UNIVERSIDAD NACIONAL AUTÓNOMA DE MÉXICO

POSGRADO EN CIENCIAS BIOLÓGICAS

ESCUELA NACIONAL DE ESTUDIOS SUPERIORES, UNIDAD MORELIA

Genética de la conservación del árbol tropical dioico *Spondias purpurea*

(Anacardiaceae)

TESIS

QUE PARA OPTAR POR EL GRADO DE:

DOCTOR EN CIENCIAS

PRESENTA:

EDSON JACOB CRISTÓBAL PÉREZ

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MORELIA, MICHOACAN, MARZO, 2021

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P r e s e n t e

Me permito informar a usted que en la reunión ordinaria del Comité Académico del Posgrado en Ciencias Biológicas, celebrada el día **30 de noviembre de 2020** se aprobó el siguiente jurado para el examen de grado de **DOCTOR EN CIENCIAS** del estudiante **CRISTÓBAL PÉREZ EDSON JACOB** con número de cuenta **51202803-1** con la tesis titulada **"Genética de la conservación del árbol tropical dioico *Spondias purpurea* (Anacardiaceae)"**, realizada bajo la dirección del **DR.MAURICIO RICARDO QUESADA AVENDAÑO,** quedando integrado de la siguiente manera:

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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 HABLARÁ EL ESPÍRITU"
Cd. Universitaria, Cd. Mx., a 25 de febrero de 2021

COORDINADOR DEL PROGRAMA

DR. ADOLFO GERARDO NAVARRO SIGÜENZA

POSGRADO EN CIENCIAS BIOLOGICAS
COORDINACIÓN

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ÍNDICE

RESUMEN

La conservación de la diversidad genética de las poblaciones es esencial para mantener el potencial evolutivo de las especies y por asegurar su persistencia en el largo plazo. Dos de los principales temas en biología de la conservación es el estudio de los factores que determinan y afectan la diversidad y estructura genética de las poblaciones. Los árboles dioicos son un grupo bien representado en ecosistemas tropicales, en los que el entrecruzamiento es obligado y dependen generalmente de insectos y vertebrados para el movimiento de polen y semillas respectivamente. En esta tesis evaluamos a) los factores que determinan la diversidad y estructura genética, el flujo génico y el fitness bajo condiciones naturales; b) las consecuencias reproductivas y genéticas de la fragmentación del hábitat y c) la diversidad y estructura genética y el patrón de apareamiento de poblaciones silvestres y cultivadas del árbol dioico *S. purpurea*. Los sitios de estudio incluyen la Reserva de la Biosfera Chamela-Cuixmala en México y el Área de Conservación Guanacaste en Costa Rica. Desarrollamos y caracterizamos marcadores moleculares tipo microsatélite para esta especie. Nuestros resultados demuestran que bajo condiciones naturales los procesos dependientes de la densidad y la distancia determinan la diversidad genética, limitan la estructuración genética a escala fina y tienen un fuerte efecto sobre el fitness masculino y femenino de la población. En ambientes fragmentados la alteración de la interacción-planta polinizador tiene consecuencias negativas sobre el fitness masculino y femenino (tasa de fructificación, los niveles de flujo génico, el número de donadores de polen) y los niveles de diversidad genética. Nuestros resultados demuestran que la diversidad y estructura genética entre poblaciones silvestres y cultivadas es similar y la producción de frutos es en gran medida mantenida por vía sexual a través del flujo de polen proveniente de árboles masculinos silvestres y por via asexual mediante la formación de semillas por apomixis. Los resultados de esta tesis indican que las interacciones bióticas que las plantas sostienen con polinizadores y dispersores de semillas, son responsables del mantenimiento de la diversidad genética de las poblaciones y en poblaciones cultivadas del aseguramiento de la producción de frutos. Sin embargo, alteraciones en las interacciones bióticas resultado de la pérdida de hábitat tienen consecuencias negativas sobre el fitness y la diversidad genética, lo cual puede comprometer la persistencia de las poblaciones en el largo plazo.

ABSTRACT

Conservation of genetic diversity is essential to maintain the evolutionary potential of populations and therefore ensure species long-term persistence. Two principal concerns in conservation biology are the study of the factors that determine population genetic diversity and the reproductive and genetic consequences of anthropogenic activities such as habitat fragmentation and plant cultivation. Dioecious trees are obligate outcrossing plants, well represented in tropical ecosystems, that depend on insects and vertebrates for pollen and seed movement respectively. In this thesis we evaluated a) the influence of density and distance dependent factors on genetic diversity and fine scale genetic structure, gene flow and fitness; b) the reproductive and genetic consequences of habitat fragmentation and c) the genetic diversity and structure and mating patterns of wild and cultivated populations of the dioecious tree *S. purpurea*. Study sites include the Chamela-Cuixmala Biosphere Reserve in Mexico and the Guanacaste Conservation Area in Costa Rica. We developed and characterized microsatellite molecular markers for *S. purpurea*. Our results show that density and distance dependent processes have a strong effect on genetic diversity, fine scale genetic structure and male and female fitness. However, habitat fragmentation alters pollinator-plant interaction resulting in negative consequences on male and female fitness (lower fruit set, lower gene flow distances and lower number of pollen donors) and lower genetic diversity. Our results show that genetic diversity and structure is similar between wild and cultivated populations. Fruit production in cultivated stands is maintained both sexual via through gene flow between female cultivated trees and male wild relatives flow and asexual via through mechanism as apomictic seed formation. Our results indicate that biotic interactions that plants maintain with pollinators and seed dispersers, are key elements determining genetic diversity, gene flow levels, fine scale genetic structure and plant fitness. However, gene flow and pollination success will be negatively affected by fragmentation, reducing male and female fitness and the genetic diversity of future generations. Thus, in the long term, population viability in small fragmented habitats will decline due to limited recruitment and evolutionary potential.

INTRODUCCIÓN GENERAL

Uno de los temas centrales en Biología de la Conservación es el estudio de los factores que determinan la diversidad y estructura genética de las poblaciones. En las plantas, rasgos como el sistema reproductivo y de apareamiento, así como los vectores de dispersión de polen y semillas son los principales factores responsables de la diversidad y estructuración genética de las poblaciones (Hamrick et al. 1992; Hamrick 2002; Vekemans y Hardy 2004). Los árboles dioicos, en los que el entrecruzamiento es obligado, son un grupo bien representado en los ecosistemas tropicales (~27 % de las especies de árboles) (Bawa et al. 1975; Bawa et al. 1985; Ibarra-Manríquez y Oyama 1992). Estos árboles comparten características de historia de vida como son la producción de flores pequeñas y frutos carnosos, la polinización por insectos pequeños y la dispersión de sus semillas por vertebrados (Ibarra-Manríquez y Oyama 1992; Renner y Rickfles 1995). Debido a estas características, se espera que los árboles dioicos presenten altos niveles de diversidad genética y bajos niveles de estructuración genética.

Bajo condiciones naturales, procesos dependientes de la densidad y la distancia han sido reconocidos como factores clave para explicar la alta diversidad de especies de plantas en los ambientes tropicales (Janzen 1970; Connell 1971; Comita et al. 2014). De acuerdo con la hipótesis planteada por Janzen-Conell (1971), la probabilidad de depredación por herbívoros y patógenos hospedero-específicos, es alta en la cercanía a árboles maternos donde la densidad de conespecíficos generalmente es mayor. De esta forma, los árboles tropicales tendrían una distancia óptima de dispersión con relación al árbol materno, conocida como "curva de reclutamiento poblacional", la cual cambiaría entre especies en función del modo de dispersión de las semillas (Janzen 1970). Los efectos de estos mecanismos sobre el reclutamiento y la abundancia poblacional han sido bien estudiados (Comita et al. 2014). Sin embargo, su papel sobre los patrones de flujo génico y la estructuración genética a escala fina de las poblaciones ha sido poco explorado. Pocos son los trabajos que hayan evaluado estructura genética a escala fina en árboles tropicales dioicos. La evidencia sugiere que en árboles tropicales dioicos dispersados por vertebrados la estructura genética espacial es ligera en comparación con otros sistemas reproductivos (Hardesty et al. 2005; Hardy et al. 2006; Riba-Hernández et al. 2014).

En la actualidad los bosques tropicales se encuentran entre los ecosistemas más amenazados por las actividades humanas. Datos para México indican que entre 2001 y 2018 la tasa de deforestación anual en estos ecosistemas fue de 5.7 % (FAO 2019). Esta conversión de áreas boscosas a zonas de cultivo y pastoreo fragmenta el hábitat original, provocando que grandes extensiones de bosque continuo sean transformadas a pequeños fragmentos de bosque

aislados entre sí (Fahrig 2003; Fischer y Lindenmayer 2007). En sitios de bosque fragmentado se espera que el tamaño poblacional se reduzca en función del tamaño del parche de bosque y que el aislamiento entre estos parches afecte la interacción con polinizadores, reflejándose en una baja producción de frutos y semillas (Aguilar et al. 2006). Los estudios de los efectos de la fragmentación de hábitat sobre el éxito reproductivo se han enfocado en la función femenina, como producción de flores, frutos y tasas de fructificación (Aguilar et al. 2006). Sin embargo, los efectos sobre la función masculina, como producción, desempeño y distancias de flujo de polen han sido muy poco analizadas. Las plantas dioicas representan un buen modelo para entender estos efectos debido a que las funciones masculina y femenina se encuentran en individuos separados.

En hábitats fragmentados la reducción en el tamaño efectivo poblacional incrementa la probabilidad de apareamiento entre individuos relacionados y la ocurrencia de eventos estocásticos como la deriva génica, lo cual resulta en la pérdida de la diversidad genética de las poblaciones (Ouborg et al. 2006; Aguilar et al. 2019). Además, en estos ambientes el flujo génico puede reducirse debido a i) incrementos en la distancia entre conespecíficos, ii) cambios en el comportamiento de polinizadores y dispersores de semillas y iii) limitado movimiento de los polinizadores y dispersores de semillas entre parches de bosque (Sih & Baltus 1987; Quesada et al. 2003, 2004; Breed et al. 2015). El flujo génico entre individuos espacialmente cercanos incrementa la estructura genética espacial, resultando en vecindarios de individuos genéticamente relacionados, lo cual a su vez puede aumentar la tasa de endogamia y reducir la diversidad genética y la viabilidad de las poblaciones en el largo plazo (Sork y Smouse 2006; Aguilar et al. 2008, 2019).

La domesticación de plantas es un proceso de selección artificial, por medio del cual los humanos han seleccionado y fijado características fenotípicas para satisfacer necesidades particulares (Aguirre-Dugua y González-Rodríguez 2016; Casas et al. 2019). Alrededor del mundo existen varios centros de domesticación, donde actualmente algunas especies cultivadas coexisten con sus parientes silvestres (Balvino-Olvera et al. 2017). Entre las plantas domesticadas un grupo importante son las plantas perennes, generalmente cultivadas por sus frutos comestibles (Van Tassel et al. 2010; Miller y Gross 2011). Estas plantas se caracterizan por presentar largos periodos en estado juvenil por lo cual son propagadas vegetativamente (Miller y Gross, 2011). Además, una alta proporción de especies presentan sistemas reproductivos y de apareamiento con entrecruzamiento obligado (i.e. especies dioicas o auto incompatibles), altas tasas de flujo génico y la capacidad para producir frutos tanto por vía sexual como asexual (Zohary y Spiegel-Roy 1975; Zohary 2004; Miller y Gross 2011). En

especies dioicas, en las que los frutos son producidos exclusivamente por los individuos femeninos, la propagación vegetativa de clones implica algunas limitaciones para la producción de frutos. Por lo cual, bajo condiciones de cultivo, la producción de frutos en plantas dioicas puede ser asegurada por a) vía sexual si individuos masculinos son plantados dentro de los cultivares o poblaciones silvestres coexisten con las cultivadas (Barghchi y Alderson 1989; Moore 2014), o por b) vía asexual si existe un reemplazo de la polinización por partenocarpia (Zohary 2004; Kislev et al. 2006). En zonas donde las especies domesticadas coexisten con sus parientes silvestres, la diversidad genética depende en gran medida de la diversidad clonal propagada en cada cultivar y del flujo génico entre individuos silvestres y cultivados (Ellstrand et al. 1999; O'Connor et al. 2015). El estudio de esta diversidad es de particular interés en términos de la conservación de recursos genéticos de especies de interés agronómico, sin embargo esta información es escasa para árboles frutales tropicales.

El análisis de los procesos que determinan la diversidad genética de las poblaciones de árboles en condiciones naturales, así como el entendimiento de las consecuencias reproductivas y genéticas de las actividades humanas, son indispensables para la conservación de los recursos genéticos y la preservación del potencial evolutivo de las especies. *Spondias purpurea* es un árbol tropical dioico, que se distribuye de manera natural en los bosques tropicales secos de Mesoamérica (Miller y Schaal 2005; Miller y Knouft 2006). Las flores de esta especie son rojas, pequeñas, y son polinizadas por insectos principalmente abejas sin aguijón (Bullock 1992; Mitchell y Daly 2015). Los frutos son rojos y carnosos, los cuales representan un importante recurso para los vertebrados que los consumen, particularmente en la época seca del año donde el agua es un factor limitante en el bosque (Mandujano et al. 1994). *Spondias purpurea* ha sido domesticada para el consumo de sus frutos (Miller y Schaal 2005). En condiciones de cultivo, los individuos femeninos son propagados vegetativamente en cultivos de traspatio, huertos pequeños o cercas vivas (Miller y Schaal 2005). A lo largo de su distribución estos cultivos coexisten con individuos silvestres que habitan en remanentes de bosque original.

En la presente tesis se presentan cuatro capítulos. El primer capítulo es un artículo publicado (Cristóbal-Pérez et al., 2020), donde se analiza el efecto de procesos dependientes de la distancia y la densidad sobre la diversidad genética, la estructura genética a escala fina y los patrones de flujo efectivo y realizado de polen y semillas. El segundo capítulo es un artículo publicado (Cristóbal-Pérez et al. 2021) donde se analiza el efecto de la fragmentación del hábitat sobre el fitness masculino y femenino, la diversidad genética, el flujo génico y los patrones de apareamiento. En el tercer capítulo se compara la diversidad y estructura genética

de poblaciones silvestres y cultivadas y se analiza el flujo génico entre poblaciones cultivadas y sus parientes silvestres. El cuarto capítulo es un artículo publicado (Cristóbal-Pérez et al. 2019) en el que se presentan los resultados del desarrollo y caracterización de los marcadores tipo microsatélite, utilizados para los análisis genéticos llevados a cabo en esta tesis.

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Janzen-Connell effects shape gene flow patterns and realized fitness in the tropical dioecious tree *Spondias purpurea* (ANACARDIACEAE)

E. Jacob Cristóbal-Pérez[1,2], Eric J. Fuchs[2,4], Ulises Olivares-Pinto[2,3] & Mauricio Quesada[1,2*]

Pollination and seed dispersal patterns determine gene flow within plant populations. In tropical forests, a high proportion of trees are dioecious, insect pollinated and dispersed by vertebrates. Dispersal vectors and density dependent factors may modulate realized gene flow and influence the magnitude of Fine Scale Genetic Structure (FSGS), affecting individual fitness. *Spondias purpurea* is a vertebrate-dispersed, insect-pollinated dioecious tropical tree. We assessed the influence of sex ratio, effective and realized gene flow on genetic diversity, FSGS and individual fitness within a 30 ha plot in the tropical dry forest reserve of Chamela-Cuixmala, Mexico. All individuals within the plot were tagged, geo-referenced and sampled for genetic analysis. We measured dbh and monitored sex expression during two reproductive seasons for all individuals. We collected seeds directly from maternal trees for effective pollen dispersal analysis, and analyzed established seedlings to assess realized pollen and seed dispersal. Nine microsatellite loci were used to describe genetic diversity parameters, FSGS and gene flow patterns among different size classes. A total of 354 individuals were located and classified into three size classes based on their dbh (<10, 10–20, and >20 cm). Population sex ratios were male biased and diametric size distributions differed among sexes, these differences may be the result of precocious male reproduction at early stages. Autocorrelation analyses indicate low FSGS (Fj <0.07) across all size classes. Long realized pollen and seed dispersal and differences among effective and realized gene flow were detected. In our study site low FSGS is associated with high gene flow levels. Effective and realized gene flow indicate a population recruitment curve indicating Janzen-Connell effects and suggesting fitness advantages for long-distance pollen and seed dispersal events.

