(−0.05, 0.1)	(−0.09, 0.14)
Multilocus outcrossing rate: t_m	0.86 (0.06)	0.90 (0.05)	0.67 (0.11)	1.0 (0.09)
B.C.I	(0.80, 0.93)	(0.87, 0.92)	(0.63, 0.73)	(0.89, 1.0)
Single-locus outcrossing rate:t_s (sd)	0.71 (0.07)	0.85 (0.06)	0.65 (0.13)	0.77 (0.11)
B.C.I	(0.60, 0.76)	(0.78, 1.0)	(0.61, 0.68)	(0.63, 0.86)
Biparental inbreeding: tm − ts (sd)	0.15 (0.05)	0.04 (0.05)	0.02 (0.03)	0.24 (0.14)
P.C.I.	(−0.05, 0.2)	(−0.05, 0.1)	(−0.04, 0.09)	(−0.02, 0.52)
Correlation paternity	Φ_{FT} Φ''_{FT}	Φ_{FT} Φ''_{FT}	Φ_{FT} Φ''_{FT}	Φ_{FT} Φ''_{FT}
	0.30* 0.49	0.10* 0.17	0.16* 0.19	0.26* 0.40
Effective pollen donors: *Nep* Φ''	1.00	2.88	2.57	1.23
Genetic Neighborhood: A_{ep} (m^2)	0.62	14.4	14.30	6.85
Pollen distance δ (m)	0.41	1.09	1.08	1.04

Notes.

F, inbreeding of progeny (estimated in GENETIX) and inbreeding coefficient of maternal parents (estimated in MLTR); t_m, multilocus outcrossing rate; t_s, single-locus outcrossing rate; $t_m - t_s$, biparental inbreeding; *sd*, standard deviation 95% bootstrap confidence interval (B.C.I) and parametric confidence interval (P.C.I.); Φ''_{FT}, Correlation of paternity corrected by inbreeding and selfing rate.

*$p < 0.05$.

RESULTS

We did not detect evidence of null alleles. Significant deviations from Hardy–Weinberg equilibrium ($p < 0.05$) were observed in all populations, likely due to heterozygotes deficit rather than null alleles. Across populations, 10 loci displayed a significant heterozygotes deficit and one (1810) displayed a significant excess. Exact test of linkage disequilibrium indicated significant deviations at six out of 55 possible primer pair's comparisons. Deviations of Hardy–Weinberg equilibrium could be explained by age structure caused by overlapping generations and patchy plant distribution that can create Wahlund-like effects.

Genetic diversity and inbreeding

Overall, levels of mean population genetic diversity of *A. aurantiaca* at Los Tuxtlas rainforest were high ($N_a = 5.5$, $H_o = 0.57$, $H_e = 0.61$) and similar among fragments. High genetic diversity values are expected for long-lived perennial plants ($H_o = 0.63$, $H_e = 0.68$; *Nybom, 2004*). Inbreeding coefficient values were low ($F_{IS} = 0.097$, $p = 0.00$), ranging −0.066–0.29 for populations, and statistically similar between fragments of different size (range: 0.05 to 0.114), but slightly lower for large fragments (Table 1). Mantel's test did

not detect a relationship between genetic and geographical distances of all populations ($r = -0.28, p = 0.94$). Geographic distances between populations are given in Table S4.

Effective population size and bottleneck analysis

Effective population sizes (N_e) are relatively large in all fragment sizes (Table 1). Average effective population size of large fragments ($N_e = 205.7$ (CI [123–480.6])) is not significantly higher than in small fragments ($N_e = 119.2$ (CI [86.4–176.7])). However, effective population size tends to decrease with reduction of fragment size. The lowest estimated N_e corresponds to one small fragment (19.2 at fragment 3 SM) while the highest value (infinite N_e estimate) corresponds to a large fragment (9 Lrg).

Under the Stepwise Mutation Model (SMM) and Two-Phase Mutation Model (TPM), the bottleneck test failed to detect a recent genetic bottleneck. The proportion of heterozygotes observed (H_O) was lower than expected (Heq), suggesting an absence of recent genetic bottlenecks in fragments of all sizes (all $p < 0.001$). All fragments sizes exhibited significant allele deficiency (Table S5).

Demographic history (ABC)

Population expansion was the best scenario and had the highest posterior probability ($p = 0.99$, 95% CI [0.9997–0.9998]). The PCA representation exhibited a good recovery of the posterior predictive distribution and the observed data (Fig. S1). Under this model, we found evidence of a small population with an effective population size of approximately 1,180 individuals that expanded to a present effective population size of approximately 68,600 individuals. We estimated that this expansion occurred approximately 2690 years before present (Table S3). We found a type I error rate of 0.03, and a type II error rate of 0.02, indicating a statistical strength of 97% and a high degree of confidence for the population expansion scenario.

Population differentiation

Populations showed a lack of genetic differentiation despite isolation by fragmentation. The hierarchical AMOVA analysis indicated weak genetic differentiation among all fragments ($R_{st} = 0.037, p = 0.00$), with most genetic variance (96.2%) within populations, while only 3.8% of the variance was among populations within fragment size classes.

Bayesian statistical modeling for clustering implemented in STRUCTURE showed the most likely number of clusters at $K = 2$ (LnP $= -11100.43$) and $K = 3$ (LnP $= -10965.43$) (Fig. S2). For $K = 2$, cluster I includes almost all sampled populations while cluster II was composed of only two populations (3 SM and 4 SM). For $K = 3$, cluster I is also composed by almost all populations (1 SM, 2 SM, 5 SM, 6 Med, 7 Med, 9 Lrg, 11 Lrg, and 12 Lrg), while cluster II and III included two main populations (8 Med, 10 Lrg and 3 SM, 4 SM, respectively) (Fig. S2).

As in the STRUCTURE analysis, the constructed network in NetStruct also detected three groups or communities with significant community partitions ($p < 0.05$). The network indicates that these communities are dispersed over the landscape without a clear pattern (Fig. S3A). The assignment of individuals to communities showed that all are composed of individuals from all populations. However, community I was mainly

composed by populations 10 Lrg, 8 Med, 6 Med, and 5 SM; community II includes 7 Med, 1 SM, 3 SM, 4 SM and 11 Lrg populations; and community III is composed by 9 Lrg, 12 Lrg and 2 SM. Moreover, the mean of SAD was low and similar for the three detected communities (I: 0.0012, II: 0.0010 and III: 0.0013), all with a wide left-skewed distribution suggesting moderate strength of association and constant gene flow levels (Fig. S3B). Community II showed slightly lower association without a very wide skewed tail in comparison with the other communities, suggesting gene flow in the past. The coefficient of variation was high for all communities, but the lowest was for community II.

DAPC showed that membership probabilities were higher for individuals in their home population, ranging from 46% to 77% (Table S6 and Fig. S4). Population 3 SM, and 4 SM had the highest membership probabilities for individuals in their home, suggesting higher isolation. DAPC detected 89 admixed individuals: 33 from small fragments, 18 from medium fragments, and 38 for large fragments. The highest number of admixed individuals belongs to population 11 Lrg (15) and population 5 SM (12). The lowest value was for population 3 SM with one admixed individual.

Spatial genetic structure, connectivity and barriers

The Population Graph consisted of 12 populations connected by 24 edges that exhibited a significant conditional covariance. The topology showed that all populations formed a single interconnected network, indicating gene dispersal (Fig. 2). No IBD (Isolation by distance) among populations was detected (Mantel $Z = 67.8$, $p = 0.805$). Conditional genetic distances (cGD) are shown in Table S4. We found extended edges (between 1 SM–2 SM, 1 SM–3 SM, 1 SM–11 Lrg, 3 SM–4 SM, and 5 SM–6 Med) whose lengths were longer than expected from the spatial distances, indicating long distance dispersal (Fig. 2). We also found compressed edges (between 2 SM–11 Lrg, 3 SM–12 Lrg, 4 SM–7 Med, 4 SM–8 Med, 4 SM–10 Lrg, 7 Med–8 Med, and 11 Lrg–12 Lrg) whose lengths were shorter than expected from the spatial distances, suggesting a reduced permeability of landscape.

Migration rates and first generation migrants

The BayesAss analysis showed that most gene flow (in the last 2–5 generations) occurred from large to both medium and small fragments (Table 3). However, the large–medium rate was higher than the large-small rate, suggesting higher connectivity between large and medium fragments. GeneClass2 identified 15 putative first-generation migrants out of a total of 388 individuals ($p < 0.01$) (Table S6). These results also indicated considerable gene dispersal from the largest fragment. We found that nine out of the 14 migrants derive from the large fragment, five reside in small fragments, two in medium fragments and two in large fragments. Three derived from medium fragments and reside in smaller populations. Two out of 14 migrants derived from small fragments, one resides in a medium fragment and the other in a large fragment (Table S6).

Mating system, pollen structure and pollen movement

Plant density of *A. aurantiaca* at Los Tuxtlas is variable among populations. Densities ranged from 0.42 to 3.18 individuals/m^2; similar values have been reported by *Calvo-Irabién*

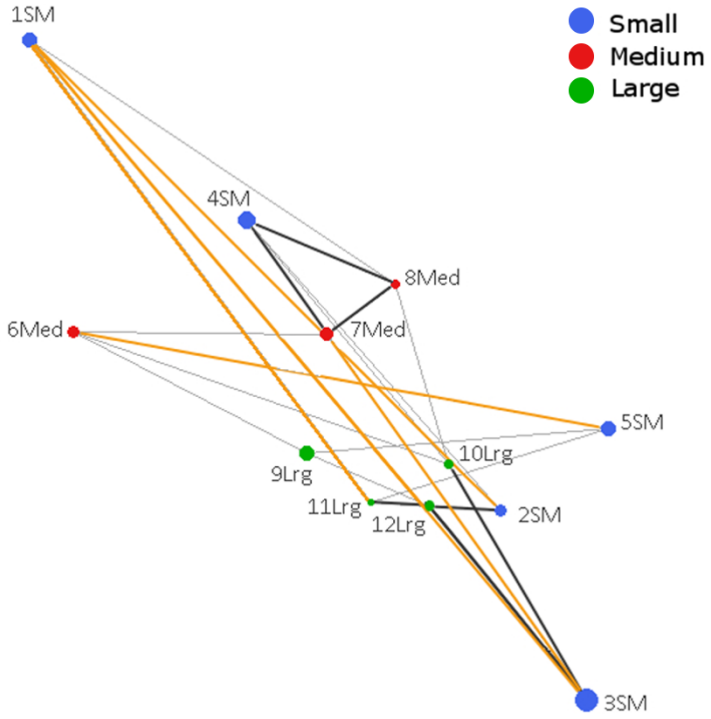

Figure 2 Genetic network obtained from Population Graph for *Aphelandra aurantiaca* populations at Los Tuxtlas. The differences in node (circles) size reflect differences in genetic variability within populations. Edge length (lines connecting nodes) represents the among population component of genetic variation. The figure shows normal edges whose length is proportional to that expected under a model of isolation by distance (thin black lines); extended edges (yellow) indicate long distance dispersal events, and compressed edges (thick black lines) indicate reduced gene permeability of the landscape.

Table 3 Gene flow estimates for *Aphelandra aurantiaca* between small, medium, and large fragments.

Pair fragmented populations		*Nm*	BayesAss
1	0	Based on *Fst*[a]	Short-term gene flow *m* (95% credible set)
Small	Medium	152	0.0459 (0.016–0.075)
	Large	38.5	0.0408 (0.011–0.070)
Medium	Small	152	0.0042 (−0.0014–0.009)
	Large	37.2	0.0149 (0.003–0.026)
Large	Small	35.5	0.2069 (0.175–0.238)
	Medium	37.2	0.2672 (0.235–0.299)

Notes.

Migration rate (*m*) estimated using BayesAss v 3.0 (*Wilson & Rannala, 2003*) for paired fragment sizes (small, medium, and large). Where *m* [1][0] is the fraction of individuals in population 0 that are migrants from population 1.

[a]Indirect measures of gene flow (*Nm*) for each paired fragment size were calculated with the formula of *Wright (1951)*: $Nm \approx \frac{1}{4}\left(\frac{1}{Fst}-1\right)$.

(1989), who found a range from 0.38 to 3.32 individuals/m^2. The density of reproductive individuals in our study ranged from 0.18 to 1.6 individuals/m^2.

Aphelandra aurantiaca has a mixed mating system ($t_m = 0.81$), altough mating system estimators of each population are not related to fragment size (Table 2). There were no significant differences among populations in multilocus outcrossing rate, single outcrossing rate, and biparental inbreeding. Bootstrapped confidence intervals showed a significantly lower multilocus outcrossing rate in one population (11 Lrg). The AMOVA of gametes indicated that most genetic variance was contained within mothers (70–90%). The correlation of paternity estimates(Φ_{FT}), were high and significantly higher than zero for all populations (Table 2). Hence, the correlation of paternity estimates,Φ_{FT}, indicated restricted pollen dispersal (part of the offspring are full-sibs), with a relatively low number of effective pollen donors (N_{ep}), and short pollen distance movement in this species (Table 2). Some populations showed a high (45%) or a low (17–19%) fraction of siblings sharing the same father. The number of effective pollen donors (N_{ep}) was relatively low (1.92, on average) (Table 2). Average inbreeding values [$F_{is}(s.d.)$] were slightly lower for adult maternal plants [0.03(0.15)] than for seedlings [0.05(0.04)]. Furthermore, we detected biparental (or uniparental) inbreeding in all populations ($t_m - t_s$ ranged from 0.02 to 0.24), that did not differ between populations. The effective pollination neighborhood ranged from less than 0.6 to 14.4 m^2, whereas pollen distance movement ranged from half a meter up to 1.1 m (Table 2). We suggest caution in the interpretation of TwoGener results due to uniform individual distribution assumptions.

DISCUSSION

Genetic structure and habitat fragmentation

Habitat fragmentation has not produced genetic differentiation or immediate reductions in genetic diversity of *A. aurantiaca* despite dozens of generations since the onset of fragmentation in the region of Los Tuxtlas. Regardless of fragment size, populations possess private alleles and high genetic diversity ($H_e = 0.61$), similar to those of long-lived perennial herbs (*Nybom, 2004*), but higher compared to other understory Acanthaceae plants [e.gr., *Graptophyllum reticularum* ($H_e = 0.31$), *Graptophyllum ilicifolium* ($H_e = 0.43$) and *Ruellia nudiflora* ($I = 0.26$) (*Shapcott, 2007*; *Vargas-Mendoza et al., 2015*). Effective population size estimates (N_e) showed that most populations are effectively large, suggesting that habitat fragmentation has not as yet reduced N_e enough to detect an impact on genetic diversity.

The weak genetic structure detected ($R_{st} = 0.037$) is supported by a number of analyses, suggesting that *A. aurantiaca* populations have remained genetically connected. Most genetic variation is contained among individuals within populations (96%) rather than between populations (3%) in fragments of different size. A similar result was obtained for other understory tropical herbs in the same region (*Cuartas-Hernández & Núñez-Farfán, 2006*; *Suárez-Montes, Fornoni & Núñez-Farfán, 2011*). Genetic clustering analyses revealed that most populations shared genetic information of one cluster or community, which explain the low genetic differentiation. However, small populations (3 SM and 4 SM) form a genetic group, suggesting ongoing isolation.

The high genetic diversity, large effective population size, and low genetic differentiation found in populations of *A. aurantiaca* could be related to historical processes of populations rather than with the present landscape configuration. Also, life history characteristics of *A. aurantiaca*, such as its mating system, generation time, vegetative reproduction, and overlapping generations, might help to diminish the impact of genetic drift, maintaining large effective population size, and buffering the loss of genetic diversity due to habitat fragmentation (*Weidema, Magnussen & Philipp, 2000*; *Hailer, Helander & Folkestad, 2006*; *Breed, Christmas & Lowe, 2014*; *Pellegrino, Bellusci & Palermo, 2015*).

Genetic structure, gene flow and past demographic change

Although low genetic differentiation and occasional long-dispersal events of *A. aurantiaca* were detected, we also found evidence of restricted gene flow. The contrasting results of restricted ecological dispersal of *A. aurantiaca* over short distances and low genetic structure could indicate a lack of population equilibrium under current demographic conditions. When populations suffer frequent extinction and re-colonization processes, low *Fst* values are expected if colonist individuals are drawn from distant populations. Besides, since dispersal could be highly variable through time, direct measures of dispersal could miss long distance dispersal events (*Coyne et al., 1982*; *Slatkin, 1985*; *Slatkin, 1994*; *Whitlock & McCauley, 1990*).

Historical data suggest ancient contraction-expansion of Los Tuxtlas rainforest. Contractions occurred during a period of low temperatures and humidity (from 20,000 to 12,000 years ago during the last glacial maximum (LGM)) followed by subsequent vegetation expansion events (*Graham, 1975*; *Toledo, 1982*; *Haffer & Prance, 2001*; *Gutiérrez-Rodríguez, Ornelas & Rodríguez-Gómez, 2011*). In *A. aurantiaca*, ABC analyses suggest a plausible past population expansion scenario at Los Tuxtlas around the end of the LGM, when warmer climatic conditions established. More recently, contraction-recolonization of Los Tuxtlas rainforest could also be related with volcanic activity (during the last 153 years ago) (*Martin Del Pozzo, 1997*; *Guevara & Laborde, 2012*), and with forest fragmentation due to human activities (only during the last 42 years) (*Dirzo & Garcia, 1992*). Further evidence indicates no relationship of geographic and genetic distances among populations of *A. aurantiaca*, suggesting a relatively recent origin. There is also evidence of ancient and recent population expansion for an abundant palm of the understory of Los Tuxtlas (J Juárez–Ramírez, 2015, unpublished data; *Martínez-Ramos et al., 2016*). Therefore, the low genetic structure of *A. aurantiaca* could be due to different historical processes at Los Tuxtlas rather than recent habitat fragmentation.

Current gene flow and habitat fragmentation

The highest rates of migrants and first-generation migrants derived from the largest and medium fragments, underscore the importance of relatively large forest patches to prevent genetic isolation. Moreover, pollinators may use a series of different fragment sizes to forage, helping to maintain connectivity across the landscape (*Llorens et al., 2012*; *Volpe et al., 2014*). Specifically, hummingbirds are effective pollinators that can fly across relatively large areas during their foraging routes, carrying pollen grains to individuals' located far

apart (*Stouffer & Bierregaard, 1995*; *Kraemer, 2001*). However, it is necessary to conduct specific studies to assess the effect of Los Tuxtlas forest fragmentation on the abundance and behavior of the hummingbird pollinator *Phaethornis longirostris,* and their consequences on reproductive output of *A. aurantiaca*.

Although both pollen and seed dispersal are relevant to the pattern of genetic structure in *A. aurantiaca,* analyses do not allow us to determine which process is the most important contributor to gene flow. Natural gene flow often follows a leptokurtic distribution, implying that most genes move over short distances and only a small fraction move over long distances. Pollen dispersal kernels are often short, resulting in self-pollination or gene exchange among closely related individuals (*Betts et al., 2014*; *Ellstrand, 2014*). However, even a small number of long distance migration events can suffice to reduce F_{st}. Unfortunately, these rare events are difficult to detect in field studies (*Nathan et al., 2003*; *Mona et al., 2014*). For *A. aurantiaca*, it is likely that current events of long distance dispersal contribute to maintain the landscape connectivity preventing genetic differentiation and increasing local genetic diversity. In contrast, restricted pollen (0.41–1.09 m) and seed dispersal (1.5 m; *Calvo-Irabién, 1989*) promote substructure within populations.

Compressed edges suggest reduced gene permeability among populations that are geographically close, even in the large fragment (Fig. 1). Within the large fragment only population 12 Lrg shows contact with three other populations, but one of them is a compressed edge (11 Lrg–12 Lrg). This could be related to the physical barrier imposed by the "Vigia" hill (ca 600 m a.s.l.) within the preserve, reducing gene dispersal. Thus, topography (elevation) of the landscape should be considered in future studies as a factor affecting gene flow.