Dispersal is a central ecological mechanism that involves the movement of genes or individuals from one location to another. In plants, gene movement occurs through pollen flow (paternal gamete dispersal) and seed dispersal, which is facilitated by animals such as pollinators and seed dispersers or abiotic agents like wind and water[1,2]. Gene flow patterns are important for population and conservation genetics since they directly influence genetic diversity within populations and determine the genetic structure among populations[3]. Within plant populations, limited gene flow could result in fine spatial genetic structure (FSGS), that is, the non-random spatial distribution of genotypes[4], resulting in neighborhoods of related individuals at a restricted spatial scale[4,5]. Other factors besides gene flow may generate FSGS, including colonization history[6,7], demographic changes[8–10] and the mating system and sexual expression of plants[3,11].

Spatial distribution patterns in plants are determined by seed dispersal patterns and post-dispersal processes such as seed predation and seedling survival. For example, in zoochorous plants[12] short-range dispersers forage near fruiting trees, resulting in higher seed deposition near maternal plants and FSGS. Further, seeds may accumulate in roosting or sleeping sites which would again result in aggregated dispersal and consequently resulting in FSGS[12]. According to the Janzen-Connell hypothesis, the establishment and survivorship of seedlings near

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maternal or conspecific plants could be affected by negative density-dependent processes such as intra-specific competition, host-specific predation, herbivory or pathogens[13,14]. Although density-dependence is commonly accepted as a factor responsible for high tropical diversity[15], its influence on FSGS patterns is poorly understood. Proximate density dependent factors may increase mortality of seedlings near conspecifics or at high densities[13,14], reducing FSGS. Dioecious trees are relatively common in tropical forests (ca. 27% trees)[16–18]. Dioecy is a reproductive system in which the male and female reproductive functions exist in separate individuals. A high proportion of dioecious species share reproductive traits such as small insect pollinated flowers and fleshy fruits dispersed by vertebrates[18,19]. Although dioecious trees are frequent in tropical forests, gene flow patterns[20,21] and FSGS have been rarely studied[11,22]. In dioecious trees the magnitude of FSGS will depend, on the one hand, on the movement ability and behaviour of pollinators and seed dispersers and, on the other hand, on demographic traits associated with the reproductive system, such as sexual ratio and spatial distribution of adults. Theory of density-dependent animal pollination assumes that tree species occurring at low densities receive pollen from fewer individuals than trees in denser populations, and from longer pollen flow distances[23–26]. Theory predicts a 1:1 sex ratio maintained by negative frequency-dependent selection[27], however intersex differences in growth, reproduction and survival may result in biases in sex ratio[28]. Specifically, males are expected to have higher growth rates, survivorship and reproduction than females, due to the higher energy costs associated with gamete and fruit production in females; resulting in male-biased sex ratios[29]. For dioecious plants, the spatial distribution of male and female trees as well as the population sex ratios could affect seed disperser behaviour playing an important role in determining FSGS patterns.

Fitness may be defined as an individual's genetic contribution to future generations[30]. The number of surviving offspring of a given individual in the population, determined through paternity analyses, is a good proxy of plant fitness[31,32]. In plants, individual fitness will depend on their ability to produce viable seeds (i.e. fecundity) and the success of seeds to disperse and establish (i.e. survival). Therefore, factors affecting individual plant fitness as pollen gene flow distances, seed or seedling predation near maternal trees may affect FSGS formation. Due to the difficulty to estimate overall fitness in natural conditions, fitness analyses frequently rely on traits associated with one component of individual fitness, generally associated to fecundity (*e.g.* fruit production, seed production)[33], ignoring the transition between seed formation and seedling emergence[34]. An approach to analyze fitness and fill the gap in this demographic transition is to estimate effective pollen flow and realized pollen and seed dispersal. Effective pollen dispersal is the pollen flow that occurs before seed dispersal, while realized pollen and seed dispersal refer to pollen and seed flow measured on established seedlings and juveniles[35]. This approach allows the evaluation of individual fitness through an estimate of the number of offspring by each parental plant, and secondly to analyse the contribution of gene flow patterns on FSGS.

In this study, we analyze fine-scale genetic structure (FSGS) in relation to gene flow patterns and fitness on the dioecious, insect pollinated and vertebrate seed dispersed tree, *Spondias purpurea* L. We analyse the effect of demography, gene flow and sex ratio on effective gene flow patterns. Specifically, we evaluate the following objectives: (1) Determine the relationship between population sex ratios, size classes and FSGS; (2) Determine the relationship between gene flow patterns via seed and pollen dispersal, with respect to FSGS, and (3) Determine the relationship of effective and realized pollen dispersal, as a proxy of plant fitness in relation to FSGS.

Results

Sexual ratios. A total of 354 *S. purpurea* individuals were mapped within the 30 ha plot, 144 individuals were included in size class I, 113 in size class II and 97 individuals in size class III (Fig. 1). During two reproductive seasons we observed 62 size class I and 32 size class II individuals that did not flower, these individuals were included as non-reproductive individuals (Fig. 2). We observed significant differences in sex ratios across size classes ($G_{heterogeneity} = 22.809$, d.f. = 2, $p = 0.0011$). A male-biased sex ratio was observed for the pooled population and for size classes I and II ($p < 0.05$; Fig. 2). For size class III, the sex ratio did not significantly deviate from unity ($p > 0.05$) (Fig. 2). We observed significant differences in diameter size distribution between sexes (Fig. 2, $D = 0.33$, $p < 0.001$).

Genetic diversity analysis. All 354 mapped individuals were genetically analysed. All nine loci were polymorphic and had similar levels of genetic diversity across size classes (Table 1). For the entire population, the average number of alleles per locus was $N_a = 6.51$ (± 0.231), the allelic richness was $A_r = 6.07$ (± 0.582), the observed and expected heterozygosities were $H_o = 0.430$ (± 0.09) and $H_e = 0.502$ (± 0.006) respectively.

Fine scale genetic structure and spatial analysis. Our results indicated relatively low levels of FSGS across different size classes. Individuals of size class I had significant but low pairwise kinship coefficient for the 20 and 40 m distance classes, $F_{ij} = 0.0531$ an $F_{ij} = 0.069$ respectively (Fig. 3A). Individuals of size class II had significant FSGS for the 20 m distance class ($F_{ij} = 0.077$) (Fig. 3B), similarly size class III individuals showed significant family structure for the 20 m distance class ($F_{ij} = 0.05$) (Fig. 3C). Although significant for the first distance classes, kinship coefficients were low ($F_{ij} \leq 0.077$) in this *S. purpurea* population. The *Sp* statistic confirms these results, with all regression slopes being statistically significant (Table 2). The between-size class FSGS analysis, showed that size class I individuals exhibited low genetic relatedness with nearby size class III individuals (Fij = 0.043 and Fij = 0.030 for 20 and 40 m respectively), than expected for parent-progeny pairs (Fij $\simeq$ 0.5) (Fig. 4). Results of spatial interaction analyses indicated spatial association between individuals of size class I and III (Fig. 5).

Parentage analysis. The exclusion probability estimated over all individuals and over nine loci was 99.99%. Out of 127 genotyped seeds for effective pollen dispersal estimates, 68 were assigned to pollen donors within the plot, 58 of which with 95% confidence. We were able to identify six pollen donors within the plot that sired between 5 and 12 seeds (mean = 9.6 seeds by pollen donor). Effective pollen dispersal distances ranged between 27.9 m

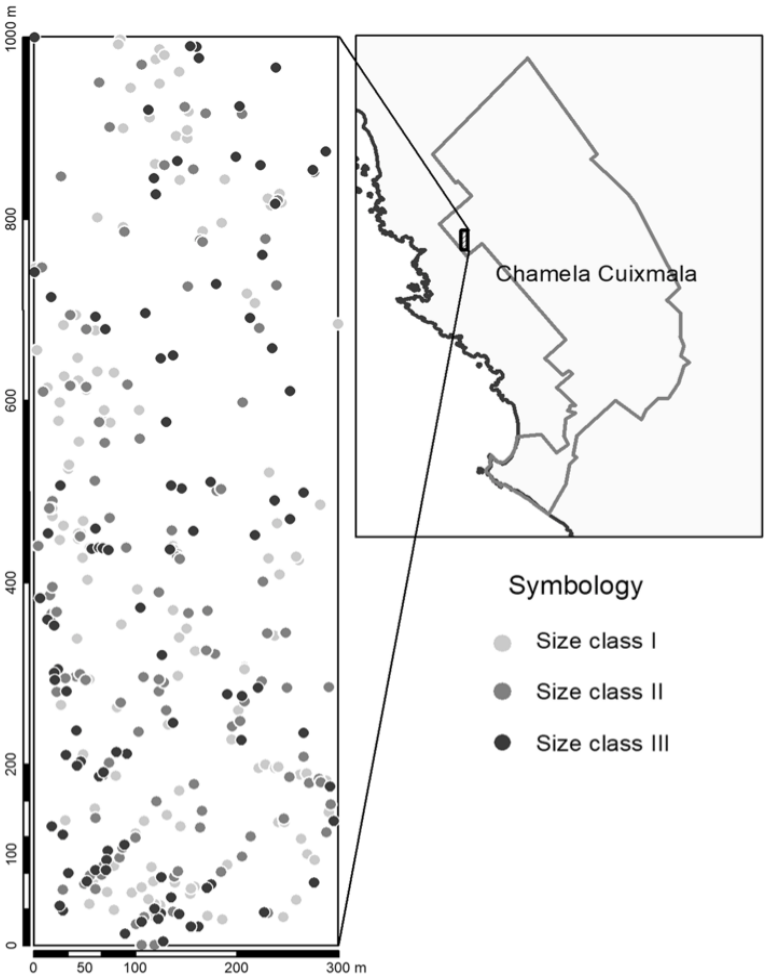

Figure 1. Spatial distribution Spondias purpurea individuals in a 30 ha plot located in Chamela, Jalisco Mexico. Size class I (DBH < 10 cm), Size class II (10 cm <DBH < 20 cm), Size class III (DBH > 20 cm). This map was created in Quantum GIS v.3.4 (Quantum GIS Development Team (2018). Quantum GIS Geographic Information System. Open Source Geospatial Foundation Project. http://qgis.osgeo.org).

and 828.2 m with an average of 306.78 m (±28.57) and a median of 263.29 m. Approximately 30% of all effective pollination events assigned through paternity analysis, occurred between trees that were less than 150 m apart, 70% occurred between trees >200 and 600 m apart and 12% occurred between trees 600 to 850 m apart (Fig. 6A).

We determined realized pollen dispersal distances on 144 individuals in size class I, 31 were assigned to a parent-pair within the plot, 29 of which were assigned with >95% confidence. We were able to identify 11 pollen donors within the plot that sired between 1 and 5 established seedlings by each donor (mean = 2.8 seedling by pollen donor). The realized pollen dispersal distance ranged from 96.6 m to 854.6 m with an average of 331.6 m (±31.14) and a median of 293 m. For realized pollen dispersal, only 6.4% of the events occurred between trees <150 m apart, 93% occurred between trees >150 m apart and 12% occurred between trees 600 to 854 m apart (Fig. 6B).

The same 144 individuals of the previous analyses were used to estimate seed dispersal distances, 77 of these seedlings were assigned to a mother (13 maternal plants) within the plot (67 with >95% confidence). Seed dispersal distances ranged between 7.2 m and 833.2 m with an average of 305 m (±20.6) and a median of 255.9 m, 19.4% of all seed dispersal events occurred at distances <150 m apart from assigned maternal trees, 80.5% occurred between 150 m and 600, and 12.9% occurred between 600 and 833 m apart from the assigned maternal trees (Fig. 6C).

We did not find any significant evidence of a relationship between the number of established progeny produced by an individual and its DBH ($r^2 < 0.44$, $p > 0.144$), nor between the number of progeny established and their distance from any given maternal tree (r 2 = 0.09, p = 0.99).

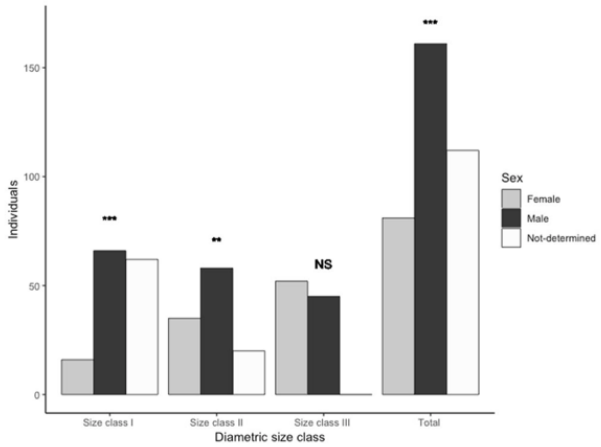

Figure 2. Number of individuals in each size class for each sex and for not-determined individuals over two reproductive seasons of *Spondias purpurea* within a 30 ha plot in Chamela Jalisco, Mexico. Notes: ***indicates $p < 0.001$, **$p < 0.05$, NS: $p > 0.05$ (G-test goodness of fit).

Size class	N	N_a	A_r	H_o	H_e	F
Size class I	144	6.333 (0.273)	6.02 (1.05)	0.41822 (0.077)	0.49947 (0.078)	0.116 (−0.0692–0.3636)
Size class II	113	6.444 (0.297)	6.08 (1.01)	0.43545 (0.071)	0.49751 (0.075)	0.072 (−0.1256–0.3179)
Size class III	97	6.778 (0.037)	6.12 (1.08)	0.43775 (0.082)	0.51023 (0.078)	0.099 (−0.1151–0.3545)

Table 1. Genetic diversity estimates for each size class within 30 ha plot of *Spondias purpurea*. N: Number of individuals; N_a: mean allele number per locus (±SD); A_r: Allelic richness (±SD); H_O: observed heterozygosity (±SD); H_e: expected heterozygosity (±SD); F: fixation index (Bootstrap estimates of confidence intervals).

Discussion

Sex ratios in the studied *S. purpurea* population were significantly male-biased, but departures from 1:1 ratio varied across sizes classes. While smaller size classes (DBH < 20 cm) were mostly male-biased, the largest size classes (>20 cm) did not deviate from a 1:1 sex ratio. In the two sampling periods of this study, we did not find differences in flowering frequency between male and female *Spondias* trees across size classes. Differences in size distributions between males and females are unlikely due to greater growth rates in males, but instead by an overrepresentation of males in the smaller size classes. The 1:1 sex ratio in the larger size classes probably indicates that many of the non-flowering individuals in the smaller size classes are likely females. Thus, *S. purpurea* females reach age at first reproduction at a minimum threshold size determined by a minimum budget of resources necessary for flower and fruit production. Flower production in males start at an early stage in individuals that are 2 cm in diameter and less than 1 m in height; while in females, flowering starts when they grow taller than 5.2 cm in diameter and >1 m in height. This result suggests precocious male reproduction in S. purpurea, as also found in other tropical species[36–41]. Female trees likely require a minimum branch size to bear fruit (i.e. >1 cm) which is related to the overall development of the plant.

We observed intermediate levels of genetic diversity in all three size classes of *S. purpurea* as expected for tropical trees[42–44] and for dioecious species with obligate outcrossing[45,46]. Our data showed significant but low fine-scale structure up to 40 meters for the smallest size class and up to 20 meters for size classes II and III. *Sp* values were comparable to estimates for animal dispersed tropical trees ($Sp \in [0.01 \ldots 0.03]$) and showed no evidence of FSGS across all size classes, which indicates that genotypes were largely randomly distributed in space. These results contradict expectations of high and significant structure in the smaller size classes of animal dispersed trees due to limited seed dispersal[20,45,47,48], and a reduction in FSGS in larger diametric size classes due to post-dispersal demographic thinning (e.g. density-dependent predation and competition)[20,45,48–50]. Our expectations were based on previous findings for other vertebrate dispersed tropical dioecious tree species such as *Pouteria reticulata* (Sapotaceae), *Simarouba amara* (Simaroubaceae), *Ficus cyrtophylla* (Moraceae), and *Protium spruceanum* (Burseraceae); where significant FSGS was detected in sapling and adult size classes at short distances, and FSGS declined in larger diametric classes[20,45,51,52]. This pattern has also been shown for dioecious species such as *F. hispida, F. exasperata, F. pumila, Ceratiola ericoides, Eurya emarginata, Myracrodruon urundeuva*[46,53–56]; however this is not the pattern that we found for *S. purpurea* at Chamela, our data shows low genetic structure and no differences among size classes.

The unexpected low FSGS in *S. purpurea* is likely a result of long-distance seed dispersal and early density-dependent factors shaping FSGS patterns. In the study area two bird species (*Ortalis poliocephala* and

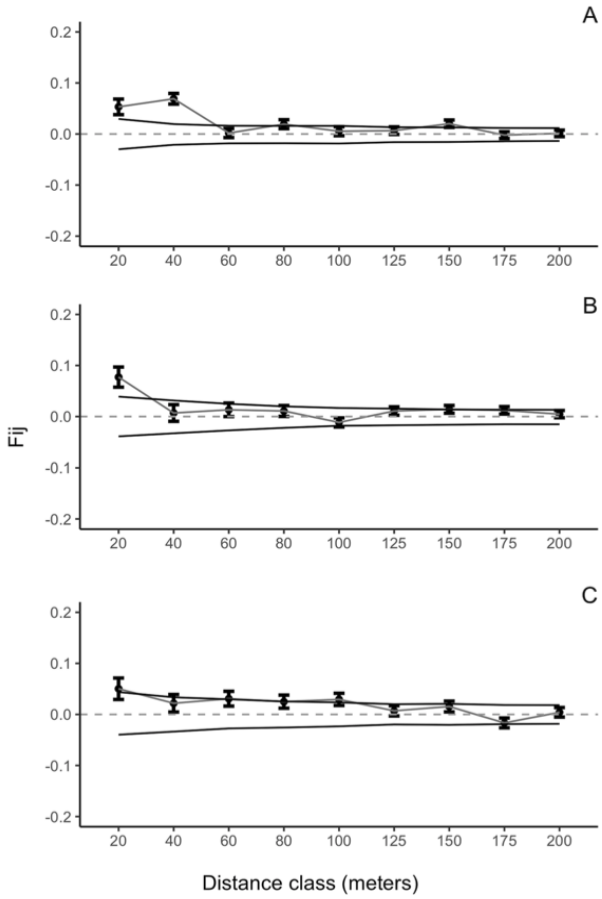

Figure 3. Autocorrelograms for estimated pairwise kinship correlation F_{ij} for three diametric size classes: (**A**) size class I (DBH < 10 cm), (**B**) size class II (10 <DBH ≤ 20 cm), (**C**) size class II (DBH > 20 cm). Each point represents calculated r_j values for each distance class. The solid lines represent upper and lower 95% confidence envelopes for the null hypothesis of no correlation ($r_j = 0$).

Size class	b_{log}	$F_{(1)}$	Sp
Size class I	-0.0121^{NS}	0.0531	0.0128
Size class II	-0.01^{NS}	0.0771	0.0108
Size class III	-0.01^{NS}	0.0502	0.0105

Table 2. Slope of the regression (b_{log}) between kinship coefficient and the log of the geographic distance between individuals, the average kinship between individuals separated by less than 20 meters ($F_{(1)}$) and the *Sp* statistic calculated for each size class within the plot. NS: Not significant.

Icterus pustulatus), eight mammal species (*Odocoileus virginianus*, *Pecari tajacu*, *Canis latrans*, *Nasua narica*, *Urocyon cinereoargenteus*, *Didelphis virginiana*, *Sciurus colliaei* and *Liomys pictus*) and one reptile (*Ctenosaura pectinata*) have been documented to feed on *S. purpurea* seeds[57]. Collared peccaries (*P. tajacu)* predate seeds[57], however the rest are all potential dispersers that are capable of long-distance seed dispersal. Only three species consumed and left seeds underneath tree crowns (i.e. *D. virginiana*, *S. colliaei* and *I. pustulatus*), while the other species moved fruits away from maternal trees[57]. Intact seeds were observed in feces of *C. latrans*, *N. narica*, *U. cinereoargenteus* and *C. pectinata*; regurgitated seeds were also observed on rest spots and roosting sites of *O. virginianus* and *O. poliocephala*, respectively[57]. Seed dispersal studies assume that most seed dispersal follows a leptokurtic distribution, in which most seeds are dispersed nearby maternal trees[58]. However, seed dispersers may collect seeds on one tree and move them to other conspecifics in their feeding routes[59–62]. For most animal dispersed plant species, ingested seeds are normally dispersed away from maternal trees[62–64]. This seed dispersal behaviour has two consequences on gene flow patterns associated with seed dispersal. First, it promotes gene movement over long distances. In our parentage analysis, we were able to assign maternity to 53% of offspring within the plot, 85% of these assignments occurred at distances over 150 m away from the maternal tree. We

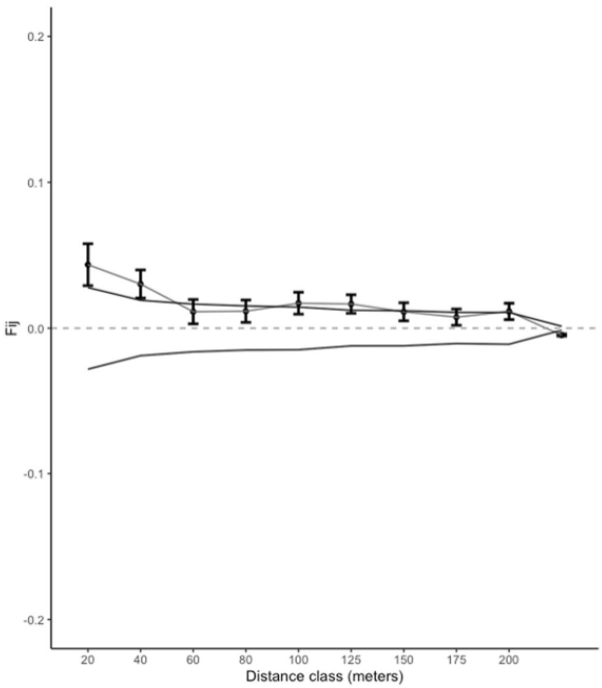

Figure 4. Kinship values (F_{ij}) of size class I - size class III pairs. Each point represents calculated r_j values for each distance class. The solid lines represent upper and lower 95% confidence envelopes for the null hypothesis of no correlation ($r_j = 0$).

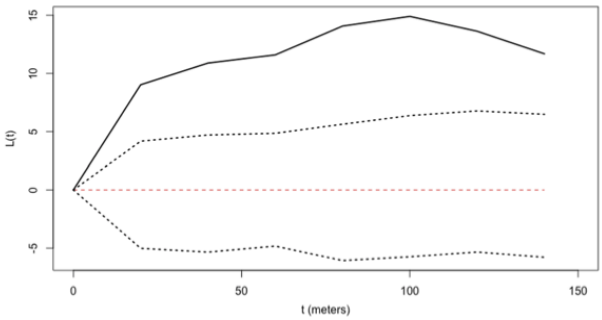

Figure 5. Results of Ripleý s bivariate L(t) analyses for size class I and size class III individuals in a 30 ha plot located in Chamela, Jalisco, Mexico. Solid lines represent the L(t) values. Dotted lines represent 95% confidence intervals. Spatial association between size classes is suggested if values of L(t)>0. Conversely, L(t) <0 indicates repulsion between size classes. If L(t) values fall within the confidence envelope, the spatial relationship between size classes is deemed random.

deduced that offspring not assigned to maternal trees within the plot may be the result of seed dispersal events by mothers located outside our plot. Our results are comparable to paternity assignments obtained for other species using microsatellite markers[10,65,66]. Seed dispersal distance is an important result, as most studies that analyze seed dispersal rarely estimate seed dispersal over such long distances[9,20,67,68]. Second, dispersers may consume seeds from multiple trees before depositing them in resting places, creating a mixture of different genotypes[64]; both factors should reduce FSGS. Similar to seed dispersal, effective and realized pollen flow occurred more frequently at distances greater than 150 meters (70% and 93% respectively), suggesting that for *S. purpurea* insects also provide long-distance gene dispersal services, comparable to other tropical trees[69].

In addition to long-dispersal gene flow, at shorter distances other factors may operate to limit FSGS and male and female fitness. Spatial association results between the smallest size class and the largest size class indicated that there is recruitment near conspecific trees, however only if the genetic relatedness between these two size classes is low (Figs. 4 and 5), suggesting a parentage recruitment effect. Furthermore, based on pre-dispersed seeds (effective pollen dispersal) paternity analysis show that 27% of pollen donors are located within 100 meters

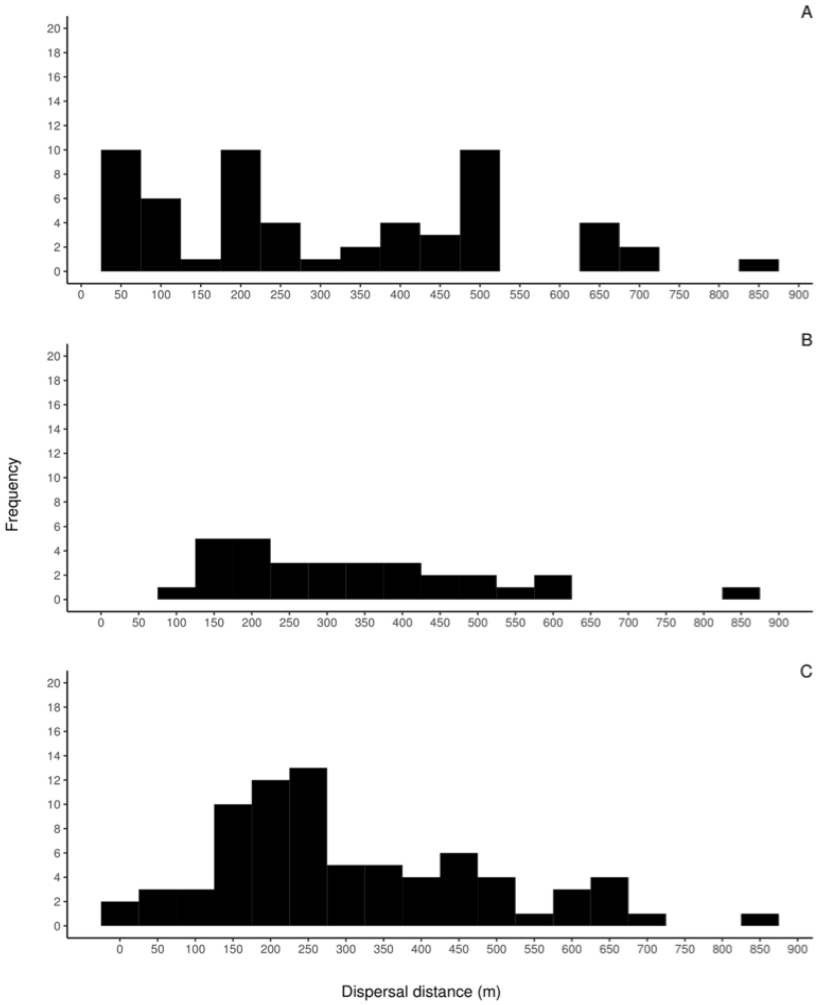

Figure 6. Frequency distribution of dispersal event distances: (**A**) Effective pollen dispersal, (**B**) Realized pollen dispersal and (**C**) Realized seed dispersal. Pollen dispersal is based on the results of a paternity assignment on seeds (n = 127) collected from 10 *Spondias purpurea* trees. Effective pollen and seed dispersal is based on parent-pair assignment on *Spondias purpurea* seedlings in the diametric size class I (n = 144) within of 30 ha plot in Chamela.

of maternal trees (Fig. 6A), meanwhile parent-pair analyses of established seedlings (realized gene flow) showed that less than 7% of all seedlings had a parent within the same distance range (100 m) (Fig. 6B,C). In other words, seeds sired by distant male trees have a higher likelihood of establishing than seeds sired by nearby males; in the same way seeds dispersed away from maternal trees have a greater probability of germination and survivorship in comparison with seed dispersed at shorter distances. This indicates that the probability of surviving and establishing in the population is a function of distance to their parental trees (both male and female) congruent with the Janzen-Connell (J-C) hypothesis[13,14]. According to J-C, recruitment is limited in nearby neighborhoods of paternal plants due to asymmetric competition and predator and pathogen transferal from related nearby individuals. Thus, dispersal away from parental genotypes should increase the survival probability of seedlings, thereby in accordance with our paternity data, lower FSGS could be indicative of strong J-C effects. In our study site, Mendez-Toribio and colleagues[70] compared recruited seedling richness and density underneath female and male *S. purpurea* trees. They found that seedling density and richness was higher underneath female trees compared to male trees; furthermore density of zoochorous species was greater underneath the canopy of *S. purpurea* females suggesting a directional dispersal bias towards them, mediated by seed dispersers[70]. Interestingly no seedlings of *S. purpurea* were recorded directly underneath conspecific trees possibly due to strong negative density dependent effects (*e.g.* seed predation, interspecific competition)[70–72] reinforcing the idea that J-C effects strongly shape *S. purpurea* seedling recruitment.

To our knowledge only two studies have previously shown J-C effects using genetic data, in *Pinus halepensis* (Pinaceae), Steinitz and colleagues[73] used parentage analysis to estimate differences among seed dispersal kernels

and effective dispersal kernels demonstrating higher seedling survival with increasing distance from maternal plants. Similar results were found by Berens and colleagues[74] in the vertebrate seed dispersed tropical tree *Prunus africana* (Rosaceae); by comparing dispersal distances among different seedling stages, they found that dispersal distances were higher in older seedlings.

Our paternity analyses also showed evidence of other factors that affected individual plant fitness in *S. purpurea* including pollen and seed dispersal distances. Previous studies have analysed individual plant fitness associating only fecundity (seed production) with tree size indicating that in several species, the size of the plant is positively related to greater fecundity[75]; however, information on the success of these seeds to survive and establish in the population is scarce[75]. Genetic markers are a useful tool to trace the number of offspring recruited in the population sired or dispersed by a particular parental tree, data that can be used as an individual fitness measure[75]. Our results indicated that there is not a relationship between the number of established progeny and tree size (DBH); larger individuals could have the potential to produce more offspring due to higher fecundity[75], however, other factors can also influence progeny success (e.g., timing of germination, seed predation, dispersal of offspring). In addition, the number of progeny assigned is not a linear function of the distance from its maternal tree. Our realized seed dispersal data suggests a population recruitment curve (PRC) between 150 and 250 meters away from the maternal tree (Fig. 6C). In accordance with the original proposal of Janzen[13] PRC is the result of the interaction between seed dispersal and seed or seedling predation[13]. In addition, our results show that the effective pollen flow distances are also comparable to the realized seed dispersal PRC. The proposal by Janzen[13] did not originally considered pollen flow in the model; interestingly, our results show that Density Dependent (DD) factors also regulate effective pollen dispersal distances (Fig. 6B). Therefore, DD factors may regulate the parental structure (both maternal and paternal) of the established progeny, also regulating the spatial distribution of genetic diversity within populations.

By comparing effective and realized pollen dispersal results, we can observe that seeds sired by males separated by at least 150 m from female trees have a greater likelihood of establishing in the population than seeds sired by nearby male trees. As shown for several high density insect pollinated tropical trees, effective pollination with nearby neighbours also occurred in *S. purpurea*[76,77]. However, our realized pollen flow data showed that 85% of established seedlings are sired by pollen donors that are >150 m away from maternal trees, indicating that long distance sires have higher male fitness. Similarly, our realized seed dispersal data revealed that seedlings are dispersed more frequently at distances over 150 m, indicating that long dispersal distances from maternal trees also had a strong effect on individual seedling fitness. It has been proposed that in order to coexist with hermaphrodites, dioecious species could have compensatory fitness mechanisms[58,78] for example in some tropical forests females of dioecious species produce more seeds than hermaphroditic species[79]. However, such greater seed production can result in greater seed deposition nearby maternal female trees due to limited seed dispersal; consequently, more aggregated seedlings should be expected and this aggregation could lead to a reduction in fitness via decreased seedling performance[15,80,81]. Under this scenario dioecious plants may allocate more resources to increase seed dispersal to enhance fitness as we show here for *S. purpurea*.