Mating system and fragmentation effects

The species' mating system is an important factor that affects the distribution of genetic diversity. *Aphelandra aurantiaca* is predominantly outcrosser ($t_m = 0.81$). The description of *A. aurantiaca* as a selfing species with a mixed mating system agrees with values found in other hummingbird-pollinated plants in the Neotropics (*Wolowski et al., 2013*). However, we found that its mating system is predominatly of outcrossing. A mixed-mating system can combine advantages of both reproductive strategies: outcrossing promotes genetic diversity when pollinators are abundant, while self-fertilization may ensure reproduction when pollinators are scarce or absent (*Goodwillie, Kalisz & Eckert, 2005*; *Ruan & Teixeira da Silva, 2012*).

Self compatible tropical herbs do not necessarily suffer of inbreeding because they may possess breeding system traits that promote outcrossing (*McDade, 1985*). In general, *A. aurantiaca* showed no signs of inbreeding, although some populations exhibited inbreeding (Table 1). This finding could be a consequence of the Wahlund effect caused by genetic structure within populations (*Murren, 2003*) due to limited seed/pollen dispersal within populations. For *A. aurantiaca* at Los Tuxtlas fragmented rainforest, maintaining a mixed mating system with a high outcrossing rate appears to help preventing the loss of genetic variation, as would be theoretically expected for smaller populations.

Forest fragmentation does not seem to affect the contemporary pollen dynamics of *A. aurantiaca*, as in other herbs at Los Tuxtlas (*Cuartas-Hernández, Núñez-Farfán & Smouse, 2010*). The pollen pool structure was variable; in some populations it was more restricted than in others. The effective pollination neighborhood estimated resulted smaller than 14 m^2, and plants received pollen from neighbors located, on average, within a radius of 1.1 m. This finding agrees with an estimation of pollen movement using fluorescent dyes (reported by *Calvo-Irabién, 1989*). The effective number of pollen donors of *A. aurantiaca* is relatively low (range 1.0–2.8), suggesting that the potential pollen donors contribute little to N_{ep}. The moderate biparental inbreeding in *A. aurantiaca* could be explained by the limited seed dispersal and mating among close relatives, which may be due to hummingbirds' moving among relatively close plants (P Suárez-Montes, pers. obs., 2014). High plant density may contribute also to increase inbreeding and shorten pollen dispersal distance in *A. aurantiaca*. However, assessing whether high plant density reduces pollen dispersal makes necessary an extensive sampling of populations with different densities. We suggest caution when interpreting results of TwoGener given that it assumes uniform individual distribution.

CONCLUSIONS

Current genetic structure of *A. aurantiaca* is the result of different factors acting simultaneously. Despite extensive forest fragmentation of Los Tuxtlas rainforest, *A. aurantiaca* maintains high genetic diversity and low genetic differentiation between populations, suggesting effective gene flow. Habitat fragmentation has not affected the outcrossing rate and pollen dynamics within populations. Demographic history and life history characteristics are important to explain the current pattern of low population structure of *A. aurantiaca*, rather than recent fragmentation effects. We propose that past demographic dynamics, large effective populations, long distance gene dispersal events, and life history characteristics of this species, such as mixed mating system, overlapping generations, and ability to re-sprout after forest disturbance (e.g., light-gaps formation), ameliorate the effects of fragmentation. In addition, higher gene flow originated from medium and large fragments favour genetic connectivity and confirm their importance as genetic reservoirs and gene sources. Conservation efforts must be directed to preserve these fragments. However, small fragments should not be overlooked, as they may act as stepping stones to increase/maintain connectivity among fragmented populations, especially for species whose gene flow is aided by animals. Our findings should contribute significantly to the development of effective conservation strategies for *A. aurantiaca* and species with similar mating systems and pollinators.

ACKNOWLEDGEMENTS

The authors thank to the staff of the Los Tuxtlas Biological Research Station and Rosamond Coates who allowed us the use of the facilities. We also thank Rosalinda Tapia López for lab advice, to Guadalupe Andraca Gómez, César A. Domínguez, Victor Parra Tabla, and Edson Sandoval for valuable suggestions to an early draft of this paper, and Helen Salazar

Arteaga and Javier Laborde Dovalí for assistance in landscape description. This study is part of the PhD Dissertation of P Suárez-Montes in the Graduate Program in Biological Sciences, UNAM.

ADDITIONAL INFORMATION AND DECLARATIONS

Funding

The project "Evolutionary ecology studies of rainforest fragmentation in Mexico" was funded by a Papiit grant, UNAM (IN 215111-3), to JNF. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Grant Disclosures

The following grant information was disclosed by the authors:
Papiit grant, UNAM: IN 215111-3.

Competing Interests

The authors declare there are no competing interests.

Author Contributions

- Pilar Suárez-Montes conceived and designed the experiments, performed the experiments, analyzed the data, contributed reagents/materials/analysis tools, wrote the paper, prepared figures and/or tables, reviewed drafts of the paper.
- Mariana Chávez-Pesqueira performed the experiments, contributed reagents/materials/analysis tools, reviewed drafts of the paper.
- Juan Núñez-Farfán conceived and designed the experiments, performed the experiments, contributed reagents/materials/analysis tools, wrote the paper, reviewed drafts of the paper.

Data Availability

The following information was supplied regarding data availability:

The raw data has been supplied as a Supplemental File.

Supplemental Information

Supplemental information for this article can be found online at http://dx.doi.org/10.7717/peerj.2764#supplemental-information.

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Supplemental Information

Table S1. Geographic coordinates for the studied populations of *A. aurantiaca* at Los Tuxtlas, Mex.

	Population	Name	Altitude masl	Coordinates Latitude	Longitude
Small	1SM	Cola Pescado	108	18.663183°	-95.145750°
	2SM	F1	82	18.577461°	-95.068416°
	3SM	Sta. Rosa	82	18.549040°	-95.055235°
	4SM	San Pedro	104	18.630437°	-95.110194°
	5SM	Playa	94	18.588617°	-95.055900°
Medium	6Med	Bambú	538	18.610050°	-95.138717°
	7Med	Ruiz Cortines	93	18.609440°	-95.096811°
	8Med	Borrego	180	18.618656°	-95.085541°
Large	9Lrg	Selva2	318	18.587717°	-95.100184°
	10Lrg	Selva1	100	18.585885°	-95.076820°
	11Lrg	Zacatal	213	18.578804°	-95.089628°
	12Lrg	Vigia	454	18.578114°	-95.080036°

Table S2. Microsatellite loci of *Aphelandra aurantiaca*

Locus name	Motif repeat	Primer sequences	Allele size	GeneBank Accession no.
5490	$(ATGC)_{24}$	F: GGTGTACGTAGCCCACAACG R:TGAAGAAGTTGTTCCAAGGTACG	174-184	SRR1816884
0432	$(AGCC)_{24}$	F:AGGCTGAAGAGATTTGCAGG R:AAGACAGGCTGATGCAGTCG	113-124	SRR1816885
4343	$(ATT)_{27}$	F: TGTAAAGGAAAGTTGAAGAAATAAGGG R: TGATTCGTTGGAGACACATGC	150-172	SRR1817142
1233	$(AT)_{22}$	F:GTTGCATTTGAGGCATGAGG R:TGTAATTGAACTAGGTCTTGTACTCGC	116-126	SRR1514097
4914	$(AT)_{22}$	F: AGGAATTGTCCGGTCTTCCC R:CCGGCTGATTCTGCTTCC	130-152	SRR1817143
1810	$(AC)_{26}$	F:TGGCACTTATAGCCACATCCC R:GAACCAGTGTTGCGTGTCC	194-207	SRR1817168
4883	$(TC)_{30}$	F:GATGGAGGCAGTGGAGATAGC R:GCAGAATCTTCTGGAACCACC	206-229	SRR1817184
5250	$(TC)_{28}$	F:TTCCTTCTTGTTGTTATTCTTGGC R:GGAACAAAGAGTCATGATTGAAGC	208-293	SRR1817169
5441	$(TC)_{30}$	F:CAAAGACCTGTAATAGATATAAGGAAGCC R:AACTTAATGGACCATGTCGGC	200-300	SRR1817260
1808	$(AGT)_{30}$	F:TGCGTGTCTTTGTTGTACTATCTGG R:AATGCTCAAGGCATGCACC	294-318	SRR1817198
4536	$(TGC)_{21}$	F: AAGAATTGTAATCCTTGAAAGCCC R:GGAAATTTATATGGAATGCCGC	187-193	SRR1817191

Table S3. Priors parameters distribution of *A. aurantiaca* used in DIYABC and posterior parameters estimated for Scenario 4.

Prior parameters		Prior distribution	Posterior parameters				
			Mean	Median	Mode	Quantile2.5%	Quantile97.5%
Population size assoc with expansion	*Ns*	UN 1000-100000	66100	68600	96000	21000	98500
Population size assoc with bottleneck	*Nb*	UN 10-10000	1810	1180	482	64.2	6990
Time since the expansion, years before present	*t*	UN 10-10000	2690	2030	712	254	8370

UN: Uniform distribution, with minimum and maximum values. We consider a generation time of approximately 1 year for *A. aurantiaca*

Table S4, Mean geographic distance (km, in lower diagonal) and Conditional genetic distances (cGD, in upper diagonal) between populations of *A. aurantiaca*.

		SMALL					MEDIUM			LARGE			
	Pop	1SM	2SM	3SM	4SM	5SM	6Med	7Med	8Med	9Lrg	10Lrg	11Lrg	12Lrg
SMALL	1SM	**0**	4.73	8.27	10.3	9.5	13.9	10.8	5.31	13.3	10.5	5.00	9.36
	2SM	12.56	**0**	11.9	5.65	9.07	12.4	11.4	10.0	8.57	11.5	4.55	4.63
	3SM	15.91	3.46	**0**	6.59	15.5	11.9	7.40	12.3	11.2	7.77	11.7	7.32
	4SM	5.28	7.3	10.72	**0**	14.7	10.2	5.78	5.77	14.1	5.91	10.2	10.2
	5SM	12.59	1.76	4.36	7.36	**0**	4.43	8.94	14.4	4.24	9.57	4.52	8.18
MEDIUM	6Med	5.95	8.29	11.16	3.81	9.11	**0**	4.50	10.0	3.89	5.13	5.13	8.95
	7Med	7.91	4.65	7.98	2.74	4.87	4.5	**0**	5.53	8.40	9.64	13.4	12.3
	8Med	8.1	4.89	8.36	2.9	4.56	5.74	1.56	**0**	13.9	5.22	10.32	14.68
LARGE	9Lrg	6.44	3.61	6.42	4.83	4.7	4.81	2.45	3.76	**0**	9.02	8.34	3.94
	10Lrg	11.3	1.31	4.75	6.07	2.25	7.12	3.33	3.73	2.47	**0**	14.0	12.9
	11Lrg	4.99	2.26	4.99	6.11	3.73	6.29	3.37	4.32	1.44	1.55	**0**	4.39
	12Lrg	11.72	1.22	4.41	6.66	2.8	7.16	3.86	4.54	2.38	0.92	1	**0**

Table S5. Tests of population bottleneck of *A. aurantiaca* in different fragment size.

fragment	Bottleneck							
	TPM				SMM			
	He excess (eq.)	*Ho excess*	*H deficit*	*P*	*He excess (eq.)*	*Ho excess*	*H deficit*	*P*
Small	6.45	1	10	0.002*	6.43	1	10	0.002*
Medium	6.49	2	9	0.004*	6.47	2	9	0.009*
Large	6.48	0	11	0.004*	6.53	0	11	0.004*

Tests were based on heterozygosity excess. TPM: two-phase model of mutation; SMM: step-wise mutation model. **P*< 0.05 indicates significance of Wilcoxon's signed –rank test.

Table S6. Membership probabilities of *A. aurantiaca* to their populations of sampling extracted from DAPC. Bold numbers indicate membership probabilities for individuals in their home population.

	1SM	2SM	3SM	4SM	5SM	6Med	7Med	8Med	9Lrg	10Lrg	11Lrg	12Lrg
1SM	**0.644**	*0.070*	5E-4	2E-4	0.031	0.019	0.009	*0.053*	0.034	*0.085*	0.021	0.028
2SM	*0.057*	**0.633**	4.3E-7	0.007	0.018	0.018	0.075	0.010	0.049	0.043	*0.065*	0.018
3SM	0.001	0.003	**0.778**	*0.243*	0.007	0.009	*0.098*	0.003	0.011	0.035	0.019	0.007
4SM	3E-4	0.034	*0.040*	**0.658**	0.020	0.013	*0.083*	0.008	0.045	*0.061*	0.016	0.016
5SM	0.033	0.012	9.6E-7	0.034	**0.537**	*0.062*	0.035	0.023	*0.109*	0.020	*0.077*	0.054
6Med	0.012	0.003	1.8E-5	0.001	0.092	**0.564**	*0.080*	0.038	0.073	0.022	*0.075*	0.034
7Med	0.029	0.049	8.3E-5	0.014	0.015	*0.073*	**0.586**	0.011	0.040	0.043	0.059	*0.075*
8Med	*0.058*	0.035	1.6E-6	0.007	0.032	0.013	0.026	**0.592**	*0.084*	*0.120*	0.022	0.007
9Lrg	0.025	0.028	1.6E-6	0.008	*0.067*	*0.101*	0.048	*0.067*	**0.465**	*0.061*	0.044	*0.081*
10Lrg	0.004	0.012	8.2E-6	0.010	0.045	0.055	0.013	*0.090*	*0.079*	**0.587**	0.057	0.041
11Lrg	0.022	*0.056*	1.1E-6	0.007	0.049	*0.063*	0.040	0.019	*0.076*	0.035	**0.555**	*0.072*
12Lrg	0.026	*0.086*	3.2E-7	0.015	0.043	0.032	0.019	0.009	*0.090*	0.025	*0.075*	**0.574**

Table S7. First-generation migrants of *Aphelandra aurantiaca* among fragment sizes identified by GeneClass2 analysis ($p < 0.01$).

Resident fragment	Resident population	Fragment source	Population source	-L(home)	-L(log)	Probability	Membership Probability *	Distance (km)
Medium	7Med	Small	2SM	14.2	13.9	0.003	0.049	4.65
Large	12Lrg	Small	1SM	17.9	14.9	0	0.026	11.72
Small	1SM	Medium	6Med	13.7	12.7	0.007	0.019	5.95
Small	3SM	Medium	7Med	11.2	8.5	0.0015	0.098	7.98
Small	3SM	Medium	8Med	13.6	10.2	0.0064	0.003	8.36
Small	2SM	Large	11Lrg	14.5	13.4	0.005	0.065	2.26
Small	1SM	Large	11Lrg	14.4	12.6	0.0001	0.021	4.99
Small	1SM	Large	10Lrg	15.3	13.3	0.004	0.085	11.3
Small	2SM	Large	11Lrg	12.5	11.0	0.0003	0.065	2.26
Small	4SM	Large	10Lrg	15.1	13.8	0.0051	0.061	6.07
Medium	6Med	Large	10Lrg	20.7	18.7	0	0.022	7.12
Medium	6Med	Large	11Lrg	15.2	15.0	0.008	0.075	6.29
Large	10Lrg	Large	12Lrg	14.9	13.1	0.009	0.041	0.92
Large	11Lrg	Large	12Lrg	14.2	13.7	0.005	0.072	1

Resident fragment refers fragment size to which an individual was found; fragment source indicates the most likely origin. L was estimated as L= -LOG (L_home), where L is the ratio of the likelihood computed from the population where the individual was sampled (L_home). Fifteen migrants were identified across the different fragment sizes. *Average membership probability to resident population was estimated in DAPC.

Figure S1. Comparison of the possible demographic scenarios for *Aphelandra aurantiaca* using Principal component analysis in DIYABC. Scenario 4 fits the observed data best.

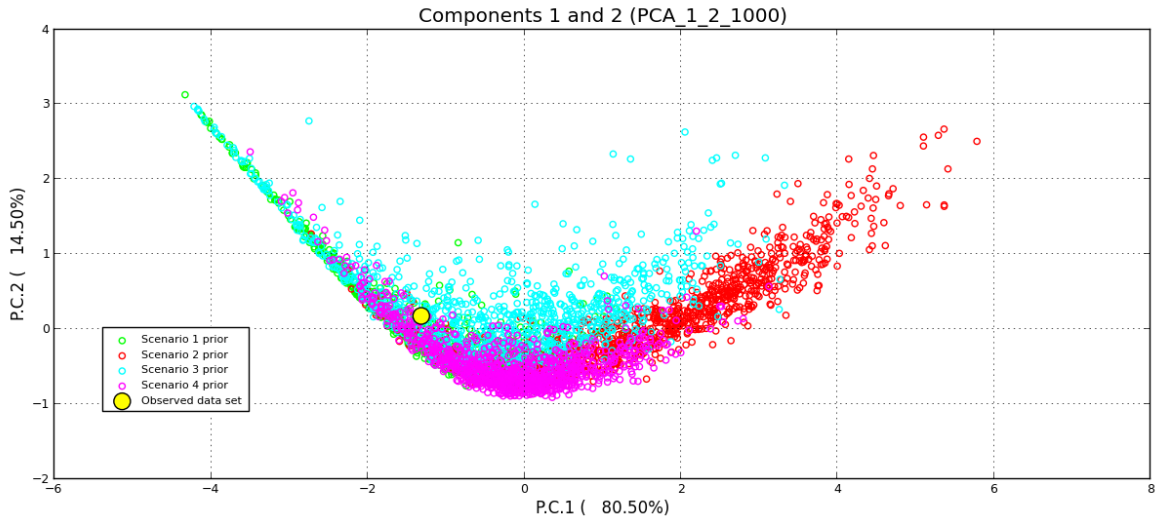


Figure S2. Genetic structure for twelve populations of *Aphelandra aurantiaca* ilustrated by Structure *K* = 2 and *K* = 3 were detected by Evanno, Regnaut & Goudet (2005) method of optimal number of genetic clusters. Each bar represents an individual and its proportional membership to clusters. Populations are ordered from small to large fragments.