In summary, we evaluated the population sex ratio and the effects of gene dispersal through effective and realized seed and pollen dispersal on FSGS across size classes of the dioecious tree *S. purpurea*. Our results indicated that the male biased sex ratio of this species is due to precocious male reproduction; and the low levels of FSGS among size classes is likely determined by a combination of factors related with long dispersal gene flow and J-C effects during early recruitment stages. J-C density dependent factors regulate realized gene flow patterns and fitness in *S. purpurea*; both long-distance pollen donors and long-distance seed dispersers have higher male and female fitness, respectively, all of which result in low FSGS. Therefore, we propose that low levels of FSGS in tropical trees may be indicative of density dependent factors regulating seedling populations of tropical trees.

Materials and Methods

Study area and study species. This study was conducted in the Chamela-Cuixmala Biosphere Reserve at Chamela Biological Station, UNAM (ChaBS) (19°30′N, 105°03′W) in the central Pacific coast of Jalisco, Mexico. This reserve protects 13,142 hectares of Tropical Dry Forest (TDF), a highly endangered ecosystem. Permission for sampling was obtained from ChaBS director.

Spondias purpurea L. (ANACARDIACEAE) is a small (3–10 m), deciduous and dioecious tree native to the tropical dry forests of Mexico and Central America[82,83]. Reproduction occurs between December and May. This tree depends on insects for cross pollination, primarily stingless bees and wasps[84]. Female trees produce small, bright red, juicy and sweet-acidic fruits that are eaten by various mammals and birds[57]. These fruits represent an important source of water and nutrients in the dry season when high temperatures, water and food scarcity are commonplace[57,70].

Experimental design and sample collection. We set a 30 ha plot (1000 × 300 m) where all *S. purpurea* individuals were tagged and geo-referenced. Plants were grouped into size classes using diameter at breast height (DBH), except for trees less than 2 m tall, for which we used the diameter at the base of the stem. We defined three size classes: class I (DBH < 10 cm), class II (10 cm < DBH < 20 cm), class III (DBH > 20 cm). Seedlings were included in size class I. Sexual expression was monitored for all individuals in two consecutive flowering seasons (2015, 2016). We collected flowers from different sections of the canopy to accurately determine the gender of all individuals.

To estimate pollen dispersal distances we collected twenty fruits from ten randomly chosen female trees within the plot. Fruits were collected directly from the canopy of each individual to guarantee that we sampled progeny from a single maternal tree.

Sex ratios. Sex ratio was expressed as the proportion of males in the population: males/ (females + males)[85]. For each sex we determined the frequency of individuals in each diametric size class. To determine deviations from 1:1 sex ratio we performed a goodness of fit G-test for each size class and for pooled size classes. To evaluate differences in diameter size distribution between sexes, we used a Kolmogorov-Smirnov two sample test. Statistical analyses were performed using the *RVAideMemoire*[86] and *stats* libraries implemented in the R computing environment[87].

DNA extraction and microsatellite amplification. We collected fresh leaf tissue from all individuals within the 30 ha plot and stored them at −20 °C until DNA extraction. For pollen dispersal analyses we dissected fruits and sampled embryos for DNA extraction. DNA from leaves and embryos was extracted using a modified Cetylmethylammonium Bromide (CTAB) protocol[88]. Nine microsatellites previously developed for *S. purpurea*[89] were amplified via multiplex PCR using QIAGEN Multiplex kit (QIAGEN, Hilden, Germany) in 12 µL reaction volumes. The first multiplex mix used primers SPUR44, SPUR40, SPUR28 and SPUR41, the second mix included the primers SPUR35, SPUR29 and SPUR33 and the third set contained the primers SPUR42 and SPUR39; in all cases primers were at a 0.2 µM concentration. The PCR amplification profile included an initial activation step of 95 °C for 15 min, followed by a touchdown PCR consisting of 32 cycles with denaturation at 95 °C for 90 s; annealing for 60 s with temperature decreasing 1 °C every two cycles from 64 °C (12 cycles), then 10 cycles at 58 °C and 10 cycles at 57 °C; elongation at 72 °C for 60 s; and a final extension at 72 °C for 5 min. Fragments were analysed on an automatic ABI-PRISM 3100-Avant sequencer (APPLIED BIOSYSTEMS, Carlsbad, California, USA) using GeneScan LIZ 600 (APPLIED BIOSYSTEMS, Carlsbad, California, USA) to determine fragment sizes. Alleles were scored manually using *GeneMarker Software* version 2.6.4 (SOFTGENETICS). To reduce genotyping error, all samples were independently genotyped by two different researchers to reach consensus in the final data set. The primer SPUR57 was excluded from the analyses because in several individuals it produced multiple peaks that could not be genotyped properly. Erroneous genotypes may critically bias paternity assignments and eliminating SPUR57 did not significantly reduce the exclusion probability of the remaining 9 markers.

Genetic diversity analysis. Genetic diversity was quantified for each size class using the following parameters: allele number averaged across loci (*Na*), Allelic richness (*Ar*), observed (*Ho*) and expected heterozygosities (*He*) and fixation indexes (*F*). These parameters were calculated using the software *ARLEQUIN*[90]. Confidence intervals for *F* values were obtained by bootstrapping over loci using the library *hierfstat*[91] implemented in R[87].

Fine scale genetic structure and spatial analysis. Fine-scale genetic structure was estimated for all size classes using the software *SPAGeDi*[92]. We estimated the mean and the confidence intervals of pairwise kinship coefficients between all pairs of individuals within each size class. For each analysis, we selected eight distance categories; the first five categories are separated by 20 m intervals up to 100 m, with the remaining categories separated by 25 m intervals up to 200 m. All categories included more than 100 pairwise comparisons. To test the null hypothesis of a random distribution of genotypes in space, estimated F_{ij} values were compared to the 95% confidence intervals generated by 10,000 random permutations of individuals in space. F_{ij} values were regressed onto ln (d_{ij}), where d_{ij} is the spatial distance between all pairs of *ij*-individuals. To quantify the FSGS intensity and to compare it with other studies the *Sp* statistic was calculated according to Vekemans and Hardy[3]. The significance of the slope was assessed by comparing the estimated value with 10 000 random shifts of individuals among locations. Standard errors (SE) for all slopes were determined by jackknifing across loci. In order to analyze parentage and conspecific effects on seedling recruitment, we tested the spatial interaction (i.e., association or repulsion) and inter-size class genetic structure between the smallest size class (size class I) and the largest size class (size class III). To evaluate relatedness patterns among plants between size class I and III we performed a "between-size class" FSGS analysis; in which relatedness is calculated for pairs of individuals including an individual from size class I and an individual from size class III, yielding the degree of relatedness of each "seedling" with their closest adults. The distances classes analysed and parameters used for to test the null hypothesis of a random distribution of genotypes in space were the same used in the FSGS within size classes. The spatial interaction (association or repulsion) between individuals of size class I and III was evaluated using Ripley's second order $K_{12}(t)$ function. $K_{12}(t)$ estimates the expected number of type 1 (size class I individuals in this study) within a distance (t) of a randomly chosen type 2 point (size class III). To reduce scale dependencies and stabilize variances, we used the L (t) square root transformation of K (t)[21,93]. Spatial interaction was evaluated for t distances between 0 and 150 m and an isotropic edge correction was also applied. We chose 'random labeling' as the null hypothesis to construct confidence envelopes[94]. Confidence envelopes were constructed from 999 permutations. Spatial association between size class I and size class III individuals is suggested if values of L(t) >0. Conversely, L (t) <0 indicates repulsion between size class I and size class III individuals. If L(t) values fall within the confidence envelope, the spatial relationship is deemed random. Spatial analysis was conducted using the spatstat library[95] implemented in the R computing environment[87].

Parentage analysis. To determine gene flow levels via pollen and seeds we performed two separate parentage analysis. We first evaluated effective pollen dispersal via paternity analysis on seed embryos as offspring genotypes. Males that reproduced in the same year when seeds were collected were considered potential fathers in the analysis. The effective pollen dispersal distance was calculated as the distance between the father assigned to the embryo and the maternal tree. In a second analysis we evaluated realized pollen and seed dispersal distances. To this end we used the genotypes of individuals in the diametric class I as offspring, while reproductive individuals (i.e. identified as male or females by flower observation) in diametric classes II and III were used as potential parents. These analyses resulted in two dispersal distances: (i) the realized pollen dispersal distance estimated as the distance between the mother and father assigned to an established seedling when both parents

occur within the plot[20]; (ii) and the effective seed dispersal distance calculated as the distance between seedlings and their assigned mother. Parentage analyses were conducted using Colony V2[96]. We used the parameter of a polygamous outcrossing dioecious species, and selected the full likelihood method with a large run. We assigned paternity using "strict" (>80%) confidence levels. We included a genotyping error probability of 0.0923 based on the number of mismatches observed between the genotypes of maternal trees and their offspring (calculated from the data set of embryos with known maternal genotypes), estimated using Cervus 3.0[97].

Data availability

The datasets generated and analysed during the current study are available from the corresponding author upon request.

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References

1. Ghazoul, J. Pollen and seed dispersal among dispersed plants. *Biol. Rev.* **80**, 413 (2005).
2. Jordano, P. What is long-distance dispersal? And a taxonomy of dispersal events. *J. Ecol.* **105**, 75–84 (2017).
3. Vekemans, X. & Hardy, O. J. New insights from fine-scale spatial genetic structure analyses in plant populations. *Mol. Ecol.* **13**, 921–935 (2004).
4. Wright, S. Isolation by distance. *Genetics* **28**, 114–138 (1943).
5. Bohonak, A. J. Dispersal, Gene Flow, and Population Structure. *Q. Rev. Biol.* **74**, 21–45 (1999).
6. Kalisz, S., Nason, J. D., Hanzawa, F. M. & Tonsor, S. J. Spatial population genetic structure in Trillium grandiflorum: the roles of dispersal, mating, history and selection. *Evolution* **55**, 1560–1568 (2001).
7. Pardini, E. A. & Hamrick, J. L. Inferring recruitment history from spatial genetic structure within populations of the colonizing tree Albizia julibrissin (Fabaceae). *Mol. Ecol.* **17**, 2865–2879 (2008).
8. Jacquemyn, H., Brys, R., Vandepitte, K., Honnay, O. & Roldán-Ruiz, I. Fine-scale genetic structure of life history stages in the food-deceptive orchid Orchis purpurea. *Mol. Ecol.* **15**, 2801–2808 (2006).
9. Jones, F. A. & Hubbell, S. P. Demographic spatial genetic structure of the Neotropical tree, *Jacaranda copaia*. *Mol. Ecol.* **15**, 3205–3217 (2006).
10. Choo, J., Juenger, T. E. & Simpson, B. B. Consequences of frugivore-mediated seed dispersal for the spatial and genetic structures of a neotropical palm. *Mol. Ecol.* **21**, 1019–1031 (2012).
11. Nazareno, A. G., Alzate-Marin, A. L. & Pereira, R. A. S. Dioecy, more than monoecy, affects plant spatial genetic structure: the case study of *Ficus*. *Ecol. Evol.* **3**(10), 3495–3508 (2013).
12. Gelmi-Candusso, T. A., Heymann, E. W. & Heer, K. Effects of zoochory on the spatial genetic structure of plant populations. *Mol. Ecol.* **26**, 5896–5910 (2017).
13. Janzen, D. H. Herbivores and the Number of Tree Species in Tropical Forests. *Am. Nat.* **104**, 501–528 (1970).
14. Connell, J. H. On the role of natural enemies in preventing competitive exclusion in some marine animals and in rain forest trees in Dy namics of Populations. (eds. Den Boer, P. J. & Gradwell, G. R.) 298–312 (Centre for Agricultural Publishing and Documentation, 1971).
15. Comita, L. S. *et al.* Testing predictions of the Janzen–Connell hypothesis: a meta-analysis of experimental evidence for distance- and density-dependent seed and seedling survival. *J. Ecol.* **102**, 845–856 (2014).
16. Bawa, K. S., Opler, P. A., Rica, D. C. & Rica, C. Dioecism in Tropical Forest Trees. *Evolution* **29**, 167–179 (1975).
17. Bawa, K. S., Perry, D. R. & Beach, J. H. Reproductive Biology of Tropical Lowland Rain Forest Trees. I. Sexual Systems and Incompatibility Mechanisms. *Am. J. Bot.* **72**, 331 (1985).
18. Ibarra-Manriquez, G. & Oyama, K. Ecological Correlates of Reproductive Traits of Mexican Rain Forest Trees. *Am. J. Bot.* **79**, 383 (1992).
19. Renner, S. S. & Ricklefs, R. E. Dioecy and its correlates in the flowering plants. *Am. J. Bot.* **82**, 596–606 (1995).
20. Zhou, H. P. & Chen, J. Spatial genetic structure in an understorey dioecious fig species: the roles of seed rain, seed and pollen-mediated gene flow, and local selection. *J. Ecol.* **98**, 1168–1177 (2010).
21. Riba-Hernández, P., Segura, J. L. & Muñoz-Valverde, J. Female fruit production depends on female flower production and crown size rather than male density in a continuous population of a tropical dioecious tree (*Virola surinamensis*). *Am. J. Bot.* **103**, 1990–1999 (2016).
22. Degen, B. & Sebbenn, A. M. Genetics and Tropical Forests in *Tropical* Forestry *Handbook* (eds. Köhl, M. & Pancel, L) (Springer, 2014).
23. Murawski, D. & Hamrick, J. The effect of the density of flowering individuals on the mating systems of nine tropical tree species. *Heredity* **67**, 167–174 (1991).
24. Bianchi, F. J. J. A. & Cunningham, S. A. Unravelling the role of mate density and sex ratio in competition for pollen. *Oikos* **121**, 219–227 (2012).
25. Castilla, A. R. *et al.* Adding landscape genetics and individual traits to the ecosystem function paradigm reveals the importance of species functional breadth. *Proc Natl Acad Sci USA* **114**, 12761–12766 (2017).
26. Grant, E. L. *et al.* Short distance pollen dispersal and low genetic diversity in a subcanopy tropical rainforest tree, Fontainea picrosperma (Euphorbiaceae). *Heredity* **123**, 503–516 (2019).
27. Barrett, S. C. H., Yakimowski, S. B., Field, D. L. & Pickup, M. Ecological genetics of sex ratios in plant populations. *Philos. T. R. Soc. B.* **365**, 2549–57 (2010).
28. Lloyd, D. G. & Webb, C. J. Secondary sex characters in plants. *Bot. Rev.* **43**, 177–216 (1977).
29. Obeso, J. R., Alvarez-Santullano, M. & Retuerto, R. Sex ratios, size distributions, and sexual dimorphism in the dioecious tree *Ilex aquifolium* (Aquifoliaceae). *Am. J. Bot.* **85**, 1602–1608 (1998).
30. Wilson, A. J. & Nussey, D. H. What is individual quality? An evolutionary perspective. *Trends Ecol. Evol.* **25**(4), 207–214 (2010).
31. Meagher, T. R. & Thompson, E. Analysis of Parentage for Naturally Established Seedlings of Chamaelirium Luteum (Liliaceae). *Ecology* **68**, 803–812 (1987).
32. Primack, R. B. & Kang, H. Measuring fitness and natural selection in wild plant populations. *Annu. Rev. Ecol. Syst.* **20**, 367–396 (1987).
33. Kingsolver, J. G., Diamond, S. E., Siepielski, A. M. & Stephanie, M. C. Synthetic analyses of phenotypic selection in natural populations: lessons, limitations and future directions. *Evol. Ecol.* **26**, 1101 (2012).
34. Campbell, D. R., Brody, A. K., Price, M. V., Waser, N. M. & Aldridge, G. Is Plant Fitness Proportional to Seed Set? An Experiment and a Spatial Model. *Am. Nat.* **190**(6), 818–827 (2017).
35. Sant'Anna, C. *et al.* Realized pollen and seed dispersal within a continuous population of the dioecious coniferous Brazilian pine [Araucaria angustifolia (Bertol.) Kuntze]. *Conserv. Genet.* **14**(3), 601–613 (2013).
36. Opler, P. A. & Bawa, K. S. Sex ratios in tropical forest trees. *Evolution* **32**, 812–821 (1978).

37. Bullock, S. H. & Bawa, K. S. Sexual Dimorphism and the Annual Flowering Pattern in Jacaratia Dolichaula (D. Smith) Woodson (Caricaceae) in a Costa Rican Rain Forest. *Ecology* **62**, 1494–1504 (1981).
38. Allen, G. A. & Antos, J. A. Sex Ratio Variation in the Dioecious Shrub *Oemeleria cerasiformis*. *Am. Nat.* **141**, 537–553 (1993).
39. Nicotra, A. B. Sex ratio variation and spatial distribution of *Siparuna grandiflora*, a tropical dioecious shrub. *Oecologia* **115**, 102–113 (1998).
40. Thomas, S. C., LaFrankie, J. V., Thomas, A. S. C. & LaFrankie, J. V. Sex, size and interyear variation in flowering among dioecious trees of the malayan rain forest. *Ecology* **74**, 1529–1537 (1993).
41. García, M. & Antor, R. Sex Ratio and Sexual Dimorphism in the Dioecious Borderea pyrenaica (Dioscoreaceae). *Oecologia* **101**(1), 59–67 (1995).
42. Hamrick, J. L., Godt, M. J. W. & Sherman-Broyles, S. L. Factors influencing levels of genetic diversity in woody plant species. *New Forest* **6**, 95–124 (1992).
43. Hamrick, J. L. Response of forest trees to global environmental changes. *Forest Ecol. Manag.* **197**, 323–335 (2004).
44. Dick, C. W., Hardy, O. J., Jones, F. A. & Petit, R. J. Spatial Scales of Pollen and Seed-Mediated Gene Flow in Tropical Rain Forest Trees. *Trop. Plant Biol.* **1**, 20–33 (2008).
45. Hardesty, B. D., Dick, C. W., Kremer, A., Hubbell, S. & Bermingham, E. Spatial genetic structure of Simarouba amara Aubl. (Simaroubaceae), a dioecious, animal-dispersed Neotropical tree, on Barro Colorado Island, Panama. *Heredity* **95**, 290–297 (2005).
46. Dev, S. A., Kjellberg, F., Hossaert-McKey, M. & Borges, R. M. Fine-scale Population Genetic Structure of Two Dioecious Indian Keystone Species, Ficus hispida and Ficus exasperata (Moraceae). *Biotropica* **43**, 309–316 (2011).
47. Loiselle, B. A., Sork, V. L., Nason, J. & Graham C. Spatial genetic structure of a tropical understory shrub, *Psychotria officinalis* (Rubiaceae). *Am. J. Bot.* **82**, 1420–1425 (1995).
48. Chung, M. Y., Epperson, B. K. & Chung, M.G. Genetic structure of age classes in *Camellia japonica* (Theaceae). *Evolution* **57**, 62–73 (2003).
49. Epperson, B. K. & Alvarez-Buylla, E. R. Limited seed dispersal and genetic structure in life stages of *cecropia obtusifolia*. *Evolution* **51**, 275–282 (1997).
50. Kitamura, K., Nakanishi, A., Lian, C. & Goto, S. Distinctions in Fine-Scale Spatial Genetic Structure Between Growth Stages of Picea jezoensis Carr. *Front. Genet.* **9**, 490 (2018).
51. Schroeder, J. W., Trana, H. T. & Dick, C. W. Fine scale spatial genetic structure in Pouteria reticulata (Engl.) Eyma (Sapotaceae), a dioecious, vertebrate dispersed tropical rain forest tree species. *Glob. Ecol. Conserv.* **1**, 43–49 (2014).
52. Vieira, F. A., Fajardo, C. G., Souza, A. M., Reis, C. A. F. & Carvalho, D. Fine-scale genetic dynamics of a dominant neotropical tree in the threatened Brazilian Atlantic Rainforest. *Tree Genet. Genomes* **8**, 1191–1201 (2012).
53. Chung, M. G. & Epperson, B. K. Clonal and spatial genetic structure in *Eurya emarginata* (Theaceae). *Heredity* **84**, 170–177 (2000).
54. Trapnell, D. W., Schmidt, J. P. & Hamrick, J. L. Spatial genetic structure of the Southeastern North American endemic, *Ceratiola ericoides* (Empetraceae). *J. Hered.* **99**, 604–609 (2008).
55. Wang, R. *et al.* Spatial genetic structure and restricted gene flow in a functionally dioecious fig Ficus pumila L. pumila (Moraceae). *Popul. Ecol.* **51**, 307–315 (2009).
56. Gaino, A. P. S. C. *et al.* Understanding the effects of isolation on seed and pollen flow, spatial genetic structure and effective population size of the dioecious tropical tree Myracrodruon urundeuva. *Conserv. Genet.* **11**, 1631–1643 (2010).
57. Mandujano, S., Gallina, S. & Bullock, S. H. Frugivory and dispersal of *Spondias purpurea* (Anacardiaceae) in a tropical deciduous forest in México. *Rev. Biol. Trop.* **42**, 107–114 (1994).
58. Heilbuth, J. C., Ilves, K. L. & Otto, S. P. The consequences of dioecy for seed dispersal: modeling the seed-shadow handicap. *Evolution* **55**, 880–888 (2001).
59. Hardesty, B. D., Hubbell, S. P. & Bermingham, E. Genetic evidence of frequent long-distance recruitment in a vertebrate-dispersed tree. *Ecol. Lett.* **9**, 516–525 (2006).
60. Wang, B. C., Sork, V. L., Leong, M. T. & Smith, T. B. Hunting of Mammals Reduces Seed Removal and Dispersal of the Afrotropical Tree Antrocaryon klaineanum (Anacardiaceae). *Biotropica* **39**(3), 340–347 (2007).
61. Sezen, U. U., Chazdon, R. L. & Holsinger, K. E. Proximity is not a proxy for parentage in an animal- dispersed Neotropical canopy palm. *Proc. R. Soc. B.* **276**, 2037–2044 (2009).
62. Solís-Hernández, W. & Fuchs, E. J. Effective gene flow patterns across a fragmented landscape in southern Costa Rica for Symphonia globulifera (Clusiaceae): a species with mobile seed and pollen dispersers. *Rev. Biol. Trop.* **67**(2), 95–111 (2019).
63. Howe, H. F. Bird Activity and Seed Dispersal of a Tropical Wet Forest Tree. *Ecology* **58**, 539–550 (1977).
64. Fuchs, E. J. & Hamrick, J. L. Spatial genetic structure within size classes of the endangered tropical tree *Guaiacum sanctum* (Zygophyllaceae). *Am. J. Bot.* **97**, 1200–1207 (2010).
65. Dow, B. D. & Ashley, M. V. Microsatellite analysis of seed dispersal and parentage of saplings in bur oak, *Quercus macrocarpa*. *Mol. Ecol.* **5**, 615–627 (1996).
66. Streiff, R. *et al.* Pollen dispersal inferred from paternity analysis in a mixed oak stand of *Quercus robur* L. and *Q. petraea* (Matt.) Liebl. *Mol. Ecol.* **8**, 831–841 (1999).
67. Nathan, R. & Muller-Landau, H. C. Spatial patterns of seed dispersal, their determinants and consequences for recruitment. *Trends Ecol. Evol.* **15**(7), 278–285 (2000).
68. Godoy, J. A. & Jordano, P. Seed dispersal by animals: exact identification of source trees with endocarp DNA microsatellites. *Mol. Ecol.* **10**, 2275–2283 (2001).
69. Dick, C. W., Etchelecu, G. & Austerlitz, F. Pollen dispersal of tropical trees (Dinizia excelsa: Fabaceae) by native insects and African honeybees in pristine and fragmented Amazonian rainforest. *Mol. Ecol.* **12**, 753–764 (2003).
70. Méndez-Toribio, M., Pierro, A. M. G.-D., Quesada, M. & Benítez-Malvido, J. Regeneration beneath a dioecious tree species in a Mexican tropical dry forest. *J. Trop. Ecol.* **30**, 265–268 (2014).
71. Sugiyama, A., Comita, L., Masaki, T., Condit, R. & Hubbell, S. Resolving the paradox of clumped seed dispersal: positive density and distance dependence in a bat-dispersed species. *Ecology* **99**(1), 2583–2591 (2019).
72. Zhu, Y. *et al.* Density-dependent survival varies with species life-history strategy in a tropical forest. *Ecol. Lett.* **21**(4), 506–515 (2018).
73. Steinitz, O., Troupin, D., Vendramin, G. G. & Nathan, R. Genetic evidence for a Janzen–Connell recruitment pattern in reproductive offspring of Pinus halepensis trees. *Mol. Ecol.* **20**, 4152–4164 (2011).
74. Berens, D. G. *et al.* Changes of effective gene dispersal distances by pollen and seeds across successive life stages in a tropical tree. *Oikos* **122**, 1616–1625 (2013).
75. Younginger, B. S., Sirová, D., Cruzan, M. B. & Ballhorn, D. J. Is biomass a reliable estimate of plant fitness? *Appl. Plant Sci.* **5**, 1600094, https://doi.org/10.3732/apps.1600094 (2017).
76. Stacy, E. A. *et al.* Pollen Dispersal in Low-Density Populations of Three Neotropical Tree Species. *Am. Nat.* **148**(2), 275–298 (1996).
77. Ward, M., Dick, C. W., Gribel, R. & Lowe, A. J. To self, or not to self… A review of outcrossing and pollen-mediated gene flow in neotropical trees. *Heredity* **95**, 246–254 (2005).
78. Bawa, K. S. Evolution of dioecy in flowering plants. *Ann. Rev. Ecol. Syst.* **11**, 15–39 (1980).
79. Bruijning, M. *et al.* Surviving in a Cosexual World: A Cost-Benefit Analysis of Dioecy in Tropical Trees. *Am. Nat.* **189**(3), 297–314 (2017).

80. Harms, K. E., Wright, J. S., Calderón, O., Hernández, A. & Herre, E. A. Pervasive density-dependent recruitment enchances seedling diversity in a tropical forest. *Nature* **404**, 493–495 (2000).
81. Lebrija-Trejos, E., Reich, P. B., Hernández, A. & Wright, S. J. Species with greater seed mass are more tolerant of conspecific neighbours: a key driver of early survival and future abundances in a tropical forest. *Ecol. Lett.* **19**, 1071–1080 (2016).
82. Miller, A. J. & Knouft, J. H. GIS-based characterization of the geographic distributions of wild and cultivated populations of the Mesoamerican fruit tree *Spondias purpurea* (Anacardiaceae). *Am. J. Bot.* **93**, 1757–1767 (2006).
83. Miller, A. & Schaal, B. Domestication of a Mesoamerican cultivated fruit tree, *Spondias purpurea*. *Proc. Natl. Acad. Sci. USA* **102**, 12801–12806 (2005).
84. Mitchell, J. D. & Daly, D. C. A revision of *Spondias* L. (Anacardiaceae) in the Neotropics. *PhytoKeys* **55**, 1–92 (2015).
85. Wilson, K. & Hardy, I. C. W. Statistical analysis of sex ratios: an introduction. (ed. Hardy, I. C. W.) *Sex Ratios*. 48–92 (Cambridge University Press, 2002).
86. Hervé, M. RVAideMemoire: testing and plotting procedures for biostatistics. R package version 0.9-69-3, https://CRAN.R-project.org/package=RVAideMemoire Computer software (2018).
87. R Core Team. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria (2018).
88. Doyle, J. J. & Doyle, J. L. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochem. Bull.* **19**, 11–15 (1987).
89. Cristóbal-Pérez, E. J., Fuchs, E. J., Harvey, N. & Quesada, M. Isolation and characterization of microsatellites loci in Spondias purpurea (Anacardiaceae) and cross amplification in congeneric species. *Mol. Biol. Rep.* **46**(5), 5581–5585 (2019).
90. Excoffier, L. & Lischer, H. E. L. Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. *Mol. Ecol. Resour.* **10**, 564–567 (2010).
91. Goudet, J. H. a package for r to compute and test hierarchical F-statistics. *Mol. Ecol. Res.* **5**, 184–186 (2005).
92. Hardy, O. J. & Vekemans, X. Spagedi: a versatile computer program to analyse spatial genetic structure at the individual or population levels. *Mol. Ecol. Res.* **2**, 618–620 (2002).
93. Besag, J. Contribution to the discussion of Dr. Ripley's paper. *J. Roy. Stat. Soc. B* **39**, 193–195 (1977).
94. Goreaud, F. & Pélissier, R. Avoiding misinterpretation of biotic interactions with the intertype K12-function: population independence vs. random labeling hypotheses. *J. Veg. Sci.* **14**, 681–692 (2003).
95. Baddeley, A. & Turner, R. Spatstat: an R package for analyzing spatial point patterns. *J. Stat. Softw.* **12**, 1–42 (2005).
96. Jones, O. R. & Wang, J. COLONY: a program for parentage and sibship inference from multilocus genotype data. *Mol. Ecol. Res.* **10**, 551–555 (2010).
97. Kalinowski, S. T., Taper, M. L. & Marshall, T. C. Revising how the computer program Cervus accommodates genotyping error increases success in paternity assignment. *Mol. Ecol.* **16**, 1099–1106 (2007).

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Author contributions

E.J.C.P., E.J.F. and M.Q. designed the study. E.J.C.P. and M.Q. collected the samples. E.J.C.P. performed lab work. E.J.C.P., U.O.P and E.J.F. performed statistical and genetic analyzes. E.J.C.P. drafted the manuscript. All authors completed and approved the final manuscript.

Competing interests

The authors declare no competing interests.

Additional information

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CAPÍTULO II. Habitat fragmentation negatively affects genetic diversity, gene flow and male and female fitness in the dioecious tree, *Spondias purpurea* (Anacardiaceae).

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Research article

Habitat fragmentation negatively affects effective gene flow via pollen, and male and female fitness in the dioecious tree, *Spondias purpurea* (Anacardiaceae)

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ABSTRACT

Habitat fragmentation is recognized as one of main threats to global biodiversity. Habitat fragmentation negatively affects population size and mutualistic interactions that directly impact plant fitness and genetic diversity; however, little is known about effects on dioecious trees. We assessed the effects of forest fragmentation on plant-pollinator interactions, male and female reproductive success, realized gene flow, genetic diversity and spatial genetic structure (SGS) in the dioecious tree *Spondias purpurea*. The study was performed in continuous and fragmented forest habitats in the region of the Chamela-Cuixmala Biosphere Reserve, Jalisco, Mexico. Stingless bees were the main pollinators followed by wasps and flies. There were no differences in pollinator assemblages between habitat conditions, however visitation rate was higher in continuous habitats. Male trees produced more flowers than female trees in both habitat conditions. Total flower production was higher in fragmented habitats, but a higher fruit-set was observed in continuous habitat. In fragmented habitats, realized pollen flow occurs at a shorter distance and correlated paternity was higher than in continuous habitats. Genetic diversity and SGS were comparable among adult trees between habitat conditions; however, juveniles and seeds had lower heterozygosity levels and higher inbreeding coefficients in fragmented habitats. Our results suggest that mating systems and pollinator dependence are both key elements influencing plant vulnerability to habitat fragmentation. We conclude that conservation efforts should focus on processes that maintain reproductive success and genetic diversity of species to ensure persistence in the long term.