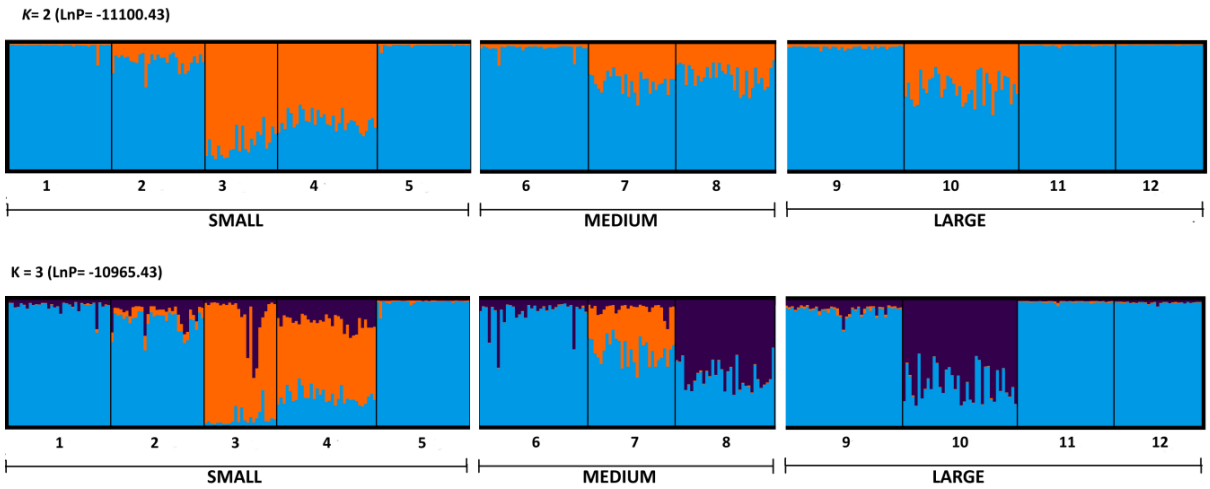
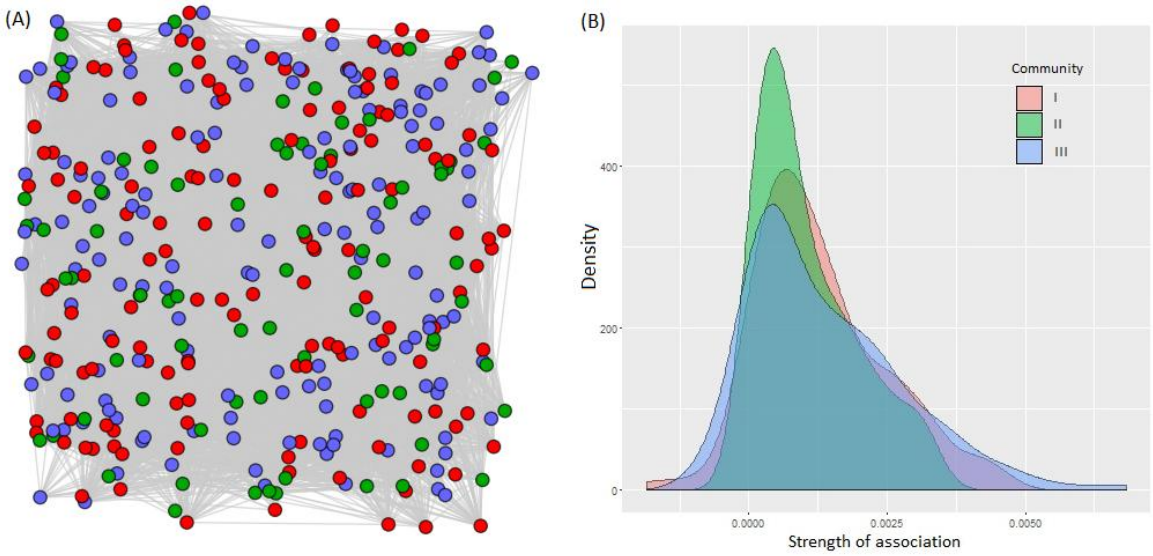
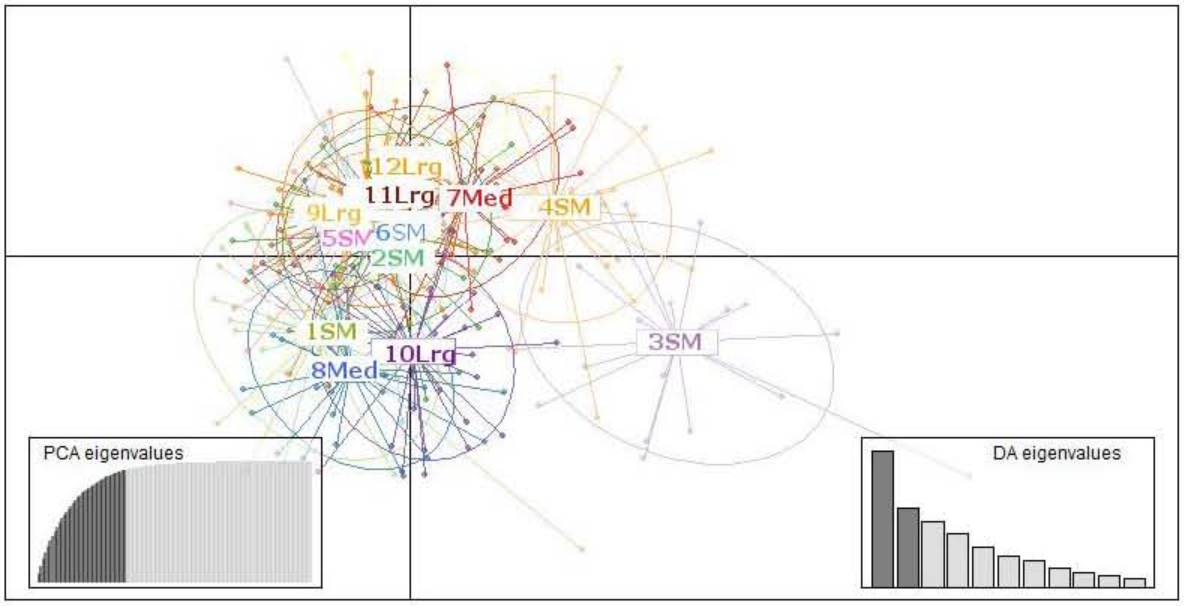


Figure S3. Network analysis



(A) Community detection on *Aphelandra aurantiaca* network with medium threshold (0.245), using Fast Greedy and geographic coordinates algorithm implemented in NetStruct. Each node represents an individual, with colors representing the three community assigned. (B) SAD Analysis for the network shows the distributions of the SA values for each community. The strength association (SA) measures how strongly the individuals are related to the community at which they were assigned to the community at which they were assigned to. The strength association distribution (SAD) analysis examines the distribution of SA values of different communities and provides information about relative gene flow. A narrow SAD indicates low gene flow, while left-skewed SAD suggests constant moderate gene flow; recent migrants will display low SA values, increasing the variance and left-skewness of distribution.

Figure S4. Discriminant Analysis of Principal Components for all populations estimated in DAPC, 50 PCA axes and 11 DA axes were retained



Capítulo III

Adaptación local en una herbácea de la selva ante la fragmentación del hábitat

Local adaptation of a tropical rainforest herb in the face of habitat fragmentation

Abstract

1. Habitat fragmentation can alter the species' genetic diversity and population structure and ultimately could influence the extent of local adaptation. These expectations have rarely been tested for organisms inhabiting fragmented ecosystems.
2. We performed a reciprocal transplant experiment between populations living in fragments and in the continuous, undisturbed, lowland tropical rainforest in southern Mexico. Full-sib families of each of four populations of the understory herb, *Aphelandra aurantiaca*, were grown at the four localities, and recorded through time to assess plant survival, vigour-related traits (plant height, number of leaves and leaf area), and damage inflicted by herbivores. Using co-dominant, neutral, molecular markers, we estimate genetic population structure and diversity, local inbreeding and gene flow among populations. Sites in the understory of the continuous forest had on average lower light intensity and higher relative humidity than sites in fragmented forests.
3. Results indicate that all populations have a shallow genetic structure, although populations in the continuous forest have larger effective population size. On average, plant mortality was significantly higher in the continuous forest than in fragments. However, local plants from the continuous forest survived better in their home habitat. Plant survival was higher in fragments regardless the origin of plants but, as a whole, plants from the continuous forest performed better in all characters measured when grown in the two environments. Herbivory was low and similar in the two environments.
4. *Synthesis***:** Results suggest local adaptation of plants from the continuous forest to the highly limiting conditions of the rainforest understory. Perhaps larger effective

population size in this habitat has maintained genetic variation in quantitative traits related to fitness, and hence the potential for local adaptation.

Key words: habitat fragmentation, genetic diversity, local adaptation, effective population size, reciprocal transplant experiment, tropical rainforest, ecological genetics, plant fitness.

Introduction

Anthropogenic global changes can create new environmental scenarios and selective regimes for plant species (Dirzo & Miranda 1991; Leimu *et al.* 2010; Matesanz, Gianoli & Valladares 2010; Matesanz & Valladares 2014; Moran & Alexander 2014; Brudvig *et al.* 2015; Valiente-Banuet *et al.* 2015). A main driver of these global changes is habitat fragmentation, which results from the division of large, continuous habitats into smaller, more isolated forest remnants. Organisms inhabiting fragments experience altered habitats in both physical and biotic environmental factors, reduction in population size, genetic diversity, increased inbreeding and loss of genetic connectivity with other populations (Aguilar et al., 2008; Quesada et al., 2013; Chávez-Pesqueira et al., 2014). Furthermore, habitat fragmentation can compromise fitness potential of plants and their ability to cope with new environments (Willi *et al.* 2007; Matesanz *et al.* 2010; Laurance *et al.* 2011; Bijlsma & Loeschcke 2012; Valladares *et al.* 2014).

While different studies have documented changes in the abiotic environment (Laurance 2004; Harper *et al.* 2005; Grimbacher, Catterall & Kitching 2006; Ewers & Banks-Leite 2013; Haddad *et al.* 2015; Stangler, Hanson & Steffan-Dewenter 2015), biotic interactions leading to changes in reproduction (Aguilar *et al.* 2006; Breed *et al.* 2012, 2013; Chávez-Pesqueira *et al.* 2015; Dáttilo *et al.* 2015; García-Guzmán, Trejo & Sánchez-Coronado 2016), and population genetic parameters (Cascante et al., 2002; Jump & Peñuelas, 2006; Quesada et al., 2013; Chávez-Pesqueira et al., 2014; Chung et al., 2014; Barr et al., 2015), few studies have considered how habitat fragmentation may influence

adaptive genetic variation (Willi *et al.* 2007; Eckert *et al.* 2010; Willi & Hoffmann 2012; Mannouris & Byers 2013; Fraser *et al.* 2014; Cheptou *et al.* 2017), and very few have assessed whether habitat fragmentation affects local adaptation (Hooftman, van Kleunen & Diemer, 2003; Bowman et al., 2008; Lopez et al., 2009; Pickup et al., 2012).

Understanding the effect of habitat fragmentation on rainforest species is of utmost importance as this ecosystem holds most global biodiversity, the highest number of plant species threatened, and the highest deforestation rates (FAO 2014; Seymour *et al.* 2014). Small populations in fragments of rainforest have a much greater chance of local extinction due to random demographic stochasticity and local environment variation (Lande 1988). The original climate of rainforest is characterized by warm temperatures, high humidity and low light penetration into the forest's understory (Chazdon & Pearcy 1991; Bailey 2014), but forest fragmentation changes the local environment to which a great number of species have adapted along evolutionary time. Although some rainforest plant species, like pioneer trees, are adapted to recurrent disturbance and high light environments due to formation of canopy gaps, species in small forest fragments are exposed to edge effects that reduce humidity, increase light incidence and greater temperature. Moreover, it is possible that plant species adapted to a high humidity regimen and low radiation at the understory cannot easily adapt to higher temperatures and lower moisture in fragmented habitats (Laurance 2004; Laurance *et al.* 2011; Ewers & Banks-Leite 2013).

Changes occurring in fragments may alter survival, birth, recruitment, and growth rates (Bruna *et al.* 2002; Neal, Hardner & Gross 2010). After fragmentation, isolated plants populations may have fewer individuals that experience reduced viability and reproduction (Lande 1988), potentially leading to further decrease effective population size. Theoretically, small population effective size in fragments will reduced genetic variation and lower the potential response to selection (Young, Boyle & Brown 1996; Willi, Van Buskirk & Hoffmann 2006; Willi *et al.* 2007). Evidence derived from quantitative reviews in plants, indicates an overall negative effect of fragmentation on genetic diversity

(Aguilar *et al.* 2008; Vranckx *et al.* 2011), and a positive correlation between population size and genetic variation (Leimu *et al.* 2006; Honnay & Jacquemyn 2007).

Local adaptation is a process where populations evolve traits that provide an advantage under their local environmental conditions, and in consequence resident genotypes should have a higher fitness in their local habitat than genotypes originating from other habitats (Kawecki & Ebert 2004; Savolainen, Lascoux & Merilä 2013; Franks, Weber & Aitken 2014). Nevertheless, local adaptation depends on extant genetic variation, on natural selection favouring local versus foreign genotypes, on the amount of gene flow between populations, and stochastic genetic drift that may cause local maladaptation (Reznick & Ghalambor 2001; Hereford 2009).

Local adaptation can determine how well a plant performs under altered environment conditions caused by habitat fragmentation (Leimu *et al.* 2010). Fragmentation affects local adaptation in at least two ways. First, if increased isolation leads to reduced gene flow between populations, fragmentation can enhance local adaptation of populations inhabiting in heterogeneous landscapes if local population sizes are large. Second, if fragmented populations are very small and genetically depauperated, local adaptation may be less likely (Swindell & Bouzat 2006; Lopez *et al.* 2009; Leimu *et al.* 2010). Likewise, a meta-analysis suggest that local adaptation in plants is more common for large than for smaller populations (Leimu & Fischer 2008); a plausible explanation is that genetic drift in smaller populations reduces standing additive genetic variation and slows the spread and fixation of beneficial mutations (Willi *et al.* 2007; Blanquart, Gandon & Nuismer 2012). Some studies found a positive relation between plant performance and large population size. For instance Pickup et al. (2012) and Bowman et al. (2008) found such a relationship in large populations of *Rutidosis leptorrhynchoides* and *Lychinis flos-cuculi,* respectively.

Reciprocal transplant experiments are commonly applied to test the hypothesis of local adaptation. To date, few studies have experimentally tested the effects of habitat fragmentation on local adaptation in plants (Hooftman, van Kleunen & Diemer, 2003;

Bowman et al., 2008; Pickup et al., 2012), and a clear pattern has not been detected as yet. Until now, experimental results suggest that local adaptation is related to environmental variation, and the differences in plant performance may be attributable to variable selection pressures and heterogeneous selection among fragments (Hooftman *et al.* 2003; Bowman *et al.* 2008; Pickup *et al.* 2012). This may be consistent with the "Variable Hypothesis" (see Willi et al., 2007; Willi & Hoffmann, 2012b), which states that habitat characteristics and resulting natural selection pressures become increasingly variable as both habitat fragment size and population decrease, while the "Directional Hypothesis", proposes that habitat characteristics shift in a consistent manner during habitat fragmentation process, resulting in directional relationships between these characteristics, population size, and the extent of adaptive genetic variation and differentiation (Fraser *et al.* 2014). However, these must be tested in natural plants populations.

For tropical rainforest species, it is still unclear whether local adaptation is reduced in fragments. This lack of local adaptation studies in tropical rain forest is due, in part, to the long lifespan of plants and the inaccessibility of study areas (Bruna *et al.* 2002; Chen & Schemske 2015). Here, we studied the extent of local adaptation in the perennial tropical understory herb *Aphelandra aurantiaca*, with a life span ranging 13-18 years, through a reciprocal transplant experiment between populations in continuous and fragmented forests. We aim to examine whether habitat fragmentation affects the pattern of local adaptation in this species. To our knowledge, this is the first study that examines the effects of habitat fragmentation on local adaptation of a rainforest understory herb. Specifically, we ask the following questions: (1) Do population size and genetic variation are related to plant performance? (2) Is there evidence of local adaptation to local conditions of the continuous or fragmented habitats? (3) Does *A. aurantiaca* maintain the ability to adapt to physical environmental changes caused by habitat fragmentation?

Methods

Species and study site

Aphelandra aurantiaca (Scheidw.) Lindl., Acanthaceae, is a perennial herb that inhabits the understory of tropical rain forests (i.e., a shade tolerant species). It is mainly pollinated by the hummingbird *Phaethornis longirostris*, has a mixed mating system (t_m= 0.81) and most seed and pollen dispersal is restricted within populations (Calvo-Irabién 1997; Suárez-Montes, Chávez-Pesqueira & Núñez-Farfán 2016). Also this herb exhibits vegetative reproduction by stolons. Its life span ranges from 13-18 years (Calvo-Irabién 1989), and is abundant in both continuous and fragmented habitats at Los Tuxtlas region (Mexico), the northernmost limit of tropical rainforest in the Americas (Dirzo & Miranda 1991). This region has been severely fragmented in the past fifty years, remaining less than 10% of the area originally covered by rainforest. Most rainforest fragments are relatively small (<100ha), surrounded by grasslands and restricted to the top of the mountains, in glens or areas of difficult access (Mendoza, Dirzo & Fay 2005; CONANP 2011; Salazar Arteaga 2015).

Reciprocal transplant experiment

Four populations of *A. aurantiaca* from Los Tuxtlas were chosen to carry out a reciprocal transplant experiment. We selected two populations in highly isolated fragments ("Bambú" (Bu), and "Cola de Pescado" (Cp)), and two populations from continuous forests ("Selva 1" (S1), and "Selva 2" (S2)). Distances among populations ranged from 2.47 to 7.12 km; the studied populations vary in plant abundance and local abiotic conditions (see below). Unlike fragments, continuous forest structure is characterized by large primary trees, higher abundance of palms, and many herb species in the understory (Arroyo Rodriguez y Mandujano, 2006). However, within continuous forest there are also differences between sites, population S1 is characterized by higher density of trees and the palm *Astrocaryum mexicanum,* there is a slightly slope and it seems that accumulates

higher woody material and leaf litter, mainly of large palm leaves that fall on the forest floor.

To determine whether phenotypic plant traits possess genetic variance we analyzed natural progenies (Lawrence 1984), from different mother plants of each population during 2012. Seeds were germinated in a greenhouse at Los Tuxtlas Research Station (UNAM). Once the seedlings produced the first true leaves, these were transplanted to their home location and to the three other foreign localities. Prior to transplanting, we measured seedlings' plant height, leaf area, and leaf number. Plants were arranged in each site under a randomized block design (Steel & Torrie 1960). In each site, seedlings of each family (i.e., fullsibs), from each population, were assigned to each of five blocks (1m × 4m). A total of 1600 seedlings belonging to 80 families (Cp =14; Bu=22; S2=23; S1=21) were transplanted. Transplant sites in fragmented populations were fenced to avoid damage by livestock. We monitored plants from December 2012 up to February 2014; censuses were carried out at 5, 116, 222, 341, and 469 days after transplanting.

We measured as fitness components plant survival, plant performance (growth), and damage by herbivores. In particular, plant performance was measured as the number of leaves and leaf area, an index value of plant growth. To estimate standing leaf area (LA), we measured the length (L) and width (W) of each leaf of a plant. We took a sample of 108 well developed leaves of *A. aurantiaca* non-experimental (wild) plants in December 2012, measured their L and W, and then scanned them using a leaf area meter (Li-Cor, model 3100, Lincoln, NE, USA) to obtain leaf area. A regression analysis showed that L × W explains a high amount of variance in leaf area (r^2=0.99, F= 19013, p<0.001). Thus, in each census, we measured length and width from each leaf for all plants in the experiment and then estimated the leaf area using the equation: LA (cm^2) = 0.051 + 0.0061(L × W). The proportion of damage by herbivores was estimated as the number of damaged leaves divided by the total number of leaves of a plant.

Environmental data collection

To characterize environmental physical conditions in the understory, two data loggers were placed in each site (HOBO-BOXCAR, Onset Computer Corporation, Bourne, MA, USA). Data loggers recorded temperature (°C), dew point (°C), percentage of humidity and light intensity (lum/m2), every hour during the experiment, from winter 2012 until the last census in autumn 2014. We used Man Whitney tests to determine differences in these variables between habitats and Kruskal Wallis tests between populations.

Genetic diversity and structure at neutral loci

To assess genetic diversity and structure of populations of *A. aurantiaca,* we collected young leaf tissue from 34 to 39 adult wild plants per population. We extracted DNA for each plant and amplified 11 SSR specific loci (Table S1 in Supporting Information) (Suárez-Montes, Tapia-López & Núñez-Farfán 2015; Suárez-Montes, Chávez-Pesqueira & Núñez-Farfán 2015). We estimated descriptive statistics of genetic diversity as allelic richness (*A*), observed and expected heterozygosity (H_o and H_e) using the software Arlequin (Excoffier, Laval & Schneider 2005), and average inbreeding coefficient (F_{IS}) with GENETIX v4.05 (Belkhir *et al.* 1996). Effective population sizes (N_e) were calculated by the linkage disequilibrium method for single-sampled, as implemented in NeEstimator V2 (Do *et al.* 2014). Genetic differentiation between populations was determined by pair-wise population differentiation estimates (R_{ST}, F_{ST}), and by analysis of molecular variance (AMOVA) using Arlequin (Excoffier *et al.* 2005). Recent migration rate between populations was assessed through a Bayesian approach with BayesAss. It estimates *m* as the fraction of individuals in population *i* per generation that are migrants derived from population *j* (Wilson & Rannala 2003). To check for consistency, we performed 10 runs with a different random seed number, and then calculated their respective Bayesian deviance (Meirmans, 2014). The run with the lowest deviance value was used to select the best-fitting model.

Detecting local adaptation

We assessed local adaptation following both "local vs. foreign" and "home vs. away" criteria. The "local vs. foreign" criterion expects that individuals form a local population exhibits higher fitness in their local environment than individuals transplanted from foreign populations. In contrast, under the "home vs. away" criterion local adaptation would occur if a population exhibits higher fitness in its own environment (at home) compared with a different environment (away) (see Kawecki & Ebert, 2004).

Statistical analyses: local adaptation

We tested local adaptation using generalized linear models (GLMMs). Seedling performance was assessed as a function of the site of origin, the site of transplant and their interaction. The response variables were plant survival, leaf area, and leaf damage. The variables leaf area, plant growth, and number of leaves were analyzed assuming a Gaussian error distribution whereas survival and leaf damage were analyzed using generalized linear mixed models assuming a binomial error distribution with logit link and using the Laplace approximation (O'Hara & Kotze 2010; Warton & Hui 2011). For each variable, we tested models at the population and habitat levels (see models in Supporting Information). Full models included the terms of transplant site, origin, along with their interaction site × origin (i.e., the genotype by environment interaction, as fixed effects), block nested within site (as random effect) and family (as random effect). A significant interaction between origin and habitat would suggest (see direction) adaptation of *A. aurantiaca* to local environmental conditions. To account for differences in leaf area and number of leaves at the transplant site, we included initial leaf area and initial leaf number to covariate in the model, respectively. We used log-likelihood tests to determine the overall significance of main effects and interactions. We assessed the significance of site × origin interaction by comparing the full model to the model without interaction. The significance of each factor and covariate was assessed by comparing the models from which the respective main factor or covariate was removed. Also the Akaike Information Criterion (AIC) was used to evaluate models fit, choosing the model with the lowest AIC.

We report the changes as delta AIC values. As a measure of goodness of fit for each model, we calculated the marginal R^2 (variance explained by fixed effects) and conditional R^2 (variance explained by both fixed and random factors). All statistical analyses were performed with the statistical package *lme4* (Bates *et al.* 2015) and post hoc contrast tests with the package *lsmeans* (Lenth 2016) in R 3.1.3 (R-Development-Core-Team 2015).

Statistical analyses: time-course of growth variables

The Cox proportional hazards model was used to analyze the survival rate through time (days), and to compare survival between sites, origins, and habitats. Survival analyses were performed in JMP Version 9 (SAS-Institute 2010). The time-course of leaf area, plant growth, and leaf damage since the beginning of the experiment in each habitat and population, were analyzed by GLM models with R 3.1.3 (R-Development-Core-Team 2015). These models (see Supplementary Material) include time, habitat of origin and habitat of transplant, time × origin, time × site transplant, block nested within site (as random effect), and family (as random effect). The same models were repeated at population level.

Results

Habitat environmental variables

There was a significant variation of temperature, humidity, dew point, and light intensity between habitats and populations (Table S2 in Supporting Information). Considering all months together, we found that temperature (χ^2= 381, *d.f.* =1, $P < 0.001$), humidity (χ^2= 109, *d.f.* =1, $P < 0.001$) and dew point (χ^2= 9.4, *d.f.* =1, $P < 0.05$) were significant higher in continuous forests than in fragments (Fig. 1a, b, c). Conversely, as was expected light intensity (χ^2= 25.2, *d.f.* =1, $P < 0.001$) was significant higher in fragmented that in continuous habitat (Fig. 1 d).

Neutral genetic diversity and population differentiation

Populations of *A. aurantiaca* in both continuous and fragmented habitats possess high levels of genetic diversity (Table 1). However, effective population size was higher for populations in the continuous habitats than in fragments. Inbreeding was higher for fragmented populations (F_{is} = 0.18) than for those in the continuous forest (F_{is} = 0.07). In general, population differentiation was very low (F_{st} = 0.01, R_{st} = 0.02). Population structure was slightly higher in continuous forest (R_{st} = 0.03) than in the fragmented forest (R_{st} = 0.02) (Table 1). Recent migration rates between populations, estimated by BayesAss, were low between most pairs of populations (Table S3 in Supporting Information); the exception was population S2 that exhibits the higher migration rates to the other populations (Table 1, Table S3). This result suggests that most migrants are derived from continuous forest.

Test for local adaptation

At the habitat level only leaf area was influenced significantly by the habitat of origin, transplant habitat and interaction (habitat of transplant × habitat of origin), suggesting local adaptation under the local vs. foreign criterion (Table S4 in Supporting Information). The post-hoc Student test showed significantly higher leaf area for local seedlings in the continuous habitat (t = 2.19, $d.f.$ = 114.8, P < 0.05), than for foreign seedlings (Fig. 2) (Table S5 in Supporting Information). Although we did not find a significant statistical effect of site × origin interaction for most variables, we detected a trend of better performance for plants derived from the continuous habitat than those derived from the fragmented habitat (Fig. 2). Moreover, we did not find evidence of local adaptation under the "home vs. away" criterion for any of the measured variables but the trends remain (Fig. 2). The habitat of transplanting had an important effect regardless plants' habitat of origin. Plants transplanted to the fragmented habitat had higher leaf area, survival, and number of leaves. However, plants derived from continuous habitat showed a trend of higher leaf area, survival rate, leaf damage, and number of leaves in comparison with plants derived from fragmented habitat at both habitats (Fig. 2).

At the population level, seedling survival, leaf area and leaf damage showed a significant effect of the transplant site, population of origin, and site × origin interaction (Table S6 in Supporting Information). The significant effects of both origin and transplant site suggest both genetic and environmental influences on plant survival, leaf area, and leaf damage. Although the site × origin interaction was significant, it not did reveal local adaptation either following the "local vs. foreign" or "home vs. away" criteria at a population level. None of the local populations showed higher average survival and leaf area than foreign populations (Fig. 3 a, b), or significant lower leaf damage (Fig. 3 c). However, there was a trend for local genotypes from S1 population to exhibit higher survival rates, higher leaf area, and lower leaf damage than foreign individuals (Fig. 3 a, b, c). On the other hand, some foreign populations outperformed local ones. Foreign genotypes S1 had significantly higher leaf area and survival than local genotypes of S2 population (Fig. 3 a, b). Likewise, foreign genotypes of S2 population had significantly higher survival than local genotypes of Cp population (Fig. 3 b). Foreign genotypes of S1 and S2 populations had significantly more leaf damage than local genotypes of S2 and Cp populations, respectively (Fig. 3 c). For leaf number at Cp population, foreign genotypes of S2 exhibited significantly higher value than local genotypes (Fig. 3 d).

Following the "home vs. away" criterion, none of the local populations exhibited a best performance at home for any of the variables: leaf area (Fig. 3 a, Fig. S1 a), survival (Fig. 3 b, Fig. S1 b), leaf damage (Fig. 3 c, Fig. S1 c), and the number of leaves (Fig. 3 d, Fig. S1 d). We detected significant reductions of leaf area and survival when seedlings were transplanted to S1 population (Fig. S1 a, b, c).

Survival analysis

Survival analysis showed that mortality was significantly higher when seedlings were transplanted to the continuous rather than to fragmented habitat (χ^2 = 304.4, *d.f*. = 1, *P* < 0.001). Along time, survival declined in all sites but faster at site S1, within continuous habitat (Fig. 4 d). The highest seedling mortality occurred when seedlings were transplanted to the S1 population, while the lowest was for Bu population (χ^2= 542.3, *d.f.* = 3, *P* < 0.001) (Fig. 4 d). There was a significantly effect of habitat of origin (χ^2 = 14, *d.f*. =1, *P* < 0.001) and population origin (χ^2 = 16.8, *d.f*. = 3, *P* < 0.001).

Time-course of plant growth variables

Leaf area, leaf number and damage significantly varied through time. For most variables there were significant differences between populations and habitats (Fig. S2). Leaf area was higher in fragmented than in continuous habitat, with the exception of the last census when values were similar in both habitats (note that these variables are measured on survivors). The highest leaf area was for Bu population, the lowest for S1, while intermediate values for Cp and S2 (Fig. S2 c). There was an increase in leaf damage along time, being the higher probability of leaf damage was at Cp site and the lower in Bu (Fig. S2). The number of leaves was variable for each transplant site, but Bu was the transplant site with higher leaf number while S1 the lowest (Fig. S2 b).

Discussion

This study provides evidence of environmental and adaptive phenotypic differentiation of *A. aurantiaca* populations at Los Tuxtlas rainforest between continuous and fragmented habitats, even though no genetic differentiation between populations was detected using neutral molecular markers. We found that local genotypes attained higher leaf area than foreign genotypes, indicating local adaptation of *A. aurantiaca* to the conditions of the understory in continuous forest. Moreover, only plants locally adapted to shade conditions could growth in continuous rainforest. We also documented both trait

plasticity and origin genetic effects; seedlings exhibited a plastic response to the changes in the understory environment, and origin effects for leaf area and leaf number, suggesting a genetic component of plant performance. In general, plants were more likely to survive and grow in the fragmented rainforest. However, the plants derived from the continuous habitat perform better in both continuous and fragmented habitat.

Neutral genetic diversity and local adaptation

It was expected that fragmented populations will exhibit lower levels of genetic diversity at neutral loci, and thus local adaptation would be less likely (Willi *et al.* 2006; Lopez *et al.* 2009; Leimu *et al.* 2010). However, we could not test this hypothesis since both fragmented and continuous populations of *A. aurantiaca* exhibited high genetic diversity (A= 8, H_o=0.53, H_e=0.65) and apparently the populations possess adaptive potential to respond to current and future environmental change. Nevertheless, unlike *A. aurantiaca* populations in fragments, continuous forest populations were locally adapted and exhibited larger effective population sizes (N_e = 528). These findings suggest that large effective population sizes of *A. aurantiaca* in continuous forest can maintain both neutral and quantitative genetic variation, thus increasing local adaptation and evolutionary potential. These results agree well with the expectation that local adaptation is more common in large populations (Leimu & Fischer 2008), above the critical effective population size to permit local adaptation (N_e > 100 individuals) (Lopez *et al.* 2009), and with large effective population sizes (*Ne* of 500 to 1000) necessary to retain evolutionary potential (Lande & Barrowclough, 1987; but see Lande, 1995; Franklin & Frankham, 1998).

Genetic differentiation of *A. aurantiaca* population was very low (R_{st} =0.02), with high indirect gene flow. However, recent migration rates in this species showed that, in general, most gene flow is low and restricted within populations, with events of long distance dispersal mostly from the continuous habitat. These results and the finding of local adaptation suggest that although long distance gene flow may homogenize populations within each habitat, it is likely that selection in the continuous habitat is

acting against genotypes or migrants derived from fragmented habitats. Furthermore, the one migrant per generation rule can prevent the negative effects of inbreeding and genetic drift, but still allowing adaptive divergence (Mills & Allendorf 1996; Lopez *et al.* 2009). On other hand, the mixed mating system of *A. aurantiaca* and past demographic fluctuations (contractions and expansions) at Los Tuxtlas rainforest may explain the maintenance of high neutral genetic diversity and low genetic differentiation among populations (Suárez-Montes *et al.* 2016).

The negative effects of inbreeding may affect traits involved in local adaptation or modify the constraints to trait evolution (Angeloni, Ouborg & Leimu 2011; Leimu *et al.* 2012). We found that populations in fragmented habitat have higher inbreeding (F_{is} = 0.18) in comparison with populations at the continuous habitat (F_{is} = 0.05). However, since *A. aurantiaca* is a self-compatible species, the deleterious alleles may be purged thus reducing the negative fitness effects of inbreeding through time. This could be related to higher outcrossing rate in continuous or strong inbreeding depression. Further studies are needed to discard these hypotheses.

Evidence of local adaptation

The reciprocal transplant experiment showed local adaptation of *A. aurantiaca* to the continuous rainforest; local plants from continuous rainforest exhibited higher leaf area than foreign plants from fragmented habitat to maintain positive growth in the shaded understory. Leaf area is related to better plant performance. Nonetheless, plants of *A. aurantiaca* generally performed better in the fragmented habitat regardless their origin. Our analysis suggests that, unlike fragments, continuous rainforest conditions are more heterogeneous and harsher, reducing plant survival. Hence, a gain in leaf area contributes to survival. It is known that light filtered through the forest canopy is the most variable physical factor in the tropical forest understory, characterized by a mosaic of shade sunflecks and small gaps (Théry 2001). We found that light availability was twice higher in fragments than in continuous habitat; higher light availability in forest fragments seems to

enhance plant survival, and production of leaf area of *A. aurantiaca*. Survival analyses showed that fragmented habitat is a better transplant site for plant survival, whereas mortality was higher for all plants, of all origins, at the continuous habitat, particularly at S1. A similar trend was detected for *Nectandra ambigens*, a tolerant canopy tree in the same study area (Chávez-Pesqueira & Núñez-Farfán 2016). Therefore, higher selection pressure may be associated with harsher environmental conditions in the continuous habitat for *A. aurantiaca* at Los Tuxtlas rainforest. Our results showed that habitat fragmentation disrupts the process of local adaptation (also see Templeton et al., 2001); however, such a disruption for *A. aurantiaca* in fragmented rainforest is mainly due to environmental changes and reduction of quantitative genetic variation.

The life history of *A. aurantiaca* and the rainforest dynamics could explain why plants of continuous forests are locally adapted, and why this species performs better in fragments. The abundance of *A. aurantiaca* is related to the forest regeneration cycle; it could survive both in shade conditions and high levels of light at or nearby forest gaps that favour plant growth (Calvo-Irabién 1989, 1997). However, although *A. aurantiaca* generally performs better in the fragmented habitat, only plants derived from continuous habitat exhibited a trend of higher performance (except for leaf damage). These results also suggest that plants from continuous forest may have a higher capacity to survive and growth in both habitats, and cope with altered environmental conditions.

Population level and local adaptation

At the population level, the statistical significance of the interaction between transplant site and population origin did not provide support for local adaptation on survival, leaf area, or leaf damage. Local plants generally did not outperform foreign plants; similarly, no population of origin exhibited its best performance at home. Although the statistical contrast suggests a lack of local adaptation at the population level, the general results are consistent with a trend of local adaptation to habitat. Continuous population S1 showed a trend that local plants performed better (in terms of survival and growth) than foreign plants. The most likely results responsible for the significant differences among population

were the harsh conditions at locality of S1 population in comparison to other populations. In this site, mortality was extremely high, but local genotypes survived better.

Previous studies (Calvo-Irabién 1989) reported that *A. aurantiaca's* herbivory rates at Los Tuxtlas are low (< 10% in the total leaf area lost), perhaps due to the absence of specialised herbivores or the presence of potent defensive secondary metabolites (Wasshausen 1975). Here, we also found low and similar levels of herbivory in the continuous and fragmented habitat (20-30%). However, most individuals received higher damage when transplanted to the fragmented habitat (Fig. 3 c). Moreover, all plants transplanted to S1 population exhibited lower leaf damage regardless their origin; this could be related with to absence of herbivores and pathogens in this site. Likewise, it may suggest that fragments favour generalized, opportunistic herbivores (but see Büchi & Vuilleumier, 2014; Chávez-Pesqueira et al., 2015).

Conclusion

Our study contributes to understand how tropical rainforest plants respond to new physical factors and selective pressures brought about by habitat fragmentation. Evidence shows that populations of *A. aurantiaca* are locally adapted to the heterogeneous environment of the continuous rainforest. Likewise, our results suggest that habitat fragmentation may modify the pattern of local adaptation due to micro-environmental changes. Physical conditions of continuous forests are harsh; the shady understory forest and gaps of forest regeneration impose selective pressures to which *A. aurantiaca* seems locally adapted. Therefore, only those plants adapted to both shady and light conditions could growth in the continuous forest. In the fragmented habitat plant growth and survival were higher due to favourable light conditions; nevertheless, plants derived from continuous forest showed a trend of better performance both in fragments and continuous forest. This suggests that plants from continuous forest have a higher potential to respond to environmental changes. The potential of *A. aurantiaca* to respond to different environments could be explained by its life history and its relation to the forest

regeneration cycle. The differential response of *A. aurantiaca* seedlings to both habitats highlights the importance of studying and preserving both continuous and fragmented populations of tropical rainforest. However, long-term monitoring of rain forest plant species inhabiting small fragments are needed to know if they could cope with future environmental changes.

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Figures and legends

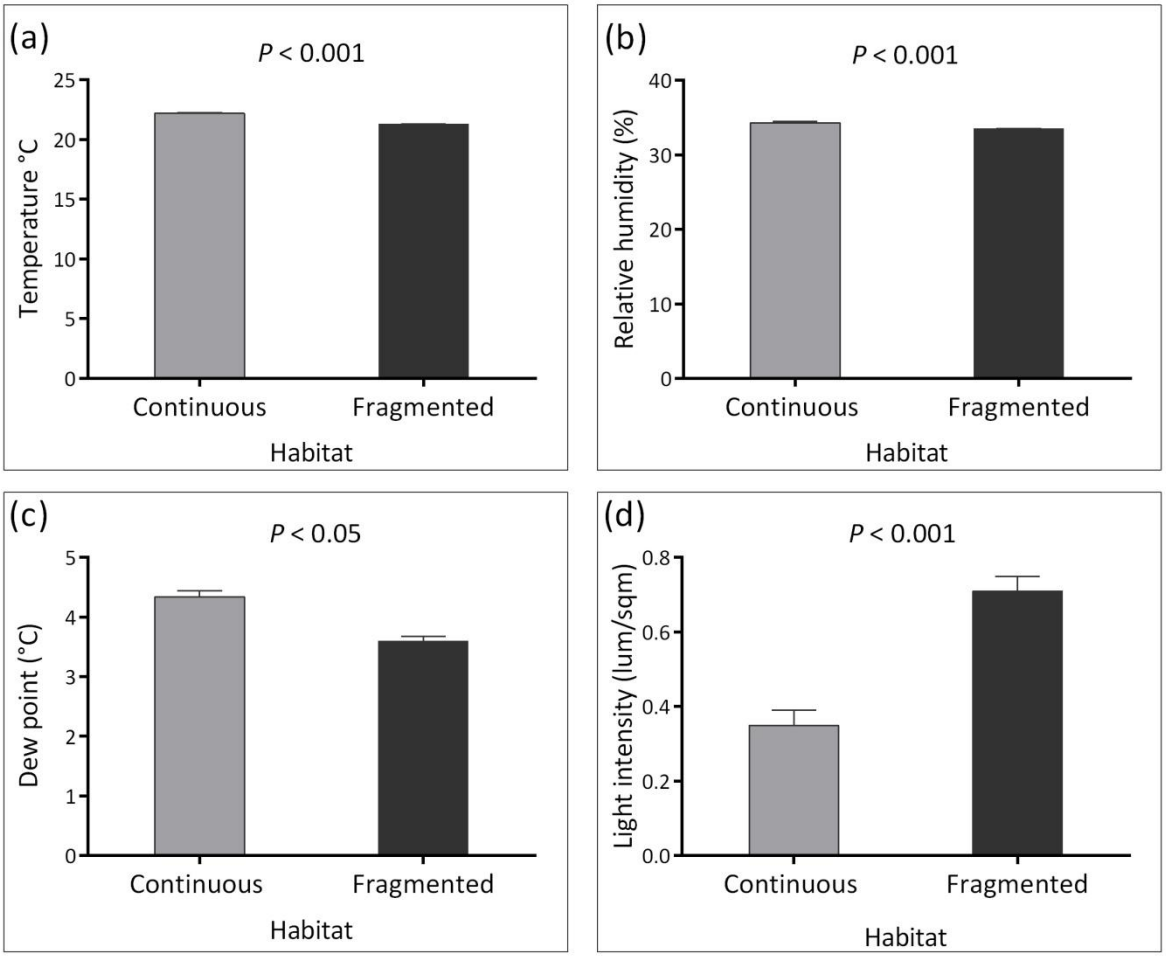

Figure 1. Environmental variables of localities of *Aphelandra aurantiaca* at Los Tuxtlas. Average values (+1 S.E.) of (a) temperature, (b) percentage of relatively humidity, (c) dew point, and (d) light intensity.