1. Introduction

In the tropics more than 80% of tree species are pollinated by biotic vectors (i.e. insects and vertebrates) and dispersed by vertebrates frugivores (Bawa et al., 1985; Bawa, 1990; Beckman and Rogers, 2013). Under natural conditions, plant-animal interactions, as well as distance and density dependent processes regulate tropical tree population dynamics and fitness (Janzen, 1970; Harms et al., 2000; Cristóbal-Pérez et al., 2020). However, human-driven habitat loss and fragmentation are likely to alter density-dependent interactions by decreasing population density and increasing isolation (Fahrig, 2003; Fischer and Lindenmayer, 2007; Legrand et al., 2017; Aguilar et al., 2019). In smaller populations, random loss of genetic variability and higher genetic structure are also more likely to occur due to genetic drift and limited gene flow among remnants (Ouborg et al., 2006; Aguilar et al., 2019), negatively affecting the long-term persistence of plant populations in fragmented habitats (Sork and Smouse, 2006; Aguilar et al., 2008, 2019).

Fragmentation also negatively affects mutualistic plant-animal interactions (Cascante et al., 2002; Harris and Johnson, 2004; Aguilar et al., 2006; González-Varo et al., 2009; Xiao et al., 2016). Changes in the abundance and composition of the pollinator community or pollinator behavior may reduce fruit-set and the quantity and quality of seeds (Fuchs et al., 2003). Altering seed dispersal mutualisms may also

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negatively affect seedling recruitment and demography (Cordeiro and Howe, 2003). In addition, habitat fragmentation may also increase antagonistic interactions, such as herbivory and seed predation, with negative consequences on plant recruitment and plant fitness (Orrock et al., 2003, 2006; Fáveri et al., 2008; Herrerías-Diego et al., 2008). Fragmentation studies have commonly focused on the effects of fragmentation on female fitness, including flower, fruit and seed production, and fruit-set (Cascante et al., 2002; Fuchs et al., 2003; Quesada et al., 2004; Aguilar et al., 2006; González-Varo et al., 2009). The effect of habitat fragmentation on plant male function, such as pollen production, pollen performance and pollen flow distances, has been rarely analyzed.

In fragmented habitats gene flow may decrease as a result of: a) longer distances between conspecifics, b) changes in the foraging behavior of pollinators and seed dispersers or, c) limited movement of pollinators and seed dispersers between forest patches (Sih and Baltus, 1987; Quesada et al., 2003, 2004; Breed et al., 2015; but see Dick, 2001; White et al., 2002; Rosas et al., 2011). Limited gene flow could also result in spatial genetic structure (i.e. the non-random spatial distribution of genetic diversity), resulting in neighborhoods of related individuals within populations, which may increase inbreeding in seedlings and juveniles, reducing the recruitment probability of these cohorts, thus reducing genetic diversity and population fitness. Most population genetic studies have been conducted in adult populations of long-lived species in relatively recent fragmented habitats, where the observed effects on genetic diversity are likely the result of recent bottlenecks (Nason and Hamrick, 1997, Oostermeijer et al., 2003, Lowe et al., 2005, Aguilar et al., 2008). However, the number of generations elapsed after fragmentation is a determining factor in the reduction of genetic diversity (Aguilar et al., 2008). For example, inbreeding coefficients in the progeny (i.e., seeds and juveniles) can increase immediately after fragmentation if mating occurs between related individuals (Aguilar et al., 2019), and spatial genetic structure can also increase due to limited dispersal (Solís-Hernández and Fuchs, 2019). Therefore, studying the effects of fragmentation on different plant cohorts provides a better understanding of the demographic genetic consequences of habitat fragmentation.

Dioecious species are obligate outcrossing because male and female functions occur in separate individuals. These species are relatively common in tropical areas (ca. 27% of all tropical trees are dioecious) (Bawa et al., 1985; Ibarra-Manriquez and Oyama, 1992). Sexual reproduction in dioecious species depends also on population sex ratios. In undisturbed conditions sex ratios are expected to be affected by intersexual differences on life history traits (Lloyd and Webb, 1977; Delph, 1999; Obeso, 2002); however, the potential reduction in population size and resource availability after deforestation can bias population sex ratios, affecting effective population sizes. Tropical dioecious trees generally bear small flowers and fleshy fruits (Renner and Ricklefs, 1995; Renner, 2014), which depend on small insects (~66% in tropical forest) and vertebrate frugivores for pollination and seed dispersal, respectively (Ibarra-Manriquez and Oyama, 1992; Renner and Ricklefs, 1995). These reproductive and ecological traits make tropical dioecious species particularly vulnerable to habitat fragmentation (Aguilar et al., 2006). However, information on the reproductive and genetic consequences of fragmentation on tropical dioecious species is still scarce (Chávez-Pesqueira et al., 2014; Riba-Hernández et al., 2014; Cascante-Marín et al., 2020).

In this study, we analyze the reproductive and genetic consequences of habitat fragmentation on the dioecious, insect pollinated and vertebrate dispersed tree, *Spondias purpurea* (Anacardiaceae). To accomplish this, we compared pollinator assemblages, pollinator visitation rates, male fitness (flower and pollen production, and gene flow via paternity analysis), female fitness (flower and fruit production), genetic diversity, and spatial genetic structure (SGS) between continuous and fragmented habitats in a tropical dry forest in Mexico. We predicted that forest fragmentation would negatively impact the fitness of male and female individuals in *S. purpurea* due to changes in pollinator visitation rates and effectiveness. We also expected fragmentation to reduce tree effective population sizes and genetic variability, alter sex ratios and increase levels of relatedness among progenies (seedlings and juveniles) and adults. Our study is the first to simultaneously evaluate direct and indirect estimates of male and female fitness with respect to habitat fragmentation in a dioecious species.

2. Materials and methods

2.1. Study area and study species

This study was conducted in the central Pacific coast of Jalisco, Mexico; in the Chamela-Cuixmala Biosphere Reserve (CCBR) (ca. 19°30′N, 105°03′W) and surrounding areas. The CCBR has an extension of 13,200 ha. The region consists primarily of tropical dry forests with a mean annual rainfall of 748 mm and a marked dry season that extends from November to June (García-Oliva et al., 2002). In this region, the tropical dry forests surrounding the CCBR have been converted into pastures, agricultural lands and forest fragments since the 1950s (Sánchez-Azofeifa et al., 2009).

S. purpurea L. (Anacardiaceae) is a neotropical dioecious tree (3–10 m high) native to the tropical dry forests of Mexico and Central America (Miller and Schaal, 2005; Miller and Knouft, 2006). Reproduction occurs between December and May (Bullock, 1992). Male flowers are smaller than female flowers (Bullock, 1992). Male and female inflorescences are visited primarily by stingless bees and wasps (Mitchell and Daly, 2015). Female trees produce small, bright red, juicy and sweet-acidic fruits dispersed by mammals and birds (Mandujano et al., 1994). *S. purpurea* is an ecologically important tree, because it represents an important source of water and nutrients for floral visitors and frugivores, particularly in the dry season when high temperatures, water and food scarcity are commonplace (Bullock, 1992; Mandujano et al., 1994).

2.2. Selection of trees and sex ratios

We sampled *S. purpurea* in two continuous and three fragmented tropical dry forest sites. Continuous sites at Chamela and Careyes, consisted of large forested areas at least 500 m away from the forest edge. Chamela was located within the official boundaries of the CCBR. Careyes is a site with continuous forest that extends beyond the official limits of the CCBR (Fig. 1). The three fragmented study sites (Mesa, Ranchitos, Nacastillo) were isolated forest remnants, located within a matrix of cultivated land, cattle pastures and secondary forest; these sites were between 4 and 14 km away from continuous forest (Fig. 1).

2.3. Pollinator visitation

The richness and abundance of floral visitors were recorded using Sony Digital Handycam DCR-SR 46 cameras (Sony, San Diego, CA, USA) on 20 adult trees (10 trees per site) in continuous habitats and 30 adult trees in fragmented habitats (10 trees per site). On each site, we sampled 5 male and 5 female trees. Video recordings were conducted for three-hour periods from 0800 to 1100 h at each site for a total of 150 h. Observations were performed during the reproductive season of 2012 in February when peak flowering occurs. On individual inflorescences we recorded the identity of floral visitors, the number of flowers visited and the number of times floral visitors made contact with sexual organs. Pollinator visitation rates were calculated by dividing the number of flowers visited per inflorescence by the number of observation hours.

2.4. Female and male fitness

To determine differences in male and female fitness between continuous and fragmented habitats, we tagged ten 50 cm long branches on 10 female individuals of *S. purpurea* per site. As a measure of female fitness we quantified the total number of female flowers and mature

Fig. 1. Map of the region of Chamela, Jalisco Mexico showing the continuous sites (Chamela and Careyes) and fragmented sites (Ranchitos, Nacastillo and Mesa).

fruits produced per branch. Fruit-set was calculated as the total number of fruits divided by the total number of flowers produced per branch.

As an indirect measure of male fitness, we quantified the number of male flowers per branch and pollen production. Differences in male flower production were quantified on ten 50 cm long branches in 10 male individuals of *S. purpurea* per site. Pollen production per flower was quantified for 15 flowers per tree per site. We excluded insect visitation by bagging inflorescences in fine-mesh nylon bags before floral anthesis. Flowers collected after anthesis were stored in 1.5 ml scintillation vials until pollen count analysis. Pollen was counted using an Elzone II 5390 particle analyzer (Micrometitics Instrument Corp., Norcross, GA, USA) using the methods described by Harder (1990).

2.5. Statistical analysis

We used generalized linear models using PROC GLIMMIX in SAS (SAS Institute Inc., 2008) to test for differences in reproductive success and pollinator visitation rates between continuous and fragmented habitats and between male and female plants. To test for differences in flower production and pollinator visitation rates, we included plant sex (female vs. male) and habitat condition (continuous vs. fragmented) as fixed factors, and their interaction (Plant sex*habitat condition). To test for differences in total pollen production and fruit-set, we included habitat condition as a fixed factor. In all cases, site was considered a random factor nested within habitat condition. We used the number of male and female flowers per 50 cm of branch, pollinator visitation rate and pollen production (Poisson distribution with a log link function) and fruit-set (Binomial distribution with a logit link function) as response variables. We used the *ilink* function to calculate back-transformed means. p-Values for multiple comparisons were Tukey-adjusted.

2.6. Sampling for genetic analysis

To determine the consequences of habitat fragmentation on genetic diversity parameters, we tagged and geo-referenced all adult and juvenile *S. purpurea* individuals in all study sites. We defined two demographic cohorts: juveniles (DBH <10 cm, height < 2 m) and adults (DBH > 10 cm). Sexual expression was monitored for all individuals in 2012 and 2015. We collected flowers from the canopy of reproductive trees to accurately determine the sex of all individuals. Sex ratio per site was expressed as the proportion of males in the population: males / (females + males) (Wilson and Hardy, 2002). To determine deviations from 1:1 sex ratio we performed a goodness of fit G-test for all sites. Statistical analyses were performed using the *stats* library implemented in the R computing environment (R Core Team, 2018). We collected fresh leaf tissue from juvenile and adult individuals and stored them at −20 °C until DNA extraction.

To estimate gene flow via pollen, we collected 15 fruits per female tree directly from the canopy of 20 female trees in continuous sites and 30 female trees in fragmented sites. We dissected fruits and sampled diploid embryos for DNA extraction.

2.7. DNA extraction and microsatellite amplification

DNA from leaves and embryos was extracted using a modified Cetyltrimethylammonium Bromide (CTAB) protocol (Doyle and Doyle, 1987). Nine microsatellites previously developed for *S. purpurea* (Cristóbal-Pérez et al., 2019) were amplified via multiplex PCR using QIAGEN ® Multiplex kit (QIAGEN, Hilden, Germany) in 12 μl reaction volumes.

Multiplex PCR amplification conditions followed Cristóbal-Pérez et al. (2020). Fragments were analyzed on an automatic ABI-PRISM 3100-Avant sequencer (Applied Biosystems, Carlsbad, California, USA)

using GeneScan LIZ 600 (Applied Biosystems, Carlsbad, California, USA) to determine fragment sizes. Alleles were scored manually using *GeneMarker Software* version 2.6.4 (SoftGenetics).

2.8. Genetic diversity and spatial genetic structure analysis

The following genetic parameters were calculated separately for juveniles and adults within each study site and for each habitat condition: allele number averaged across loci (*Na*), N_e: mean number of effective alleles per locus (± SD) observed (*Ho*) and expected heterozygosities (*He*) and fixation indexes (*F*). These parameters were calculated using GeneAlEx 6.5 (Peakall and Smouse, 2012). Differences in genetic diversity between habitat conditions and developmental stages were tested using FSTAT 2.9.3.2 (Goudet, 2005), assuming Hardy-Weinberg equilibrium and using 10,000 permutations to test for the significance of inbreeding coefficients.

Spatial genetic structure (SGS) was estimated for adults using the software *SPAGeDi* (Hardy and Vekemans, 2002). Due to the reduced number of juveniles in fragmented sites, spatial genetic structure was not estimated for this developmental stage. We estimated the mean and confidence intervals of pairwise kinship coefficients between all pairs of individuals (Loiselle et al., 1995). We selected seven distance categories; the first five categories were separated by 20 m intervals up to 100 m, with the remaining categories separated by 25 m intervals up to 150 m. All categories included more than 100 pairwise comparisons. To test the null hypothesis of a random distribution of genotypes in space, estimated F_{ij} values were compared to the 95% confidence intervals generated by 10,000 random permutations of individuals in space. F_{ij} values were regressed onto ln (d_{ij}), where d_{ij} is the spatial distance between all pairs of *ij*-individuals to estimate the *Sp* statistic as suggested by Hardy and Vekemans (2002).

2.9. Effective pollen dispersal and number of male sires

To determine effective pollen dispersal distances, we performed paternity analysis on dissected embryos. Males that reproduced on the year when seeds were collected were considered potential fathers in the analysis. The effective pollen dispersal distance was calculated as the distance between the assigned father and the maternal tree. Paternity analyses were conducted using Colony V2 (Jones and Wang, 2010). We used the parameters suggested by Jones and Wang (2010) for a polygamous outcrossing dioecious species and selected the full likelihood method with a large run. We included a genotyping error probability of 0.09 based on the number of mismatches observed between maternal and offspring genotypes, calculated using Cervus 3.0 (Kalinowski et al., 2007). We estimated the average effective number of pollen donors per maternal plant (N_{ep}) using multilocus-correlated paternity (r_{pm}) estimated for each progeny array in MLTR (Ritland, 2002) as a direct measure of male fitness. The reciprocal of the r_{pm} corresponds to the effective number of pollen donors ($1/r_{pm}$) (Ritland, 2002). The standard error of all estimates was calculated by bootstrapping with 1000 repetitions.

3. Results

3.1. Pollinator visitation rates

We observed a total of 1171 insect visits in the continuous habitat and 489 in the fragmented habitat. Flowers were visited mainly by three species of bees (*Apis mellifera*, *Trigona nigra*, *Trigona fulviventris*), three species of wasps (two *Polistes* spp. and one *Mischocyttarus* sp.) and two species of flies (from the Tachinidae and Syrphidae families); with bees performing 65.7% of all visits. Floral visitor species composition did not change between habitat conditions nor with plant sex. In continuous habitats, bees were the most abundant group visiting *S. purpurea* flowers, accounting for 68.9% of all visits, followed by flies and wasps representing 15.9 and 15.11% of the visits, respectively. In fragmented habitats, bees were also the most common taxon with 58.3% of the visits, followed by flies and wasps accounting for 28.6% and 13% of the visits, respectively.

Total visitation rates, estimated as the mean number of visits per inflorescence per hour, were significantly higher in continuous habitats than in fragmented habitats ($F_{1,4} = 89.25$, $p \leq 0.0025$; Fig. 2A). Male inflorescences received significantly more visits per hour than female inflorescences ($F_{1,43} = 155.01$, $p \leq 0.0001$; Fig. 2A), regardless of habitat conditions ($F_{1,43} = 0.01$, $p = 0.99$; Fig. 2A).

3.2. Male and female fitness

Trees in fragmented habitats produced more flowers per branch than trees from continuous populations ($F_{1,3} = 10.75$, $p < 0.0001$; Fig. 2B), and male trees produced significantly more flowers than female trees regardless of habitat condition ($F_{1,4} = 47{,}236.2$, $p < 0.0001$; Fig. 2B). However, a significant interaction term (plant sex * habitat condition) indicates that female trees produced a similar number of flowers in both habitat conditions ($F_{1,4} = 71.47$, $p < 0.0001$; Fig. 2B). Pollen grain production per flower was independent of habitat condition ($F_{1,3} = 1.55$, $p = 0.301$, Fig. 2C). Fruit production per branch did not differ between habitat conditions (mean ± SEM) (CH = 22.9 ± 9.02, FH = 12.69 ± 9.95; $F_{1,3} = 5.54$, $p = 0.09$). However, fruit-set was highest in trees from continuous habitats ($x^2_{1,3} = 50.26$, $p = 0.0058$; Fig. 2D). On average 40% (±0.9% SEM) of the flowers produced fruit on trees from continuous habitats, whereas approximately half of the flowers (20% ± 1.08%) developed into fruits in fragmented habitats.

3.3. Genetic diversity and spatial genetic structure

We sampled 308 adults (150 males, 158 females) and 172 juveniles individuals of *S. purpurea* across continuous sites and 174 adults (79 males and 95 females) and 70 juveniles in fragmented habitats. The sex ratio for all study sites did not significantly deviate from 1:1 ratio (see online Appendix A). All loci analyzed were polymorphic. All genetic diversity parameters in adult trees did not statistically differ between habitat conditions ($p > 0.39$; Table 1, Appendix B). The mean allele number per locus of the progeny (Na) (seeds and juveniles) was similar between habitat conditions ($p = 0.12$), observed heterozygosities (Ho) were higher in continuous habitats ($p = 0.007$), and the average inbreeding coefficient (F) was higher in fragmented habitats ($p = 0.001$; Table 1, Appendix B).

Our results indicated a lack of SGS between fragmented and continuous habitats. The highest pairwise kinship coefficients in both habitat conditions were both found in the 20 m distance class ($F_{ij} > 0.042$ for continuous, and $F_{ij} > 0.062$ for fragmented habitat). Kinship estimates decreased with geographic distance; however, these estimates did not differ from those expected from a random distribution of genotypes in space (Fig. 3). The *Sp* statistic, which measures the strength of SGS (Hardy et al., 2006), confirms the lack of SGS, with non significant regression slopes across all sites (Table 2).

3.4. Effective pollen dispersal and number of male sires

Out of 727 genotyped seeds for effective pollen dispersal estimates, 312 (Continuous habitat = 104, fragmented habitat = 208) were assigned with >80% confidence to pollen donors within the analyzed populations. All paternity assignments were the result of matings within populations. Average (±SEM) pollen flow distances were 209.15 ± 19.28 m in continuous habitats and 44.91 ± 1.98 m in fragmented habitats. In continuous habitats effective pollen dispersal distances ranged between 7.7 and 828.2 m, while in fragmented habitats distances were lower and ranged between 2.98 m and 134 m (Fig. 4). In continuous habitats > 75% of all effective pollination events assigned by paternity analysis occurred between trees that were more than 75 m apart,

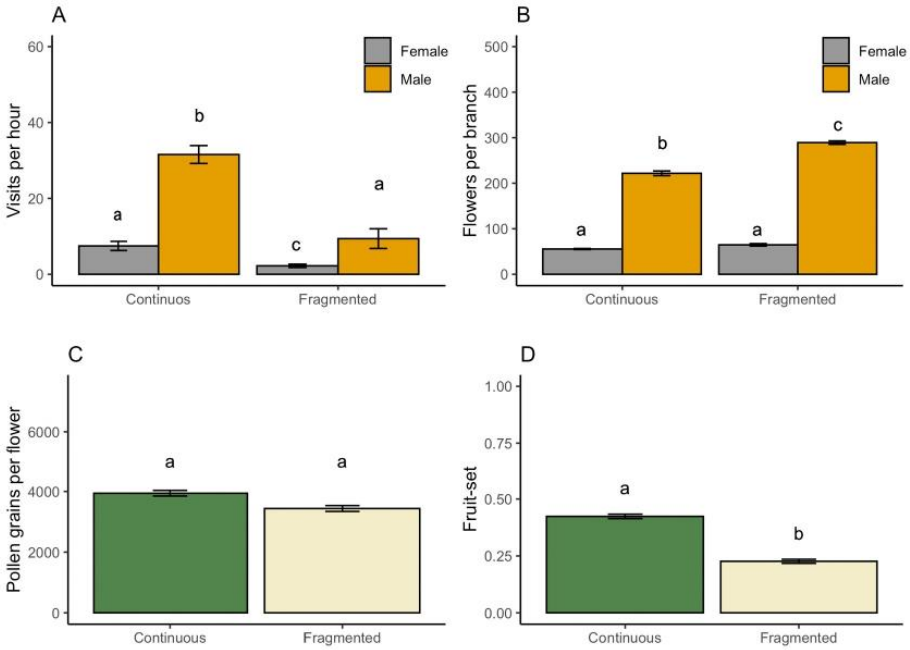

Fig. 2. Mean (±SEM) of pollinator visitation rates (visits per flower per inflorescence per hour) (A), flower production per branch (B), pollen grain production per flower (C) and fruit set (D) of dioecious tree *S. purpurea* in continuous and fragmented habitats in the region of Chamela, Jalisco. Different letters represent significant differences among groups ($p < 0.05$).

Table 1
Genetic diversity estimates for each habitat condition and developmental stage of *Spondias purpurea* in two habitat conditions in the region of the Chamela-Cuixmala Biosphere Reserve, Jalisco Mexico. N: number of individuals; N_a: mean allele number per locus (±SD); N_e: mean number of effective alleles per locus (± SD); H_O: observed heterozygosity (±SD); H_e: expected heterozygosity (±SD); F: fixation index (±SD).

Habitat type	Developmental stage	N	Na	Ne	Ho	He	F
Continuous habitat	Adults	308	6.277 (1.18)	2.653 (0.30)	0.434 (0.01)	0.536 (0.05)	0.138 (0.08)
	Juveniles	172	5.609 (1.02)	2.501 (0.09)	0.465 (0.07)	0.515 (0.02)	0.06 (0.07)
	Seeds	277	4.833 (0.24)	2.652 (0.90)	0.462 (0.16)	0.499 (0.17)	0.059 (0.01)
	Mean	757	5.574 (0.95)	2.603 (0.43)	0.454 (0.07)	0.517 (0.08)	0.086 (0.06)
Fragmented habitat	Adults	174	5.481 (0.34)	2.814 (0.34)	0.372 (0.06)	0.555 (0.05)	0.309 (0.12)
	Juveniles	70	3.815 (0.28)	2.499 (0.1)	0.272 (0.03)	0.533 (0.02)	0.461 (0.09)
	Seeds	450	4.851 (0.61)	2.892 (0.22)	0.311 (0.04)	0.581 (0.02)	0.379 (0.08)
	Mean	694	4.716 (0.82)	2.736 (0.27)	0.318 (0.05)	0.556 (0.03)	0.338 (0.1)
Grand mean		1451	5.059 (0.22)	2.683 (0.1)	0.373 (0.01)	0.541 (0.01)	0.266 (0.03)

whereas in fragmented habitat only ~32% occurred in this distance category (Fig. 4).

Table 3 shows paternity correlations (r_p) and mean effective number of parents (N_{ep}) within seed groups for both habitat conditions. A paternity correlation value of 1 indicates that the progeny are full-sibs and a value of 0 indicates that the progeny do not share sires. Paternity correlations were lower in continuous habitats (average ± SEM) (CH = 0.55 ± 0.3) than in fragmented habitats (FH = 0.639 ± 0.06 respectively) (Table 3). Therefore, in continuous habitats, the number of sires was higher than in fragmented habitats (Average N_{ep} ± SEM) (2.58 ± 1.4; 1.58 ± 0.15 respectively).

4. Discussion

Analyses of reproductive success and genetic diversity are fundamental to understand the potential of plant populations to persist in fragmented habitats. In this study, we used paternity analysis to estimate the effects of forest fragmentation on pollen flow patterns, which has been rarely used to directly estimate male fitness in dioecious species. It has been recently proposed that that a group of small habitat fragments can sustain as much biodiversity as a continuous large forest (Fahrig, 2017; Fahrig et al., 2019); however, this premise is based on comparisons of species richness, ignoring the role of demographic and genetic traits (i.e. population size, population recruitment, population growth rate) or biotic interactions (i.e. pollination, herbivory, predation, pollen and seed dispersal) on plant fitness and the evolutionary potential of plant lineages (Aguilar et al., 2019). In our study, trees within fragmented habitats had lower pollinator visitation rates, lower fruit-set, shorter effective pollen dispersal distances, less effective pollen donors and lower genetic diversity estimates than trees in continuous habitats. These elements are likely to compromise population persistence and viability in the long term (Aguilar et al., 2019). Our study is the first to simultaneously demonstrate negative effects of forest fragmentation on male and female fitness in a dioecious species.

Our results show that males produce more flowers in comparison to female trees. This sexual dimorphism is similar to other dioecious

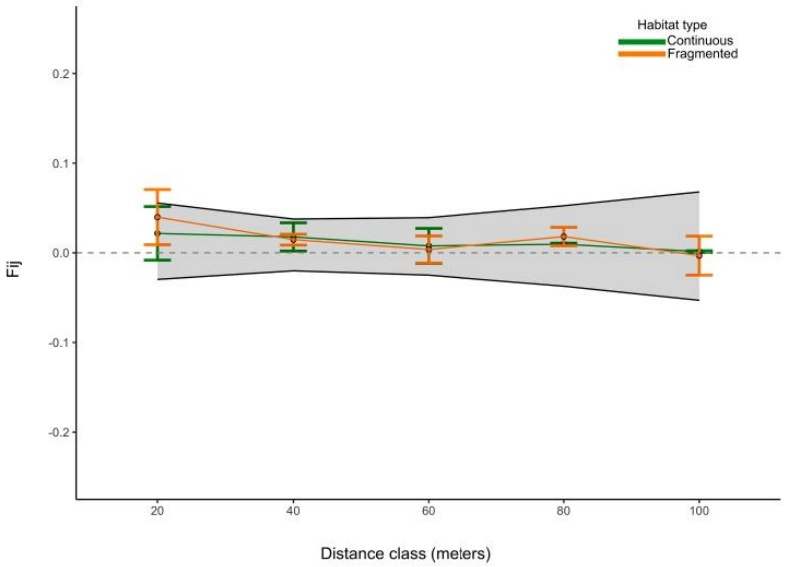

Fig. 3. Autocorrelograms for estimated pairwise kinship correlation F_{ij} for continuous and fragmented habitats. Each point represents the average F_{ij} value for individuals separated by each distance class, calculated for all study sites and averaged across habitat conditions. Solid black lines represent upper and lower 95% confidence envelopes for the null hypothesis of no correlation ($F_{ijj} = 0$).

Table 2
Spatial genetic structure (SGS) statistics for *Spondias purpurea* adults in two habitat conditions in Chamela, Jalisco Mexico. $F_{(1)}$: kinship coefficient for the first distance category (20 m), b_{log}: regression slope between kinship coefficient and the log of the distance category, *Sp*: strength of spatial structure. Standard errors are in parenthesis. ^{NS}: Not significant ($p > 0.05$).

Habitat type	Population	b_{log}	$F_{(1)}$	*Sp*
Continuous habitat	Careyes	-0.004^{NS}	0.0006	0.0043
		(0.002)	(0.007)	(0.008)
	Chamela	-0.009^{NS}	0.0428	0.0094
		(0.001)	(0.011)	(0.009)
Fragmented habitat	Mesa	-0.0036^{NS}	0.0048	0.0036
		(0.003)	(0.017)	(0.023)
	Nacastillo	-0.0098^{NS}	−0.1688	0.0084
		(0.007)	(0.162)	(0.124)
	Ranchitos	-0.00029^{NS}	0.0625	0.0003
		(0.002)	(0.094)	(0.122)

species, and it is associated with the greater costs incurred by females in the process of sexual reproduction (Niesenbaum, 1992; Delph, 1999; Eckhart, 1999; Obeso, 2002; Barrett and Hough, 2013). Trees in fragmented habitats produced significantly more flowers than trees in continuous habitats, similar to what has been found in other tropical trees (Fuchs et al., 2003; Herrerías-Diego et al., 2006). Trees in fragmented habitats develop larger crowns with more branches and therefore are exposed to greater solar radiation, which may increase flower production (Fuchs et al., 2003). Niesenbaum (1992) analyzed light influences on flower production in the dioecious shrub *Lindera benzoin* (Lauraceae), and showed that plants with greater light exposure produced more flowers than shaded plants, possibly associated with greater plant growth before reproduction (Niesenbaum, 1992). Bullock (1992) analyzed the storage and expenditure of non-structural carbohydrates in branches of *S. purpurea* before and after the reproductive period, and found that branches maintain a high concentration of non-structural carbohydrates prior to reproduction, which are then used for flower production. Thus, in *S. purpurea* it is possible that individuals with a greater number of branches are able to accumulate more resources and produce more flowers in fragmented habitats than in continuous sites.

Although flower production was greater in fragmented habitats, fruit-set was negatively affected by fragmentation. We did not find differences in total pollen production per flower or pollinator assemblage composition between habitat conditions. Therefore, if sex ratios are unbiased and pollen availability is not a limiting factor for fruit production, changes in fruit-set are more likely explained by differences in pollinator abundance within fragmented habitats. *Spondias purpurea* is mainly pollinated by stingless bees, wasps and flies that differ in abundance between habitat conditions. Winfree et al. (2009, 2011) showed that habitat loss is one of the most important factors affecting richness and abundance of native bees and other pollinator groups. Similar conclusions were later reached for other pollinator groups (Winfree et al., 2011). The few studies of bee- and wasp-pollinated plants that have analyzed the effects of forest fragmentation have found negative effects on reproductive success and plant fitness (Cascante et al., 2002; Aguilar et al., 2019).

In addition, fruit production can be negatively affected by a reduction in the quality of pollen loads. Higher and genetically diverse pollen loads are expected to increase fruit-set (Schemske and Pautler, 1984; Winsor et al., 1987; Janse and Verhaegh, 1993; Quesada et al., 2001; Cascante et al., 2002; Johnson and Nielsen, 2014), while short-distance pollination events can reduce fruit-set and increase the probability of biparental inbreeding (Schemske and Pautler, 1984; De Jong et al., 2005). Our results show that progeny in fragmented habitats is sired by a lower number of effective pollen donors located at shorter distances from maternal trees, resulting in seeds and juveniles with greater inbreeding coefficients than in continuous habitats. Larger floral displays are likely to reduce pollinator movement between reproductive adults, reducing the diversity of sires and increasing the relatedness of maternal progeny arrays (Karron et al., 2004; Karron and Mitchell, 2012). In *S. purpurea*, a previous study showed that seeds produced by pollination events from neighboring parents have lower recruitment probability (Cristóbal-Pérez et al., 2020), which jeopardizes seedling recruitment in fragmented habitats. A meta-analysis by Aguilar et al. (2019) showed that in fragmented habitats, progeny fitness was negatively affected by an increase in correlated paternity and inbreeding

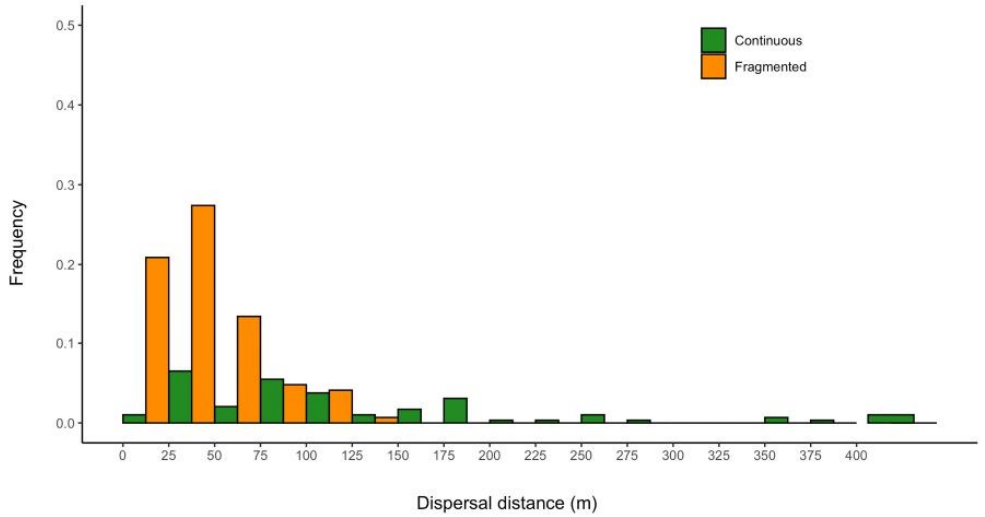

Fig. 4. Paternity assignment frequency according to the distance between maternal trees and assigned male trees. Bars represent seeds collected directly from maternal trees in continuous and fragmented habitats.