Table 1. Genetic structure of *Aphelandra aurantiaca* in fragmented and continuous forest at Los Tuxtlas rainforest.

Population	Forest size (ha)	N_e	A	H_e	H_o	F_{is} (C.I.)	R_{st}	m (95% credible set)
CP	8	68.2	5.72 ± 2.10	0.57 ± 0.12	0.66 ± 0.12	0.13(0.03,0.20)	-----	0.07(-0.1,0.2)
Bu	17	35.7	6.63 ± 2.94	0.50 ± 0.14	0.64 ± 0.13	0.21(0.09,0.31)	-----	0.07(-0.1,0.3)
$\bar{x}$ Fragments		99	8.09 ± 3.36	0.53 ± 0.13	0.65 ± 0.12	0.18(0.11, 0.23)	0.02	0.07 (-0.4,0.2)
S1	320	510	6.27 ± 2.32	0.55 ± 0.18	0.59 ± 0.12	0.06(-0.03,0.13)	-----	0.01(-0.002,0.02)
S2	320	infinite	6.27 ± 2.86	0.611 ± 0.18	0.64 ± 0.16	0.04(-0.03,0.10)	-----	0.08(-0.1,0.3)
$\bar{x}$ Continuous		528.5	7.54±3.56	0.58±0.16	0.63±0.13	0.07(0.01, 0.11)	0.03	0.04(-0.3,0.21)

N_e= effective population size; A= number of alleles per locus; H_e= expected heterozygosity; H_o= observed heterozygosity; F_{is}= inbreeding coefficient; R_{st}=population differentiation; Nm= gene flow estimated through R_{st}; m = is the fraction of individuals in each population that are migrants from other population.

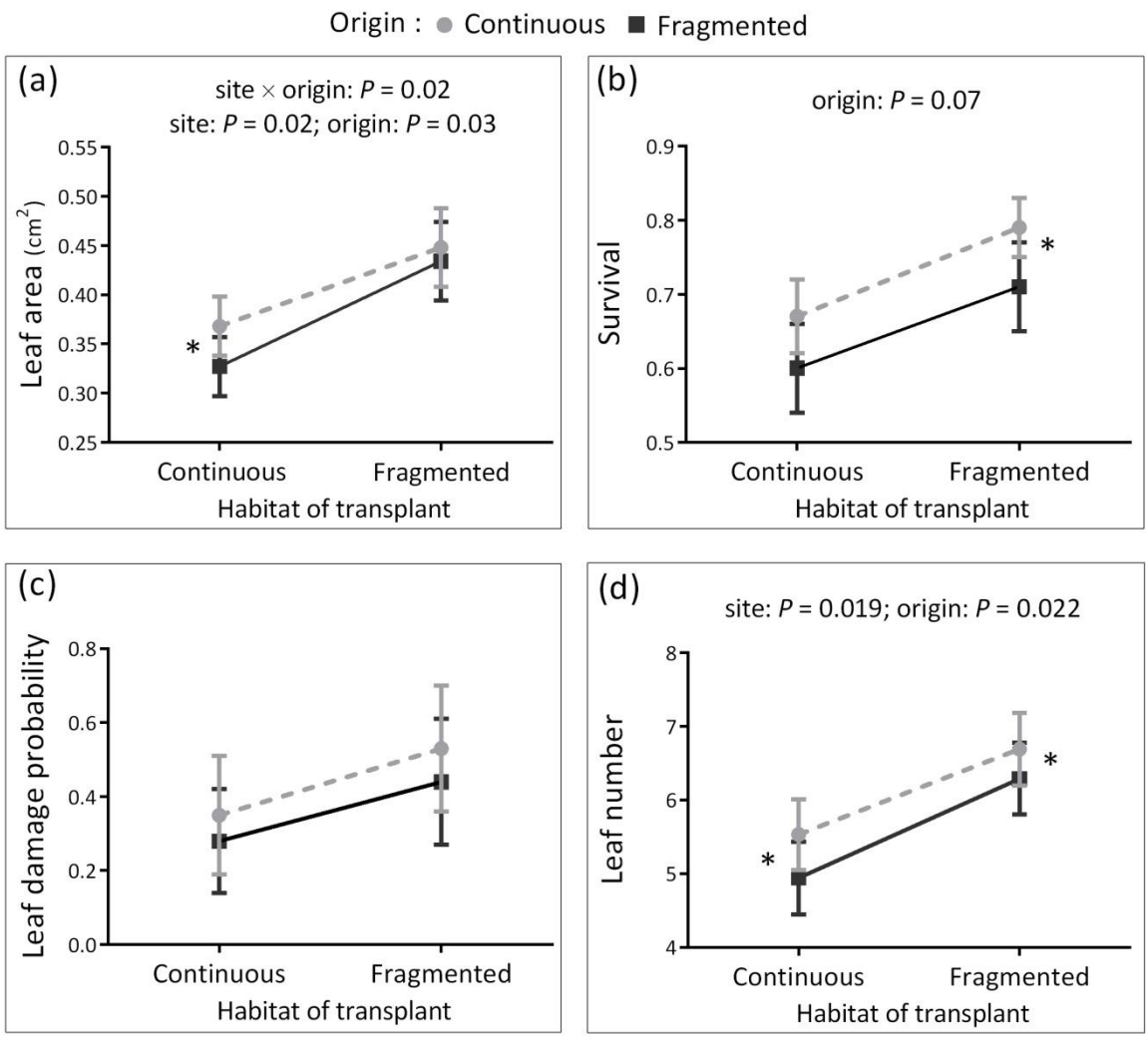

Figure 2. Performance of *Aphelandra aurantica* seedlings from continuous (circle) and fragmented (square) forest grown at their local or foreign sites. (a) Leaf area, (b) survival, (c) leaf damage, and (d) number of leaves for continuous and fragmented following the "local-foreign" and "home away" criteria. Only significant effects of site (habitat of transplant: Continuous and Fragmented), origin (habitat of origin: Continuous and Fragmented) and their interaction site × origin are listed in each panel. Error bars show standard errors. *, significant pairwise contrast tests $P < 0.05$. See full statistical results in Table S4 and TableS5.

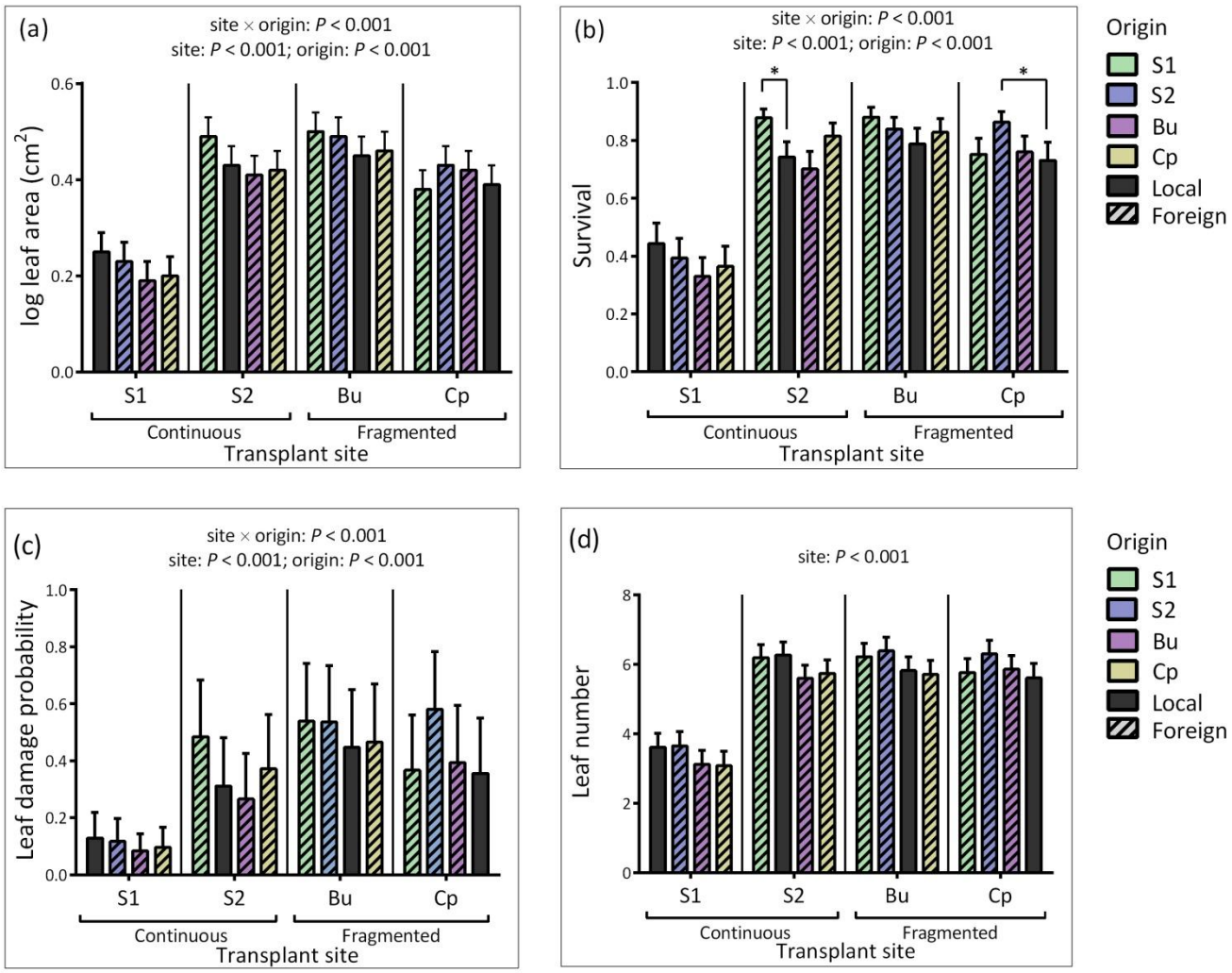

Figure 3. Local vs. foreign and home vs. away criteria analyses of (a) leaf size, (b) survival, (c) leaf damage, and (d) leaf number of *Aphelandra aurantiaca* seedlings reciprocally transplanted to fragmented and continuous forests habitats. Smooth bars indicate local population while striped bars indicate foreign populations. Only significant effects of site (transplant site: S1, S2, Bu, Cp), origin (site of origin: S1, S2, Bu, Cp) and their interaction site × origin are listed in each panel Error bars show standard errors. See full statistical results in Table S6. *, significant difference $P < 0.05$.

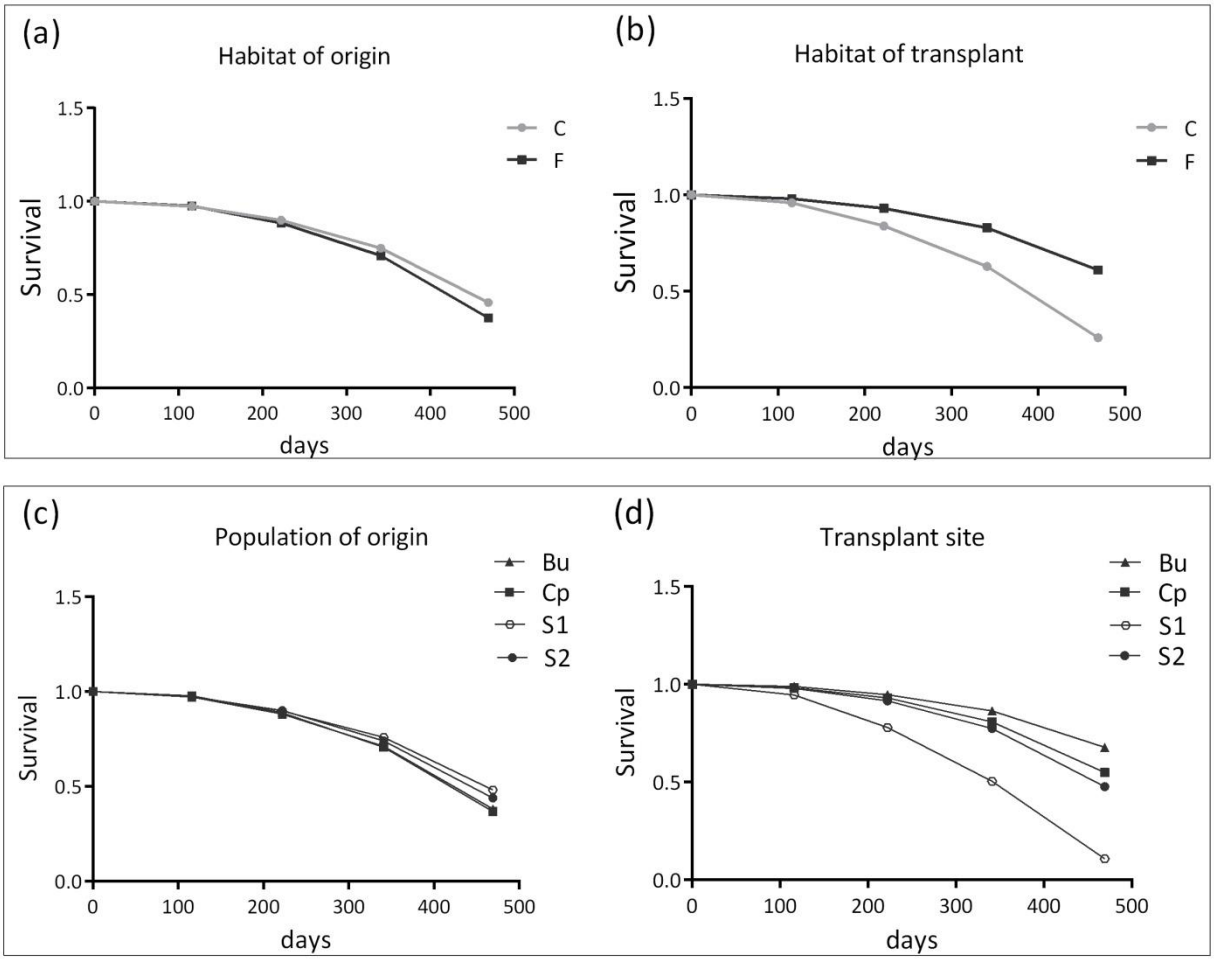

Figure 4. Survival of *Aphelandra aurantiaca* seedlings through time (days) in a reciprocal transplant experiment between (a) habitat of origin and, (b) habitat of transplant, (c) population of origin, and (d) transplant site.

Supporting Information

Appendix

Table S1. Microsatellite loci of *Aphelandra aurantiaca* used for genetic analyses.

Locus name	Motif repeat	Primer sequence	Allele size	GeneBank Accession no.
5490	$(ATGC)_{24}$	F: GGTGTACGTAGCCCACAACG	174-184	SRR1816884
		R:TGAAGAAGTTGTTCCAAGGTACG		
0432	$(AGCC)_{24}$	F:AGGCTGAAGAGATTTGCAGG	113-124	SRR1816885
		R:AAGACAGGCTGATGCAGTCG		
4343	$(ATT)_{27}$	F: TGTAAAGGAAAGTTGAAGAAATAAGGG	150-172	SRR1817142
		R: TGATTCGTTGGAGACACATGC		
1233	$(AT)_{22}$	F:GTTGCATTTGAGGCATGAGG	116-126	SRR1514097
		R:TGTAATTGAACTAGGTCTTGTACTCGC		
4914	$(AT)_{22}$	F: AGGAATTGTCCGGTCTTCCC	130-152	SRR1817143
		R:CCGGCTGATTCTGCTTCC		
1810	$(AC)_{26}$	F:TGGCACTTATAGCCACATCCC	194-207	SRR1817168
		R:GAACCAGTGTTGCGTGTCC		
4883	$(TC)_{30}$	F:GATGGAGGCAGTGGAGATAGC	206-229	SRR1817184
		R:GCAGAATCTTCTGGAACCACC		
5250	$(TC)_{28}$	F:TTCCTTCTTGTTGTTATTCTTGGC	208-293	SRR1817169
		R:GGAACAAAGAGTCATGATTGAAGC		
5441	$(TC)_{30}$	F:CAAAGACCTGTAATAGATATAAGGAAGCC	200-300	SRR1817260
		R:AACTTAATGGACCATGTCGGC		
1808	$(AGT)_{30}$	F:TGCGTGTCTTTGTTGTACTATCTGG	294-318	SRR1817198
		R:AATGCTCAAGGCATGCACC		
4536	$(TGC)_{21}$	F: AAGAATTGTAATCCTTGAAAGCCC	187-193	SRR1817191
		R:GGAAATTTATATGGAATGCCGC		

Table S2. Environmental characteristics of localities and habitats used for reciprocal transplant experiments of *Aphelandra aurantiaca* at Los Tuxtlas rainforest. Average values and ± 1 S.E. are shown.

	Population	Temperature °C	% Relatively humidity	Dew point °C	Light intensity(lum/m^2)
Fragment	Bu	22±2	29.7±11	2.8±3	1.18±4
	Cp	20.7±5	36.1±14	4.2±7	0.36±2
	Total	21.3±4	33.5±13	3.6±6	0.71±3
Continuous	S1	22.7±6	38.3±22	6.0±8	0.36±2
	S2	21.7±5	29.5±14	2.2±5	0.34±3
	Total	22.2±5	34.3±19	4.3±7	0.35±2

Table S3. Migration rate (*m*) between pairs of populations of *Aphelandra aurantiaca* in fragments and continuous sites at Los Tuxtlas.

Pair populations		**BayesAss**	**95% credible set**	***Nm***
j	***i***	***m* (S.D.)**		**Based on *Fst***
Cp	Bu	0.006(0.006)	-0.006,0.018	_____
S1	Bu	0.006(0.006)	-0.005,0.018	_____
S2	Bu	**0.203(0.025)**	0.152,0.254	_____
Average *m* from Bú population		0.072(0.113)	-0.150,0.294	_____
Bu	Cp	0.006(0.006)	-0.005,0.019	_____
S1	Cp	0.006(0.006)	-0.006,0.020	_____
S2	Cp	**0.219(0.022)**	0.174,0.263	_____
Average *m* from Cp population		0.077(0.122)	-0.162,0.317	_____
Average for fragmented habitat		0.074(0.105)	-0.447,0.282	0.12
S2	S1	**0.217(0.023)**	0.171,0.262	_____
Bu	S1	0.020(0.011)	-0.0006,0.042	_____
Cp	S1	0.006(0.006)	-0.005,0.018	_____
Average *m* from S1 population		0.010(0.006)	-0.006,0.019	_____
S1	S2	0.006(0.006)	-0.006,0.019	_____
Bu	S2	0.018(0.010)	-0.002,0.038	_____
Cp	S2	0.006(0.006)	-0.006,0.019	_____
Average *m* from S2 population		0.010(0.006)	-0.002,0.023	_____
Average for continuous habitat		0.045(0.084)	-0.328,0.210	0.08

m [*i*][*j*]: is the fraction of individuals in population *i* that are migrants derived from population *j* (per generation). Indirect measures of gene flow (*Nm*)

Table S4. Effect of habitat of transplant (continuous, fragments), habitat of origin (continuous, fragments) and their interaction on leaf area, plant survival, proportion of leaf damage, leaf number of *Aphelandra aurantiaca* seedlings reciprocally transplanted at Los Tuxtlas rainforest.

Response variable	Leaf area-habitat			Survival-habitat			Leaf damage-habitat			Leaf number-habitat		
	dAIC	χ^2	*P*	dAIC	χ^2	*P*	dAIC	χ^2	*P*	dAIC	χ^2	*P*
hab. site× hab. origin	-2.7	4.72	**0.02**	1.3	0.76	0.38	0	0	1	0	1.47	0.22
site	-3.6	7.63	**0.02**	1	3.02	0.22	0	3.04	0.21	9	7.859	**0.019**
origin	-3	6.99	**0.03**	1.1	5.31	0.07	0	4.35	0.11	4	7.572	**0.022**
covariable	211.6	213.5	**<0.001**	---------	---------	---------	---------	---------	---------	124	126	**<0.001**
Random effects	SD (Intercept)			SD (Intercept)			SD (Intercept)			SD (Intercept)		
Family	0.07			0.73			0.72			0.82		
Block/site	0.12			0.95			0.88			0.32		
Residual	0.21			0.96			0.97			2.62		
Marginal R^2	0.084			0.021			0.01			0.04		
Conditional R^2	0.36			0.32			0.60			0.23		
AIC(full model)	-840.4			6585.8			6674			8908		

Log-likelihood ratio test was used to obtain χ^2 test statistic. We report delta AIC (dAIC) values for model comparison

Table S5. Pairwise contrast tests of leaf area, survival, leaf damage and number of leaves of seedlings of *Aphelandra aurantiaca* in fragments and continuous forest at Los Tuxtlas.

	Habitat of transplant			
	Continuous		**Fragmented**	
Habitat of origin	**Leaf area (SE)**	**Pairwise differences of contrast**	**Leaf area (SE)**	**Pairwise differences of contrast**
Continuous	0.36 (0.03)	t = 2.91, ***P* = 0.03**	0.44 (0.04)	t = 0.76, P = 0.44
Fragmented	0.32 (0.04)		0.43 (0.04)	
Habitat of origin	**Survival (SE)**	**Pairwise differences of contrast**	**Survival (SE)**	**Pairwise differences of contrast**
Continuous	0.67 (0.05)	Z = 1.77, P = 0.07	0.79 (0.04)	Z=2.31, ***P* = 0.02**
Fragmented	0.60 (0.06)		0.71 (0.06)	
Habitat of origin	**Leaf damage (SE)**	**Pairwise differences of contrast**	**Leaf damage (SE)**	**Pairwise differences of contrast**
Continuous	0.35 (0.16)	Z = 1.91, ***P* = 0.05**	0.53 (0.17)	Z = 2.0, P = **0.04**
Fragmented	0.28 (0.14)		0.44 (0.17)	
Habitat of origin	**Number of leaves (SE)**	**Pairwise differences of contrast**	**Number of leaves (SE)**	**Pairwise differences of contrast**
Continuous	5.53 (0.48)	t = 2.76, ***P* = 0.006**	6.69 (0.49)	t = 1.95, ***P* = 0.05**
Fragmented	4.94 (0.49)		6.29 (0.49)	

Table S6. Transplant site, population of origin and their interaction on leaf area, survival, proportion of leaf damage and leaf number of reciprocally transplanted *Aphelandra aurantiaca* seedlings at Los Tuxtlas rainforest.

Response variable	Leaf area			Survival			Leaf damage			leaf number		
	dAIC	χ^2	*P*	dAIC	χ^2	*P*	dAIC	χ^2	*P*	dAIC	χ^2	*P*
site × origin	-12.4	30.45	**<0.001**	33	51.23	**<0.001**	28.3	46.24	**<0.001**	12	7.8716	0.6414
site	-24	47.99	**<0.001**	49.7	73.624	**<0.001**	41.5	65.57	**<0.001**	11	34.62	**<0.001**
origin	-9.63	33.68	**<0.001**	32.8	56.76	**<0.001**	26.9	50.83	**<0.001**	9	14.84	0.250
covariable	-211.4	213.4	**<0.001**	---------	---------	---------	---------	---------	---------	124	125.8	**<0.001**
Random effects	SD (Intercept)			SD (Intercept)			SD (Intercept)			SD (Intercept)		
Family	0.075			0.76			0.74			0.81		
Block/site	0.095			0.51			0.52			0.74		
Residual	0.216			0.96			0.97			2.46		
Marginal R^2	0.17			0.16			0.07			0.10		
Conditional R^2	0.36			0.33			0.67			0.22		
AIC(full model)	-790.1			6537.5			6634.8			8945.2		

Log likelihood ratio test were used to obtain χ^2 test statistic. We report delta AIC (dAIC) values for model comparison.

R code models used to test for local adaptation and change through time for seedlings. a) leaf area, b) survival rate, c) leaf damage, d) number of leaves. R code models used to test change a long time e) leaf area, f) survival rate, g) leaf damage, and h) number of leaves.

a) glmer (survival~site*origin +(1|families)+(1| block/ site), family="binomial")

b) glmer (leaf damage~ site*origin +(1|families)+(1| block/ site), family="binomial")

c) lmer (leaves~initial leaves+site*origin+ (1|families) +(1| block/ site))

d) lmer (leaf area ~ initial area + site*days +origin*days + (1|families)+(1|bloc/site))

e) glmer (survival ~ site*days +origin*days + (1|families)+(1|bloc/site), family="binomial")

f) glmer (leaf damage~ site*days +origin*days +(1|families)+(1| block/ site), family="binomial")

g) lmer (leaves~ initial leaves + site*days +origin*days + (1|families) +(1| block/ site))

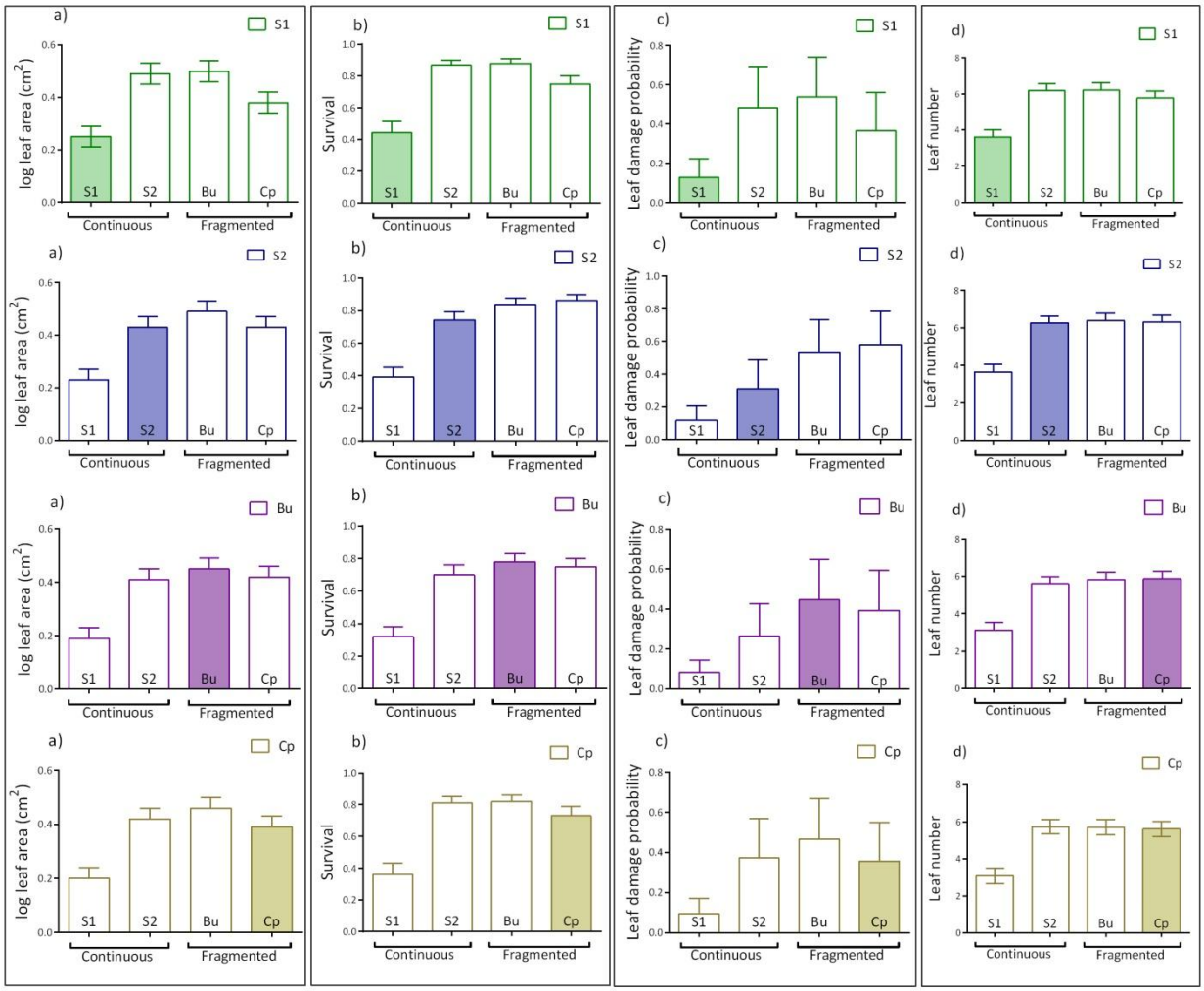

Figure S1. Home-Away criterion contrasts for *Aphelandra aurantiaca* plants reciprocally transplanted to four populations at the continuous and fragmented habitats: (a) leaf size, (b) survival, (c) leaf damage and, (d) leaf number. The "home" population (native to the site in question) is indicated by colors bars and "away" populations are indicated by open bars. Small rectangle in the upper-right corner of each panel indicates "home" population. Error bars show standard errors.

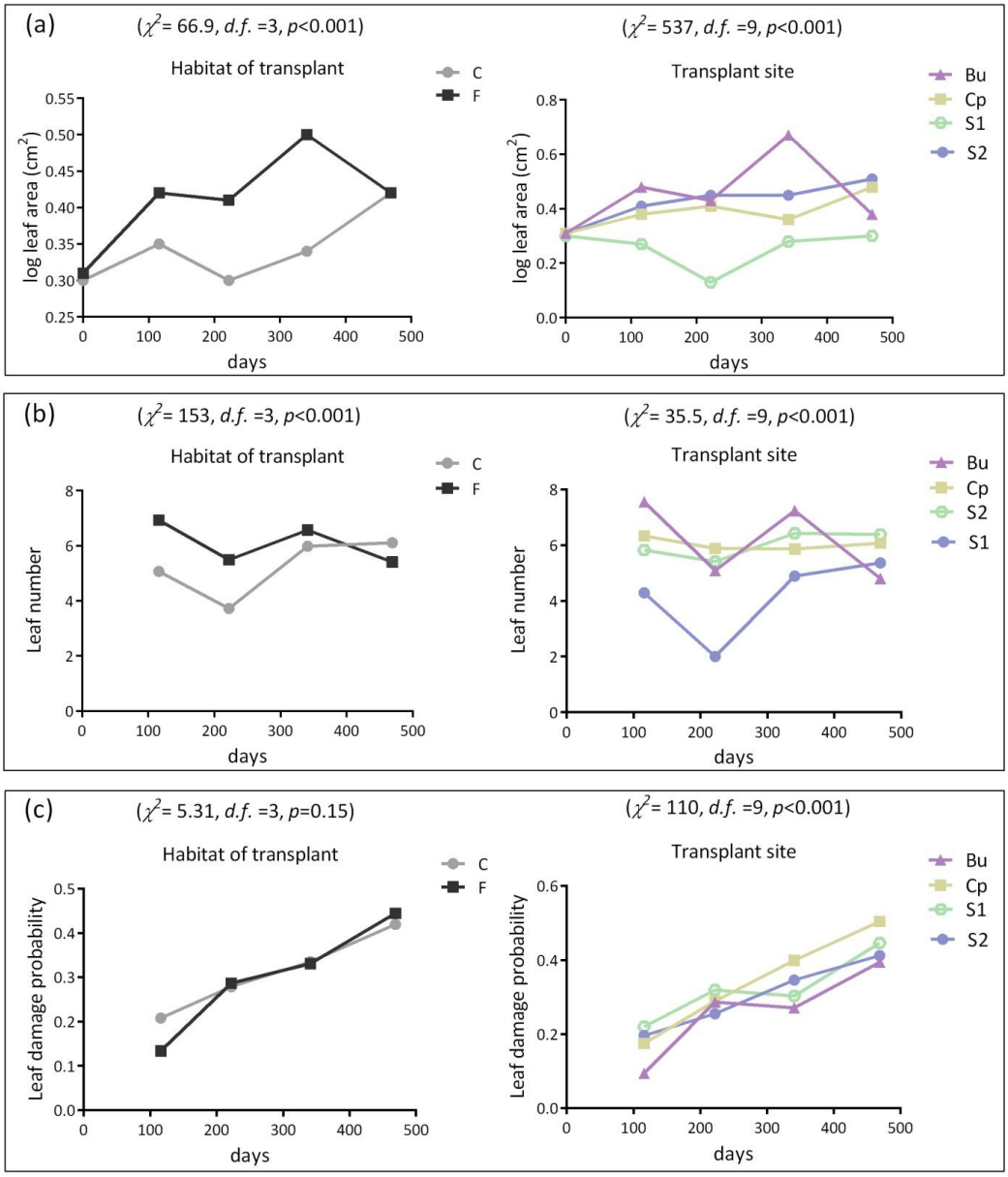

Figure S2. Relationship among time (days) in habitat of transplant and site of transplant for time –course of (a) leaf area, (b) leaf number, (c) and leaf damage probability of *Aphelandra aurantiaca* seedlings transplanted to fragments and continuous habitats.

Discusión general

La fragmentación del hábitat crea nuevos escenarios ambientales y presiones selectivas que influyen en las especies, principalmente en aquellas propias de hábitats continuos. Actualmente, muchas áreas naturales están severamente fragmentadas y reducidas a pequeñas poblaciones aisladas. Una pregunta fundamental en ecología evolutiva es cómo las plantas responden a estos escenarios y los mecanismos relacionados en el proceso. Por lo tanto, estudiar el impacto ecológico y genético, así como las implicaciones evolutivas de la fragmentación del hábitat es de suma importancia para la conservación de la diversidad biológica. La presente tesis evalúa los efectos de la fragmentación en la diversidad genética de *A. aurantiaca* en la selva de Los Tuxtlas, así como la respuesta evolutiva de esta especie ante los cambios ambientales debidos a la fragmentación.

En el Capítulo 2 evaluamos los efectos de la fragmentación en la estructura genética de *A. aurantiaca* en fragmentos de diferentes tamaños, el flujo génico, la tasa de polinización cruzada y los patrones de movimiento de polen. Encontramos que a pesar del alto grado de fragmentación en la selva de Los Tuxtlas, las poblaciones de *A. aurantiaca* tienen altos niveles de variación genética neutral (H_e = 0.61), incluso en los fragmentos más pequeños y aislados. Los altos niveles de variación genética coinciden con los altos valores reportados para especies perennes de vida larga (Nybom, 2004) y otras especies de acantáceas que también habitan el sotobosque (Shapcott 2007; Vargas-Mendoza *et al.* 2015). En general, los tamaños efectivos (N_e) son grandes, lo cual sugiere que la fragmentación no ha disminuido suficientemente los tamaños poblacionales para detectar un impacto en la diversidad genética neutral. Sin embargo, los tamaños efectivos tienden a decrecer conforme disminuye el área de las poblaciones, siendo menor en los fragmentos más pequeños.

La diferenciación genética poblacional es baja (R_{ST} = 0.037) y los análisis de agrupamiento indican que la mayoría de las poblaciones comparten información genética de un grupo o comunidad, sugiriendo flujo génico y conectividad entre las poblaciones. Los altos niveles de diversidad genética, los tamaños efectivos relativamente grandes y la baja diferenciación genética en las poblaciones de *A. aurantiaca* están más relacionados con los procesos históricos que con la

fragmentación del hábitat actual. Probamos varias hipótesis sobre el pasado demográfico de las poblaciones de *A. aurantiaca* en Los Tuxtlas, y detectamos evidencia de procesos de extinción y expansión poblacional. Esto podría explicar los bajos niveles de diferenciación poblacional.

Un resultado relevante para la conservación de la selva de Los Tuxtlas es que la mayor tasa de migración se origina partir de los fragmentos grandes y medianos. Este resultado es consistente con lo reportado para *Carica papaya* en Los Tuxtlas, un mayor porcentaje de genes se mueve del continuo hacia los fragmentos (Chávez-Pesqueira et al 2015). Por lo tanto, la conservación de estas áreas es vital pues actúan como reservorios genéticos que ayudan a evitar el aislamiento genético. Sin embargo, los fragmentos pequeños también deben ser considerados en los planes de conservación, los polinizadores pueden utilizar una serie de fragmentos pequeños para forrajear ayudando a mantener la conectividad en el paisaje (Stouffer & Bierregaard 1995; Kraemer 2001). En particular, se sabe que algunas especies de colibríes pueden volar grandes distancias, sin embargo, aún es necesario evaluar el movimiento de los colibríes en la zona fragmentada de Los Tuxtlas y evaluar su relación directa con el éxito reproductivo de *A. aurantiaca*.

El sistema de apareamiento es uno de los factores más importantes para determinar los patrones de variación genética, en específico, la incapacidad de auto-fertilizarse juega un papel mayor en la vulnerabilidad de las especies a la fragmentación (Honnay & Jacquemyn 2007; Aguilar *et al.* 2008). Sin embargo, *A. aurantiaca* no es una especie tan vulnerable a los efectos de la fragmentación ya que es una especie autocompatible (Calvo-Irabién 1989; Islas Luna 1995), además la estimación de las tasas de polinización cruzada indican que presenta un sistema de apareamiento mixto. Esto sugiere que las poblaciones estudiadas de *A. aurantiaca* en Los Tuxtlas tienen una alta tasa de polinización cruzada, pero que también pueden autofertilizarse y garantizar el éxito reproductivo en la ausencia de polinizadores.

El movimiento de polen, junto con la dispersión de semillas son factores que conducen la distribución de la diversidad genética en las poblaciones de plantas, y son importantes para generar la estructura genética dentro y entre las poblaciones (Ottewell *et al.* 2012). En particular, evaluamos la dinámica de movimiento de polen en *A. aurantiaca* en poblaciones de hábitat continuos y hábitat fragmentado a partir de familias maternas. Esperábamos encontrar mayor estructura de polen y menor número de donadores de polen en las poblaciones fragmentadas. Sin embargo, no detectamos un patrón claro sobre los efectos de la fragmentación en la dinámica de movimiento de polen en *A. aurantiaca*. En general, el movimiento de polen contemporáneo

dentro de las poblaciones es limitado y ocurre entre individuos cercanos en distancias relativamente cortas. Además, se ha reportado que la distancia de dispersión de semillas de *A. aurantiaca* también está restringida a unos metros de la planta madre (Calvo-Irabién 1989). La limitada dispersión de polen y de semillas puede promover la subestructuración dentro de las poblaciones, sin embargo también es importante resaltar que los análisis genéticos indican eventos de dispersión de genes a distancia.

En suma, en el Capítulo 2, mostramos que varios factores como el pasado demográfico de *A. aurantiaca*, los caracteres de historia de vida (sistema de apareamiento mixto), el solape generacional, los tamaños efectivos relativamente grandes y los eventos de dispersión a distancia parecen amortiguar los efectos negativos de la fragmentación del hábitat sobre la variación genética neutral y la diferenciación genética poblacional.

En el Tercer Capítulo 3, evaluamos los efectos de la fragmentación en la variación genética cuantitativa de *A. aurantiaca*. En particular, evaluamos la existencia de adaptación local en poblaciones de hábitat continuo y hábitat fragmentado, así como los posibles efectos de la fragmentación sobre la adaptación local a través de un experimento de trasplantes recíprocos.

Las condiciones ambientales del hábitat fragmentado favorecen el desempeño de *A. aurantiaca,* en términos de supervivencia y crecimiento. Por el contrario, crecer y sobrevivir es más difícil en las condiciones de la selva continua, en especial por la limitada cantidad de luz que llega hasta el sotobosque y la gran acumulación de ramas y hojarasca. En las selvas tropicales, la luz es el mayor recurso limitante para el crecimiento, supervivencia y reproducción de las plantas (Chazdon & Pearcy 1991). La mayoría de las especies que habitan el sotobosque son tolerantes a la sombra, debido a que poseen adaptaciones que maximizan la captura de luz e incrementan el fitness que les permite persistir (Valladares & Niinemets 2008). En particular, aumentan la captura luz asignando mayor biomasa en las hojas (Veneklaas & Poorter 1998; Poorter 2005), responden velozmente a los destellos de luz con una rápida inducción fotosintética (Valladares, Allen & Pearcy 1997). También acumulan mayor biomasa en las raíces (Paz 2003), y minimizan la pérdida de carbono a través de bajas tasas de respiración, hojas longevas, almacenaje de carbono y defensas contra herbívoros y patógenos (Veneklaas & Poorter 1998).

Sin embargo, algunas especies tolerantes también pueden responder positivamente a la apertura de claros y a la mayor disponibilidad de luz (Martínez Ramos, 1985; Denslow 1987; Dirzo

et al., 1992; Westerband & Horvitz 2017). Este puede ser el caso de *A. aurantiaca,* ya que está altamente relacionada con la dinámica de regeneración de la selva. Si bien la mayor parte de su ciclo de vida transcurre en zonas con baja disponibilidad de luz, se sabe que con la apertura de claros y el incremento de luz puede aumentar su crecimiento, producir mayor número de óvulos e incrementar su abundancia (Calvo-Irabién 1989). Además, el experimento de transplantes recíprocos muestra que *A. aurantiaca* tiene una respuesta fenotípicamente plástica ante cambios ambientales de corto plazo.

Para entender las diferencias en el crecimiento de *A. aurantiaca* en el hábitat continuo y fragmentado, es necesario considerar otras características foliares que permiten la adaptación a los diferentes niveles de luz, principalmente aquellas relacionadas con la tasa de crecimiento relativa (RGR), una medida que resume la ganancia y pérdida de carbono (Poorter 2002, 2005). También es necesario comparar componentes morfológicos relacionados con la arquitectura y el despliegue de las hojas (Valladares, Skillman & Pearcy 2002; Poorter 2005), así como determinar si la plasticidad que exhibe *A. aurantiaca* es adaptativa.

A pesar de las condiciones favorables para el desempeño de *A. aurantiaca* en el hábitat fragmentado, los resultados del experimento de trasplantes recíprocos indican que las plantas del hábitat continuo tienen mayor capacidad para responder ante las difíciles condiciones de la selva continua. La interacción del genotipo × ambiente fue significativa para el área foliar, y el área foliar de los individuos locales fue significativamente mayor en la selva continua que en los individuos foráneos de los fragmentos. Esto sugiere que las plantas del hábitat continuo están localmente adaptadas a las presiones de selección impuestas por las condiciones ambientales de la selva continua.

La adaptación local puede deberse a que las poblaciones de *A. aurantiaca* en la selva continua mantienen la variación genética adaptativa necesaria para responder ante las fuertes presiones de selección del hábitat continuo. Sólo aquellas plantas adaptadas a las condiciones de sombra podrán crecer y potencialmente sobrevivir en la selva continua. Asimismo, es posible que las plantas del continuo posean la variación genética necesaria para responder ante cambios ambientales. Se ha sugerido que la adaptación local tiende a promover variación genética biológicamente relevante debido a que ha sido probada y favorecida por selección en al menos un ambiente, esto provee un reservorio de variación genética que puede ser más benéfico en contextos nuevos en comparación con la variación debida a mutaciones nuevas (Hoffmann & Sgrò

2011; Whitlock & Lotterhos 2015). Debido a que la adaptación local es más común en poblaciones grandes que en poblaciones pequeñas (Leimu & Fischer 2008), sugerimos que los grandes tamaños poblacionales del continuo pueden estar relacionados con el mantenimiento de la diversidad genética cuantitativa en caracteres relacionados con la adaptación y con el potencial de adaptación local de *A. aurantiaca.*

Aunque detectamos adaptación local en un solo componente de vigor, es importante resaltar que el área foliar es una característica estrechamente relacionada con el crecimiento, la supervivencia, y el requerimiento de luz de las plantas (Poorter & Bongers 2006). En particular, tiene que ver con la captura de luz, fotosíntesis, acumulación de biomasa y transpiración (Blanco y Folegatti, 2005), siendo muy importante para sobrevivir en el sotobosque de la selva. En el caso de *A. aurantiaca,* el área foliar refleja una mayor capacidad de las plántulas del continuo para crecer y potencialmente sobrevivir ante la limitada disponibilidad de luz de la selva continua (ver Figura 2 a, b, Capítulo 3). Sugerimos que sin esta capacidad, la supervivencia de las plantas del continuo sería mucho menor.

Una de las dificultades de medir adaptación local en especies de plantas perenes como *A. aurantiaca,* es obtener el fitness a lo largo de su vida (Pickup *et al.* 2012). Para poder estimarlo se necesita realizar estudios a largo plazo y estimar la adaptación local a partir de caracteres con consecuencias demográficas (Peterson, Kay & Angert 2016). Una medida directa de fitness ideal para indicar la viabilidad poblacional a largo plazo es la tasa de crecimiento poblacional que integra la supervivencia, reproducción y crecimiento que varían entre las etapas de historias de vida (Stearns 1992). Sin embargo ha sido poco utilizada, en una revisión reciente Gibson *et al.* (2016) encontraron que de los 1046 estudios revisados sólo el 5% considera la tasa de crecimiento poblacional y que la mayoría de los estudios (96%) evalúan adaptación local en sólo una generación. Por lo tanto, es necesario realizar estudios y mediciones a largo plazo en hábitats fragmentados.

Los resultados del Capítulo 3 muestran que la fragmentación de la selva está generando diferenciación en la variación genética cuantitativa de *A. aurantiaca,* debido principalmente a la diferenciación ambiental entre los hábitats, a pesar de los bajos F_{st} y el alto flujo génico entre las poblaciones. Estas diferencias entre las plántulas de los fragmentos y la selva continua sugieren posibles patrones de divergencia a largo plazo, debido a las contrastantes presiones de selección en cada hábitat.

Para comprender mejor la respuesta adaptativa de *A. aurantiaca* en el paisaje fragmentado, es necesario realizar un diseño experimental que permita poner a prueba la hipótesis direccional y variable, incluyendo la variación genética de muchas poblaciones con diferente tamaño efectivo y que incorpore la variabilidad ambiental de las poblaciones(Willi *et al.* 2007; Willi & Hoffmann 2012; Fraser *et al.* 2014; Wood 2014). Si bien los experimentos de transplantes recíprocos son efectivos para probar adaptación local, también son logísticamente demandantes y están limitados a un reducido número de poblaciones (Kawecki & Ebert 2004; Lascoux, Glémin & Savolainen 2016). Además, la variabilidad ambiental detectada en las poblaciones sugiere que *A. aurantiaca* no habita únicamente dos tipos de hábitats; también hay que considerar que un gran número de variables pueden contribuir a la diferenciación adaptativa, incluyendo el clima, suelos, composición de la vegetación, competencia, e interacciones bióticas (Raabová, Münzbergová & Fischer 2007; Turner *et al.* 2010; Kalske *et al.* 2012; Chen & Schemske 2015; Siepielski *et al.* 2016). Por lo tanto, los efectos de la fragmentación en las condiciones micro ambientales y las interacciones bióticas pueden ser variables, generando gradientes a lo largo del paisaje fragmentado. En estos casos, los experimentos de jardín común y gradientes ambientales son alternativas que permiten incorporar un mayor número de poblaciones y ambientes (Lascoux *et al.* 2016).

En general, los resultados encontrados en esta tesis indican que (1) la fragmentación del hábitat no ha afectado los niveles de variación genética neutral, la diferenciación genética poblacional y la tasa de polinización cruzada de *A. aurantiaca*, (2) la falta de efectos negativos de la fragmentación en la estructura genética puede deberse a que los caracteres de historia de vida amortiguan los efectos negativos, además la estructura genética está más relacionada con el pasado demográfico que con la fragmentación actual del hábitat, y finalmente, (3) las plántulas de *A. aurantiaca* se ven favorecidas por las condiciones ambientales del hábitat fragmentado. Sin embargo, en el hábitat continuo, los genotipos locales tienen ventaja sobre los foráneos, solo las plántulas locales pueden acumular mayor área foliar. El incremento del área foliar podría aumentar la adecuación ante las presiones de selección en el sotobosque de la selva continua, en particular ante la limitada disponibilidad de luz. Por el contrario, las plántulas del hábitat fragmentado tienen menor posibilidad de aumentar su área foliar ante las presiones de selección en la selva continua. Esto puede estar relacionado con la reducción de los tamaños poblacionales y la pérdida de variación genética adaptativa en los fragmentos.

Perspectivas

Actualmente, las aproximaciones que se han utilizado por décadas para entender la adaptación local están cambiando. Si bien los experimentos de transplantes recíprocos son el estándar fundamental para demostrar adaptación local, los avances en secuenciación han aumentado el estudio de la adaptación local a través de datos genéticos en lugar de datos fenotípicos como primera observación (Hoban *et al.* 2016). Actualmente, uno de los principales retos de la biología evolutiva es entender las bases genéticas y evolutivas de la adaptación local (Savolainen, Lascoux & Merilä 2013), en este caso las herramientas genómicas son de mucha utilidad porque permiten la identificación de regiones genéticas relacionadas con la adaptación local (Tiffin & Ross-Ibarra 2014). Se espera que estas investigaciones aporten información relevante para la conservación de recursos genéticos ante los efectos de la fragmentación del hábitat y el cambio climático, así como en la producción de cultivos y especies domesticadas (Savolainen *et al.* 2013; Weigel & Nordborg 2015).

Para entender los mecanismos evolutivos se requiere identificar patrones de selección en caracteres que contribuyan a la adaptación local y a las bases genéticas de ese carácter (Anderson *et al.* 2013; Savolainen *et al.* 2013; Tiffin & Ross-Ibarra 2014). Los estudios sobre las bases genéticas tienen dos enfoques principales, caracteres cuantitativos poligénicos, específicamente en la arquitectura genética del carácter, como el número de loci y la distribución de sus efectos. El segundo se basa en los posibles trade-offs a nivel de loci (pleiotropía antagonista, neutralidad condicional) que controlan caracteres bajo adaptación local (Savolainen *et al.* 2013; Tiffin & Ross-Ibarra 2014; Lascoux *et al.* 2016). Existe controversia en cuanto al número de genes y los efectos que tienen sobre la adaptación local, algunos estudios sugieren que muy pocos genes tienen efectos importantes en la adaptación local (Yeaman & Whitlock 2011; Yeaman 2015; Ferris *et al.* 2016), mientras que otros proponen que múltiples genes tienen efectos pequeños y que la suma de los efectos contribuye de manera importante a la adaptación local (Le Corre & Kremer 2012; Savolainen *et al.* 2013; Lascoux *et al.* 2016).

Las bases genéticas se pueden evaluar directamente a través de la identificación de los caracteres que confieren ventaja en el fitness y la adaptación local en las poblaciones. El mapeo de locus cuantitativos (QTLs) es una de las aproximaciones directas más utilizadas para evaluar la arquitectura genética ya que se basan en combinación de análisis del fenotipo y el genotipo (Savolainen *et al.* 2013; Lascoux *et al.* 2016). Regularmente se generan líneas recombinantes (RIL)

a través de cruzas que son genotipificadas o secuenciadas y estudiadas en múltiples ambientes (Savolainen *et al.* 2013; Agren *et al.* 2016; Ferris *et al.* 2016). Asimismo, a partir de los experimentos de jardín común y el uso de marcadores genéticos (SNPs) también se pueden detectar directamente alelos que confieren mayor adecuación en ambientes diferentes (Savolainen *et al.* 2013; de Villemereuil *et al.* 2016; Lascoux *et al.* 2016).

También se pueden estudiar de manera indirecta, con los métodos de escaneo genómico que son utilizados ampliamente para probar loci bajo selección, principalmente en organismos no modelo (Ellegren 2014; Manel *et al.* 2016). La evidencia de que un gen contribuye a la adaptación local se basa en la alta diferenciación del gen entre las poblaciones, así como en la correlación entre las diferencias en las frecuencias alélicas del gen entre las poblaciones y las variables ambientales (Savolainen *et al.* 2013; Hoban *et al.* 2016; Lascoux *et al.* 2016). Por ejemplo, la detección de valores outliers de diferenciación entre poblaciones con diferentes ambientes es una prueba muy utilizada para identificar genes candidatos de adaptación local. Otro ejemplo es la correlación de las frecuencias alélicas (SNP) y las variables ambientales (Fournier-Level *et al.* 2011; Hoban *et al.* 2016; Pais, Whetten & Xiang 2016). Sin embargo, los métodos indirectos son susceptibles a falsos positivos (Lotterhos & Whitlock 2015; Hoban *et al.* 2016). Además, el uso exclusivo de datos genéticos, como el escaneo genómico, no es suficiente para detectar firmas de selección y es mejor aplicarlos en combinación con datos ambientales y fenotípicos (Le Corre & Kremer 2012).

Pocos estudios han evaluado la respuesta adaptativa ante la fragmentación del hábitat a nivel genómico (Fraser *et al.* 2014; Somervuo *et al.* 2014; Fountain *et al.* 2016; Hanski *et al.* 2017), estos son trabajos pioneros y se enfocan en especies bastante estudiadas. Por ejemplo, la mariposa *Melitaea cinxia* ha sido estudiada debido a su disminución poblacional y extinción local en zonas fragmentadas. Se han realizado estudios genómicos a partir de muestras de poblaciones extintas y existentes, utilizaron métodos indirectos de detección de outliers y posibles blancos de selección. Detectaron selección en genotipos asociados con la capacidad de vuelo y colonización en hábitats fragmentados, antes de la extinción de las poblaciones. Sugieren que la respuesta adaptativa ante los rápidos cambios ambientales no es suficientemente fuerte para rescatar a las poblaciones de la extinción (Somervuo *et al.* 2014; Fountain *et al.* 2016; Hanski *et al.* 2017).

Por otra parte, los estudios realizados por Fraser et al 2014 examinan los patrones de variación adaptativa de la trucha de arroyo *Salvelinus fontinalis* bajo un escenario de fragmentación del hábitat natural, ponen a prueba la hipótesis direccional y variable. Utilizan SNPs de regiones

codificantes y QTLs de diferentes caracteres fenotípicos relacionados con el crecimiento, reproducción y estrés. Sus resultados apoyan tanto la hipótesis direccional como la hipótesis variable, encontraron que la fragmentación afecta a la selección natural y que los cambios provocados en la composición genética adaptativa y diferenciación de los fragmentos varían con el tamaño poblacional.

Finalmente, falta mucho por hacer en el estudio de la variación adaptativa en paisajes fragmentados. En estudios futuros, es importante considerar un mayor número de poblaciones, aumentar el conocimiento de factores ecológicos, realizar estudios a largo plazo, así como evaluar los mecanismos y bases genéticas de la adaptación que permitan identificar respuestas específicas de las plantas ante cambios ambientales, esto permitirá proponer planes de manejo de conservación de recursos génicos.

Conclusiones

(1) Evaluamos los efectos de la fragmentación de la selva tropical en plantas herbáceas, en particular, sobre los efectos en la estructura genética y la adaptación local de *Aphelandra aurantiaca*, una de las hierbas dominantes del sotobosque en Los Tuxtlas.

(2) Comparamos la diversidad genética neutral y cuantitativa en poblaciones de hábitat continuo y fragmentado.

(3) *A. aurantiaca* exhibe una alta diversidad genética neutral, con poca diferenciación entre las poblaciones. La mayor parte del flujo génico se origina de fragmentos grandes y medianos funcionando como reservorio de genes.

(4) La fragmentación de la selva está generando diferenciación en la variación genética cuantitativa de *A. aurantiaca,* debido principalmente a la diferenciación ambiental entre los hábitats.

(5) Los genotipos locales del hábitat continuo tienen mayor área foliar que los genotipos foráneos del hábitat fragmentado. Esto sugiere adaptación local en el hábitat continuo y la posible pérdida de variación genética adaptativa en los fragmentos.

(6) Es posible que el aumento del área foliar en la selva continua contribuya al incremento de la supervivencia ante la baja disponibilidad de luz. Planteamos que la baja supervivencia de plantas de los fragmentos trasplantadas en la selva continua se debe a las fuertes presiones de selección en el sotobosque.

(7) Sugerimos que la pérdida de variación genética adaptativa en los fragmentos debido a la reducción del tamaño efectivo poblacional.

(8) En general, *A. aurantiaca* se ve favorecida por las condiciones ambientales del hábitat fragmentado, sin embargo, sólo las plantas originarias del hábitat continuo muestran una tendencia de mejor desempeño en ambos hábitats. Sugerimos que las plantas del continuo tienen mayor capacidad para sobrevivir y crecer en ambos hábitats, así como enfrentar cambios ambientales.

(9) Resaltamos la importancia de combinar experimentos cuantitativos y genética de poblaciones en poblaciones naturales para comprender de manera más integral los efectos de la fragmentación.

(10) Destacamos la conservación de recursos genéticos en selvas fragmentadas.

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APÉNDICE

Efecto de la fragmentación en el éxito reproductivo y el daño en

caracteres reproductivos de *Aphelandra aurantiaca*

Introducción

La fragmentación del hábitat es una de las principales causas de interrupción funcional en las interacciones planta animal. Existe evidencia de que la fragmentación tiene un efecto negativo en la polinización y reproducción de las plantas (Aguilar *et al.* 2006) ya que puede disminuir la diversidad y abundancia de polinizadores, puede promover la limitación por polen y reducir el éxito reproductivo de las plantas (Ashman *et al.* 2004; Newman *et al.* 2013).

Sin embargo, el efecto de la fragmentación en el éxito reproductivo de las plantas es variable y está relacionado con el grado de dependencia y especialización de los polinizadores. Las especies de plantas auto-incompatibles son más vulnerables ante los efectos negativos de la fragmentación debido a la estrecha dependencia con los polinizadores (Ashman *et al.* 2004; Aguilar *et al.* 2006). Además, otros factores pueden afectar la polinización y el éxito reproductivo, incluyendo la competencia de los polinizadores, la densidad de individuo, la baja compatibilidad o viabilidad de polen, así como la depredación por herbívoros (Brown & Kephart 1999; Althoff *et al.* 2013).

Los cambios en interacciones bióticas producto de la fragmentación pueden ejercer presiones de selección sobre caracteres relevantes para las plantas (Jacquemyn *et al.* 2012). Por ejemplo, en casos severos de limitación por polen, se espera que los caracteres florales sean seleccionados para promover el entrecruzamiento o auto fertilización autónoma. En contraste, si los polinizadores varían en espacio tiempo se espera que las plantas evolucionen a un sistema de apareamiento mixto con un mecanismo de autofertilización retardada para garantizar la reproducción (Eckert *et al.* 2010; Jacquemyn *et al.* 2012).

Por otra parte, la interacción entre las plantas y sus enemigos naturales es importante porque influyen en la dinámica y estructura de los ecosistemas. En particular, los herbívoros que consumen las flores, frutos y semillas pueden limitar el reclutamiento y la dinámica poblacional de las plantas. La fragmentación del hábitat puede alterar las interacciones antagonistas al afectar a la comunidad y riqueza de herbívoros.

Un metanálisis reciente indica que las plantas en hábitats fragmentados tienen menor daño causado por insectos herbívoros folívoros pero no existe un efecto de la fragmentación en la florivoría y la depredación de semillas (Chávez-Pesqueira *et al.* 2015).

En el caso del género Aphelandra (R. Brown) existen estudios sobre la biología reproductiva, polinizadores y sistema de apareamiento. Se ha reportado que algunas especies son polinizadas por colibríes y murciélagos (Wasshausen 1975; McDade 1984, 1985; Daniel 1991; Mcdade 1992; Muchhala & Thomson 2012). En México la especie más estudiada es *Aphelandra aurantiaca*, la cual tiene flores hermafroditas, proterogíneas, productoras de néctar, es auto-compatible y es polinizada por colibríes.

En cuanto al daño por herbívoros de *A. aurantiaca,* se sabe que la depredación de sus flores, frutos y semillas impacta sobre su abundancia y distribución en el sotobosque de la selva de Los Tuxtlas. La depredación predispersión en *A. aurantiaca* varía entre el bosque maduro y los claros, se ha reportado mayor daño en las flores y en los frutos en el bosque maduro (Calvo-Irabién & Islas-Luna 1999). Conocer la biología reproductiva de las poblaciones de plantas en hábitats fragmentados permite inferir el efecto de las interacciones bióticas relacionadas con la reproducción, los cambios en el sistema de apareamiento y la persistencia de las especies. Sin embargo, hasta ahora no se ha evaluado el efecto de la fragmentación en la biología reproductiva, depredación por herbívoros en caracteres reproductivos y el éxito reproductivo de *A. aurantiaca*.

El objetivo de este trabajo es evaluar el éxito reproductivo de *A. aurantiaca*, describir características florales, así como estimar el daño en las estructuras reproductivas en poblaciones de hábitat continuo y fragmentado. En específico, tratamos de responder si el éxito reproductivo (flores, frutos y semillas) de *A aurantiaca* disminuye en los fragmentos, y si existen diferencias significativas en el daño causado por herbívoros en las estructuras reproductivas (Inflorescencias, frutos y semillas) entre ambos hábitats.

Método

Se midió la altura de la planta, el diámetro del tallo, la longitud de la inflorescencia (bráctea), se cuantificó el número de flores, frutos y semillas de *A. aurantiaca* en el hábitat continuo y el fragmentado. La presencia de daño se registró en la inflorescencia y en los frutos, para ello se cuantificó el número de flores dañadas y número de semillas. Los muestreos y colectas de semillas se realizaron en varias poblaciones en marzo de 2011 y febrero de 2012. La colecta de semillas durante ambos años sólo se realizó en cuatro poblaciones (Lyell, Selva II, Bambú y Cola Pescado). Para determinar el vigor de las semillas del año 2011 se cuantificó su área y su peso. El área de las semillas se obtuvo a través de fotografías y el programa Image-Pro Plus 6.0.0260 (Media Cybernetics, Carlsbad,CA).

Inicialmente, en esta tesis se planteó determinar la magnitud de la depresión por endogamia en *A. aurantiaca* en el hábitat continuo y fragmentado, se llevaron a cabo cruzas

controladas en el invernadero para producir progenies derivadas de (1) polinización cruzada (polen de otro individuo), (2) auto-polinización (automática), y (3) se obtuvo el tratamiento control (polinización natural), a partir de la colecta directa de infrutescencias maduras en el campo y la cuantificación del número de semillas producidas. Sin embargo, la mayoría de las flores utilizadas en el tratamiento de polinización cruzada (1) no produjeron frutos y no se pudo obtener la progenie derivada de entrecruzamiento, el fallo en este tratamiento posiblemente se deba a una incorrecta manipulación de las flores. Por lo tanto, los datos solo permiten comparar el éxito reproductivo natural con el éxito de la polinización automática o autofertilización.

El tratamiento de polinización automática consistió en embolsar las inflorescencias para evitar la visita de polinizadores y promover auto fertilización, todas las polinizaciones automáticas se llevaron a cabo en plantas extraídas del campo que fueron llevadas al invernadero. Una vez que las infrutescencias maduraron se cuantificaron las semillas. Se estimó la autofertilización (AF) que es el potencial de garantía reproductiva (RA) a través de la autofertilización autónoma. La autofertilización (AF) es estimada con la fracción de semillas derivadas de las plantas embolsadas (polinización automática) (F_{IC}) y las semillas derivadas de la polinización natural (F_{IN}) (Eckert et al., 2010).

Análisis de datos

Se compararon las variables entre poblaciones y hábitats a través de modelos lineales generalizados. Analizamos las variables de largo de inflorescencia, número de flores, número de frutos, el peso y número de las semillas asumiendo una distribución gaussiana. Se asumió una distribución binomial para el daño en las inflorescencias, se asumió un error con distribución Poisson para el daño en el número de frutos, flores y semillas depredadas. Se corrigió por el tamaño usando el largo de la inflorescencia, la altura y el diámetro de la planta. Los modelos incluían la variable, las covariables, las poblaciones y las poblaciones anidadas en el hábitat. Se estimó el *fruit-set* como la proporción de frutos entre el número de flores. Para comparar el número de frutos por tratamiento (natural y automático) el modelo incluye el tratamiento, el hábitat y la interacción. Todos los análisis se realizaron en JMP.

Resultados

Los resultados indican que a diferencia de los fragmentos, las inflorescencias del continuo son de mayor tamaño (χ^2 =12.8, *g.l.*=1, p<0.001), pero en ambos hábitats se producen el mismo número de flores (χ^2=0.47, *g.l.*=1, p=0.4) (Tabla 1). Se detectaron diferencias significativas entre las poblaciones dentro de los hábitats (Tamaño de las inflorescencias: χ^2=19.6, *g.l.*=1, p<0.001), (Número de flores: χ^2=17.1, *g.l.*=4, p<0.001). El número de frutos producidos por inflorescencia difiere significativamente entre los hábitats (χ^2=16.61, *g.l.* =1, p<0.0001) en donde el número de frutos es mayor para los fragmentos. También existen diferencias significativas entre poblaciones dentro de hábitats (χ^2=16.4, *g.l.* =4, p<0.05). Asimismo, el fruit-set difiere significativamente entre

hábitats (χ^2=31.62, *g.l.* =1, *p*>0.001) y poblaciones (χ^2=13.2, *g.l.* =4, *p*<0.05). Las poblaciones de fragmento (56%) tienen mayor *fruit-set* que las poblaciones de la selva continua (43%) (Tabla 1).

A pesar de lo anterior, no se detectaron diferencias significativas en el número de semillas entre los hábitats en cada año de muestreo (2011: χ^2=1, *g.l.*=1, *p*>0.05; 2012: χ^2=1, *g.l.*=0.45, *p*>0.05) (Tabla 2). Sin embargo, existen diferencias significativas entre poblaciones dentro los hábitats (2011: χ^2=22.6, *g.l.*=7, *p*<0.05; 2012: χ^2=11.8, *g.l.*=1, *p*<0.05). Los tratamientos de polinización automática parecen producir un mayor número de semillas que el tratamiento de polinización natural. La interacción hábitat × tratamiento es significativa (χ^2=16.7, *g.l.*=1, *p*<0.05), indicando que el tratamiento automático produce más semillas que el tratamiento natural en ambos hábitats. Los contrastes de Tukey muestran diferencias significativas en el tratamiento de polinización cruzada entre los hábitats (Tabla 2). Sin embargo, las diferencias entre los tratamientos de polinización natural y polinización cruzada se deben al efecto de la depredación en flores y frutos (Figura 1). La proporción de autofertilización (RA) indica que las plantas del hábitat continuo producen un poco más de semillas derivadas de autofertilización que las poblaciones de fragmento (Tabla 2).

El peso de las semillas difiere significativamente entre los hábitats (χ^2=6.86, *g.l.*=1, *p*<0.05) y entre poblaciones dentro de hábitats (χ^2=36.42, *g.l.*=4, *p*<0.001) (Tabla 3). El peso de las semillas es mayor en el hábitat fragmentado. En cuanto a el área de las semillas detectamos diferencias significativas entre hábitats (χ^2=23.2, *g.l.*=1, *p*<0.001), el área es mayor en las poblaciones de la selva continua, también es significativamente diferente entre poblaciones (χ^2=2080, *g.l.*=5, *p*<0.001).

El daño en las inflorescencias, flores, frutos y semillas de *A. aurantiaca* no difiere significativamente entre hábitats. Sin embargo, existen diferencias significativas entre las poblaciones para el daño en las inflorescencias (χ^2=73.3, *g.l.*=4, *p*<0.0001), el número de flores (χ^2=60.4, *g.l.*=4, *p*<0.0001), en el número de frutos (χ^2=21.2, *g.l.*=4, *p*<0.001) y el número de semillas con daño (χ^2=43.3, *g.l.*=4, *p*<0.0001) (Tabla 4).

Discusión

En general, no se detectó un efecto de la fragmentación del hábitat sobre el éxito reproductivo de *A. aurantiaca*. En el hábitat continuo, las plantas producen inflorescencias más grandes pero tienen el mismo número de flores que las plantas de los fragmentos. El tamaño de las inflorescencias en la selva continua podría estar relacionado con la atracción de los polinizadores en el sotobosque. Sin embargo, es necesario probar está hipótesis mediante tasas de vista de polinizadores y el éxito reproductivo de las plantas usando inflorescencias de diferentes tamaños en ambos hábitats.

En todas las poblaciones, el número de frutos es menor al número de flores producidas. Se ha sugerido que la sobreproducción de flores puede asegurar la visita de los polinizadores debido a una mayor recompensa de polen y néctar. También, podría estar relacionado con la variación de

recursos disponibles para la maduración de los frutos, y la aborción selectiva de frutos (Sutherland 1987). Encontramos que el número de frutos producidos es mayor en plantas del hábitat fragmentado, sin embargo, el número de semillas no varía significativamente entre hábitats. Esto sugiere que la fragmentación no tiene un efecto sobre el número de semillas y coincide con lo esperado para plantas autocompatibles que pueden reproducirse sin la intervención de polinizadores (Aguilar *et al.* 2006). Asimismo, el tratamiento de polinización automática confirma que *A. aurantiaca* posee la capacidad de reproducirse sin la intervención de los polinizadores (autofertilización), sugiriendo que una parte de las semillas producidas se pueden derivar de la autofertilización, así esta especie garantiza el éxito reproductivo ante la ausencia de polinizadores. Sin embargo, la fracción de semillas potencialmente derivadas de autofertilización es mayor en las poblaciones de la selva continua en comparación con los fragmentos. Para explicar este resultado es importante realizar estudios de biología floral que incluyan la morfología floral, el tiempo de receptividad estigmática y dehiscencia de las anteras para cada población y hábitat.

Los resultados muestran que el tratamiento de polinización automática produce más semillas que la polinización natural en ambos hábitats (Figura 1). Sin embargo, esta diferencia no se debe al tipo de tratamiento de polinización, sino a la intensa depredación en las flores, frutos y semillas que ocurre en el tratamiento de polinización natural. La depredación en el tratamiento automático se evita al embolsar las inflorescencias.

Estos resultados coincide con el alto daño predispersión en *A. aurantiaca* reportado por Calvo-Irabién & Islas-Luna 1999. El daño predispersión comienza con la pérdida de óvulos en las flores, pero las capsulas de las infrutescencias inmaduras y las semillas tienen mayor probabilidad de daño (Calvo-Irabién 1989). Por lo tanto, para poder comparar los tratamientos es necesario evitar la depredación en el tratamiento natural. Asimismo, es necesario repetir el experimento para comparar el desempeño de las plantas derivadas de autopolinización y polinización cruzada.

El peso y la forma de las semillas están relacionados con la capacidad de dispersión. En particular, la dispersión de semillas balística está relacionada con el ángulo de proyección, el peso y el arrastre de la semilla (Garrison, Miller & Raspet 2000). En *A. aurantiaca* el peso de las semillas difiere entre los hábitats, las semillas de fragmento tienen mayor peso. Sin embargo, en la selva continua el área de las semillas es mayor. Para entender por qué difieren estas medidas entre los hábitat es necesario llevar a cabo un estudio que evalué el desarrollo de las semillas, la morfología de los frutos y la distancia de dispersión.

En cuanto a la depredación de las inflorescencias, flores, frutos y semillas, esperábamos encontrar menor daño en los fragmentos debido a la alteración en la comunidad y reducción en la diversidad de insectos. Sin embargo, no encontramos diferencias significativas entre los hábitats, lo que sugiere que la depredación en las estructuras florales y en las semillas no está relacionada con la fragmentación del hábitat.

Finalmente, es necesario resaltar que los datos registrados en este estudio son meramente descriptivos por lo que es de vital importancia realizar experimentos específicos en *A. aurantiaca* que contrasten más tratamientos de polinización, principalmente de entrecruza y evitando la

depredación. También se necesita realizar estudios específicos de biología floral, de morfología de las semillas y capacidad de dispersión, así como la evaluación de los niveles de daño y los cambios en la comunidad de herbívoros entre los hábitats.

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Tablas y Figuras

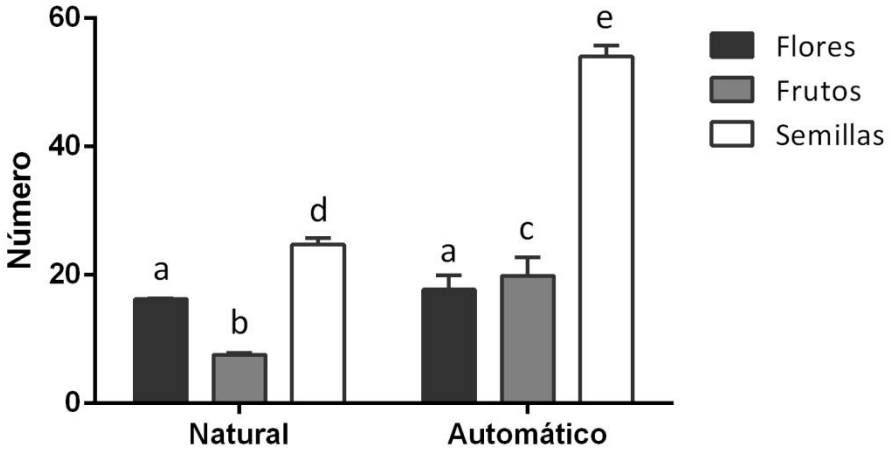

Figura 1. Número promedio de flores, frutos y semillas de *A. aurantiaca* en el tratamiento de polinización natural y de polinización automática.

Tabla 1. Medidas florales y vegetativas de *A. aurantiaca* en hábitat fragmentado y continuo en Los Tuxtlas.

	Altura (cm)	Diámetro (mm)	Largo inflorescencia (cm)	Número de flores por inflorescencia	Número de frutos	Fruit set Natural 2012
Lyell	38.6±10.4	6.0±1.2	94±29.5	16.10±0.3	6.79±5.2	0.40±0.27
Selva II	29±13.2	6.6±6.2	94.5±25.1	17.8±0.34	9.08±5.8	0.47±0.24
Continuo	33.7±12.8	6.6±5.7	94.2±27.4	16.9±0.23	7.9±5.6	0.43±0.26
Cola pescado	27.8±16.3	5.7±1.3	92.3±30	16.1±0.49	10.6±7.3	0.62±0.26
Bambú	37.6±19.5	5.8±1.1	80.1±26.7	16.3±0.32	------	0.54±0.22
Ruíz Cortines	------	------	95.4±26.9	17.4±0.51	9.76±7.0	0.53±0.28
Santa Rosa	------	------	58.4±15.6	16.6±1.28	7.28±3.2	0.78±0.18
Fragmentado	32.4±18.5	5.7±1.2	85.2±28.3	16.6±0.37	8.7±5.7	0.56±0.25

Se reporta el promedio y error estándar de cada variable

Tabla 2. Número promedio de semillas de *A. aurantiaca* derivadas de polinización natural y tratamiento de auto-polinización automática por población y hábitat.

Población Hábitat			Número de semillas por tratamiento de polinización				Potencial de garantía reproductiva (AF)
	Flores	Frutos	Natural	*n*	Automático	*n*	
Lyell	15.1±0.3	6.1±0.4	25.3±1.1	90	41.1±4.5	33	1.62
Selva II	16.5±0.3	9.3±0.4	22.9±1.0	95	68.9±3.9	44	3.00
Continuo	15.8±0.2	6.9±0.3	24.0±0.7	185	57.2±3.3	77	2.38
Cola pescado	16.2±0.6	8.4±0.8	30.0±2.0	25	57.4±5.6	23	1.91
Bambú	16.6±0.3	7.0±0.5	22.9±1.0	71	44.1±4.9	27	1.92
Fragmentado	16.5±0.3	9.0±0.4	25.8±1.1	96	49.8±4.1	50	1.93

Se reporta el promedio y error estándar de cada variable, *n*: número de plantas en estado reproductivo que se utilizaron en cada tratamiento, Flores: el número promedio de flores en cada población y hábitat en las plantas a las que se les aplico el tratamiento, corregidas por tamaño con el largo de las inflorescencias, Frutos: el número promedio de frutos que desarrollaron las plantas de ambos tratamientos, corregida por tamaño con el largo de las inflorescencias y número de flores

Tabla 3. Número promedio de semillas de *A. aurantiaca* en cada población de la selva continua y fragmentada colectadas en el año 2011 y 2012. Se reporte el peso y área promedio de las semillas colectadas en el año 2011.

		Peso semillas (mg) 2011	Área semilla (cm^2) 2011	Número semillas 2011	Número semillas 2012	Promedio del total de semillas 2011-2012
Población	Circuito	10.6±2.9	0.20±0.03	29.3±11.9	------	28.4±5.4
	Lyell	7.9±2.3	0.16±0.02	25±16.8	22±20.5	22.5±1.7
	Zacatal	13±5.7	0.24±0.03	43.8±20.9	------	43.8±2.8
	Selva II	11.8±6.4	0.20±0.03	41.2±24.6	27.6±19.8	30.2±1.7
Hábitat	Continuo	10.8±5.1	0.21±0.04	36.7±21.2	24.7±20.3	34±4.17
Población	Cola pescado	12.8±13.2	0.20±0.03	26.6±17.5	34.3±25.4	31.6±2.3
	Bambú	12.4±13.9	0.20±0.04	24.7±10.7	22.7±13.7	22.9±1.7
	Ruíz Cortines	------	------	------	28.4±23.3	28.2±2.9
	Santa Rosa	------	------	------	21.1±6.8	21.1±7.3
Hábitat	Fragmentado	12.6±13.5	0.20±0.03	25.9±15.3	26.3±19.3	25.9±2.1

Se reporta el promedio y desviación estándar de cada variable

Tabla 4. Número promedio de semillas, frutos, inflorescencias y flores dañadas de *A. aurantiaca* en la selva continua y fragmentada.

		Semillas con daño	Núm. frutos depredados	Núm inflorescencias dañadas	Núm. flores dañadas
		2011	2012	2012	2012
Población	Circuito	0.35±0.63	------	------	------
	Lyell	0.10±0.55	0.12±0.58	0.12±0.32	0.07±0.06
	Zacatal	1.23±2.29	------	0	------
	Selva II	0.28±1.49	0.32±1.0	0	0.19±0.06
Hábitat	Continuo	0.75±1.7	0.22±0.83	0.05±0.02	0.13±0.48
Población	Cola pescado	0.08±0.28	0.15±0.55	------	0.07±0.12
	Bambú	0	0.20±0.6	0.02±0.16	0
	R. Cortines	------	0.50±1.3	0.52±0.5	0.60±0.10
	Sta Rosa	------	0.42±1.13	------	0.57±0.24
Hábitat	Fragmento	0.05±0.22	0.26±0.08	0.16±0.02	0.20±0.82

Se reporta el promedio y desviación estándar de cada variable