Table 3
Paternity correlation (*rp*) and number of sires (*Nep*), estimated as Nep = 1 / *rp*, for seeds *Spondias purpurea* in two habitat conditions in Chamela, Jalisco Mexico. Standard errors are given in parentheses.

Habitat type	Population	*rp*	*Nep*
Continuous habitat	Careyes	0.849 (0.053)	1.17
	Chamela	0.251 (0.031)	3.98
	Mean	0.55 (0.29)	2.58 (1.4)
Fragmented habitat	Mesa	0.736 (0.049)	1.35
	Nacastillo	0.625 (0.044)	1.6
	Ranchitos	0.557 (0.046)	1.79
	Mean	0.63 (0.06)	1.58 (0.15)

coefficients. Frequent endogamous matings can reduce effective population sizes, which in turn, will increase local inbreeding and genetic drift (Loveless and Hamrick, 1984).

A main concern in conservation biology is the ability of species to maintain genetic diversity within fragments and disturbed habitats. In plant species, literature reviews have shown that genetic diversity is negatively affected by habitat fragmentation (Aguilar et al., 2008; Vranckx et al., 2012; González et al., 2020). Our results show that although there is no difference in allelic richness between habitat conditions, fragmented habitats had lower observed heterozygosities and higher inbreeding coefficients in contrast to continuous habitats. These negative genetic consequences are exacerbated in seeds and juveniles. Fragmented habitats may sustain as much allelic richness as continuous habitats, analogous to a pattern described in analyses of species richness (Fahrig, 2017); however, fragmentation negatively affects the mating structure of plant populations, reducing heterozygosity and increasing inbreeding in fragments. Thus, fragmented landscapes may maintain allelic richness, but not the long-term genetic diversity of populations.

Our results show a lack of SGS in adult trees regardless of habitat condition, which can be the result of demographic processes that occurred before habitat fragmentation (Hardy et al., 2006; Cristóbal-Pérez et al., 2020). In our study, the transformation of natural habitats to agricultural lands occurred since the 1950s, therefore it is likely that adult trees were recruited before fragmentation. However, future studies on SGS in juvenile stages, and gene flow via seeds are necessary to determine the effects of habitat fragmentation on seed dispersal and plant recruitment.

5. Conclusions

In conclusion, mating patterns and the dependence on animal pollination are key elements that determine reproductive and genetic susceptibility of plants to habitat fragmentation (Aguilar et al., 2006; Aguilar et al., 2008; Aguilar et al., 2019). In dioecious species, such as *S. purpurea*, habitat fragmentation has negative consequences on plant-pollinator interactions, plant reproductive success, genetic diversity, and mating patterns. In fragmented habitats, a limited number of sires is likely to reduce progeny size and fitness, which may compromise the long term viability of plant species. Small habitat fragments can harbour moderate levels of diversity, but gene flow and pollination success will be negatively affected by fragmentation, reducing male and female fitness and the genetic diversity of future generations. Thus, in the long term, population viability in small fragmented habitats will decline due to limited recruitment and evolutionary potential.

CRediT authorship contribution statement

E. Jacob Cristóbal-Perez: Conceptualization, Investigation, Formal analysis, Writing - Original draft preparation. **Mauricio Quesada:** Conceptualization, Investigation, Writing - Reviewing and editing. **Eric Fuchs:** Conceptualization, Formal analysis, Writing - Reviewing and editing. **Silvana Martén-Rodríguez:** Conceptualization, Formal analysis, Writing - Reviewing and editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A and B. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.biocon.2021.109007.

References

Aguilar, R., Ashworth, L., Galetto, L., Aizen, M.A., 2006. Plant reproductive susceptibility to habitat fragmentation: review and synthesis through a meta-analysis. Ecol. Lett. 9, 968–980. https://doi.org/10.1111/j.1461-0248.2006.00927.x.

Aguilar, R., Quesada, M., Ashworth, L., Herrerias-Diego, Y., Lobo, J., 2008. Genetic consequences of habitat fragmentation in plant populations: susceptible signals in plant traits and methodological approaches. Mol. Ecol. 17, 5177–5188. https://doi.org/10.1111/j.1365-294X.2008.03971.x.

Aguilar, R., Cristóbal-Perez, E.J., Balvino-Olvera, F.J., Aguilar-Aguilar, M.J., Aguirre-Acosta, N., Ashworth, L., Lobo, J.A., Martén-Rodríguez, S., Fuchs, E.J., Sanchez-Montoya, G., Bernardello, G., Quesada, M., 2019. Habitat fragmentation reduces plant progeny quality: a global synthesis. Ecol. Lett. 22, 1163–1173. https://doi.org/10.1111/ele.13272.

Barrett, S.C.H., Hough, J., 2013. Sexual dimorphism in flowering plants. In: Journal of Experimental Botany 64:67–82. Academic, Oxford. https://doi.org/10.1093/jxb/ers308.

Bawa, K.S., 1990. Plant-pollinator interactions in tropical rain forests. Annu. Rev. Ecol. Syst. 21, 399–422. https://doi.org/10.1146/annurev.es.21.110190.002151.

Bawa, K.S., Bullock, S.H., Perry, D.R., Coville, R.E., Grayum, M.H., 1985. Reproductive biology of tropical lowland rain forest trees. II. Pollination systems. Am. J. Bot. 72, 346–356. https://doi.org/10.2307/2443527.

Beckman, N.G., Rogers, H.S., 2013. Consequences of seed dispersal for plant recruitment in tropical forests: interactions within the seedscape. Biotropica 45, 666–681. https://doi.org/10.1111/btp.12071.

Breed, M.F., Ottewell, K.M., Gardner, M.G., Marklund, M.H.K., Dormontt, E.E., Lowe, A. J., 2015. Mating patterns and pollinator mobility are critical traits in forest fragmentation genetics. Heredity 115, 108–114. https://doi.org/10.1038/hdy.2013.48.

Bullock, S.H., 1992. Seasonal differences in nonstructural carbohydrates in two dioecious monsoon-climate trees. Biotropica 24, 140. https://doi.org/10.2307/2388667.

Cascante, A., Quesada, M., Lobo, J.J., Fuchs, E.A., 2002. Effects of dry tropical forest fragmentation on the reproductive success and genetic structure of the tree Samanea saman. Conserv. Biol. 16, 137–147. https://doi.org/10.1046/j.1523-1739.2002.00317.x.

Cascante-Marín, A., Trejos, C., Madrigal, R., Fuchs, E.J., 2020. Genetic diversity and reproductive biology of the dioecious and epiphytic bromeliad Aechmea mariae-reginae (Bromeliaceae) in Costa Rica: implications for its conservation. Bot. J. Linn. Soc. 192 (4), 773–786. https://doi.org/10.1093/botlinnean/boz083.

Chávez-Pesqueira, M., Suárez-Montes, P., Castillo, G., Núñez-Farfán, J., 2014. Habitat fragmentation threatens wild populations of Carica papaya (Caricaceae) in a lowland rainforest. Am. J. Bot. 101 (7), 1092–1101. https://doi.org/10.3732/ajb.1400051.

Cordeiro, N.J., Howe, H.F., 2003. Forest fragmentation severs mutualism between seed dispersers and an endemic African tree. Proc. Natl. Acad. Sci. 100, 14052–14056. https://doi.org/10.1073/pnas.2331023100.

Cristóbal-Pérez, E.J., Fuchs, E.J., Harvey, N., Quesada, M., 2019. Isolation and characterization of microsatellites loci in Spondias purpurea (Anacardiaceae) and cross amplification in congeneric species. Mol. Biol. Rep. 46, 5581–5585. https://doi.org/10.1007/s11033-019-04968-4.

Cristóbal-Pérez, E.J., Fuchs, E.J., Olivares-Pinto, U., Quesada, M., 2020. Janzen-Connell effects shape gene flow patterns and realized fitness in the tropical dioecious tree Spondias purpurea (Anacardiaceae). Sci. Rep. 10, 4584. https://doi.org/10.1038/s41598-020-61394-4.

De Jong, T.J., Van Dijk, H., Klinkhamer, P.G.L., 2005. Hamilton's rule, imprinting and parent-offspring conflict over seed mass in partially selfing plants. J. Evol. Biol. 18, 676–682. https://doi.org/10.1111/j.1420-9101.2004.00856.x.

Delph, L.F., 1999. Sexual dimorphism in life history. In: Geber, M.A., Dawson, T.E., Delph, L.F. (Eds.), Gender and Sexual Dimorphism in Flowering Plants. Springer, Berlin, Heidelberg, pp. 149–173. https://doi.org/10.1007/978-3-662-03908-3_6.

Dick, C.W., 2001. Genetic rescue of remnant tropical trees by an alien pollinator. Proc. R. Soc. Lond. B Biol. Sci. 268, 2391–2396. https://doi.org/10.1098/rspb.2001.1781.

Doyle, J.J., Doyle, J.L., 1987. A rapid DNA isolation procedure for small amounts of fresh leaf tissue. Phytochem. Bull. 11–15.

Eckhart, V.M., 1999. Sexual dimorphism in flowers and inflorescences. In: Geber, M.A., Dawson, T.E., Delph, L.F. (Eds.), Gender and Sexual Dimorphism in Flowering Plants. Springer, Berlin, Heidelberg, pp. 123–148. https://doi.org/10.1007/978-3-662-03908-3_5.

Fahrig, L., 2003. Effects of habitat fragmentation on biodiversity. Annu. Rev. Ecol. Evol. Syst. 34, 487–515. https://doi.org/10.1146/annurev.ecolsys.34.011802.132419.

Fahrig, L., 2017. Ecological responses to habitat fragmentation per se. Annu. Rev. Ecol. Evol. Syst. 48, 1–23. https://doi.org/10.1146/annurev-ecolsys-110316-022612.

Fahrig, L., et al., 2019. Is habitat fragmentation bad for biodiversity? Biol. Conserv. 230, 179–186. https://doi.org/10.1016/j.biocon.2018.12.026.

Fáveri, S.B., Vasconcelos, H.L., Dirzo, R., 2008. Effects of Amazonian forest fragmentation on the interaction between plants, insect herbivores, and their natural enemies. J. Trop. Ecol. 24, 57–64. https://doi.org/10.1017/S0266467407004592.

Fischer, J., Lindenmayer, D.B., 2007. Landscape modification and habitat fragmentation: a synthesis. Glob. Ecol. Biogeogr. 16, 265–280. https://doi.org/10.1111/j.1466-8238.2007.00287.x.

Fuchs, E.J., Lobo, J.A., Quesada, M., 2003. Effects of forest fragmentation and flowering phenology on the reproductive success and mating patterns of the tropical dry forest tree Pachira quinata. Conserv. Biol. 17, 149–157. https://doi.org/10.1046/j.1523-1739.2003.01140.x.

García-Oliva, F., Camou, A., Mass, J.M., 2002. El clima de la región central de la costa del Pacífico mexicano. In: Noguera, F., Vega, J., García, A., Quesada, M. (Eds.), Historia natural de Chamela. Universidad Nacional Autónoma de México, México, pp. 3–10.

González, A.V., Gómez-Silva, V., Ramírez, M.J., Fontúrbel, F.E., 2020. Meta-analysis of the differential effects of habitat fragmentation and degradation on plant genetic diversity. Conserv. Biol. 34 (3), 711–720. https://doi.org/10.1111/cobi.13422.

González-Varo, J.P., Arroyo, J., Aparicio, A., 2009. Effects of fragmentation on pollinator assemblage, pollen limitation and seed production of Mediterranean myrtle (Myrtus communis). Biol. Conserv. 142, 1058–1065. https://doi.org/10.1016/j.biocon.2009.01.017.

Goudet, J., 2005. hierfstat, a package for r to compute and test hierarchical F-statistics. Mol. Ecol. Notes 5, 184–186. https://doi.org/10.1111/j.1471-8286.2004.00828.x.

Harder, L.D., 1990. Pollen removal by bumble bees and its implications for pollen dispersal. Ecology 71, 1110–1125. https://doi.org/10.2307/1937379.

Hardy, O.J., Vekemans, X., 2002. spagedi: a versatile computer program to analyse spatial genetic structure at the individual or population levels. Mol. Ecol. Notes 2, 618–620. https://doi.org/10.1046/j.1471-8286.2002.00305.x.

Hardy, O.J., et al., 2006. Fine-scale genetic structure and gene dispersal inferences in 10 Neotropical tree species. Mol. Ecol. 15, 559–571. https://doi.org/10.1111/j.1365-294X.2005.02785.x.

Harms, K.E., Wright, S.J., Calderón, O., Hernández, A., Herre, E.A., 2000. Pervasive density-dependent recruitment enhances seedling diversity in a tropical forest. Nature 404, 493–495. https://doi.org/10.1038/35006630.

Harris, L.F., Johnson, S.D., 2004. The consequences of habitat fragmentation for plant–pollinator mutualisms. Int. J. Trop. Insect Sci. 24, 29–43. https://doi.org/10.1079/IJT20049.

Herrerías-Diego, Y., Quesada, M., Stoner, K.E., Lobo, J.A., 2006. Effects of forest fragmentation on phenological patterns and reproductive success of the tropical dry forest tree Ceiba aesculifolia. Conserv. Biol. 20, 1111–1120. https://doi.org/10.1111/j.1523-1739.2006.00370.x.

Herrerías-Diego, Y., Quesada, M., Stoner, K.E., Lobo, J.A., Hernández-Flores, Y., Sanchez, Montoya G., 2008. Effect of forest fragmentation on fruit and seed predation of the tropical dry forest tree Ceiba aesculifolia. Biol. Conserv. 141, 241–248. https://doi.org/10.1016/j.biocon.2007.09.017.

Ibarra-Manriquez, G., Oyama, K., 1992. Ecological correlates of reproductive traits of Mexican rain forest trees. Am. J. Bot. 79, 383–394. https://doi.org/10.2307/2445150.

Janse, J., Verhaegh, J.J., 1993. Effects of varying pollen load on fruit set, seed set and seedling performance in apple and pear. Sex. Plant Reprod. 6, 122–126. https://doi.org/10.1007/BF00227656.

Janzen, D.H., 1970. Herbivores and the number of tree species in tropical forests. Am. Nat. 104, 501–528. https://doi.org/10.1086/282687.

Johnson, K.M., Nielsen, S.E., 2014. Demographic effects on fruit set in the dioecious shrub Canada buffaloberry (Shepherdia canadensis). PeerJ 2, e526. https://doi.org/10.7717/peerj.526.

Jones, O.R., Wang, J., 2010. COLONY: a program for parentage and sibship inference from multilocus genotype data. Mol. Ecol. Resour. 10, 551–555. https://doi.org/10.1111/j.1755-0998.2009.02787.x.

Kalinowski, S.T., Taper, M.L., Marshall, T.C., 2007. Revising how the computer program cervus accommodates genotyping error increases success in paternity assignment. Mol. Ecol. 16, 1099–1106. https://doi.org/10.1111/j.1365-294X.2007.03089.x.

Karron, J.D., Mitchell, R.J., 2012. Effects of floral display size on male and female reproductive success in Mimulus ringens. Ann. Bot. 109, 563–570. https://doi.org/10.1093/aob/mcr193.

Karron, J.D., Mitchell, R.J., Holmquist, K.G., Bell, J.M., Funk, B., 2004. The influence of floral display size on selfing rates in Mimulus ringens. Heredity 92, 242–248. https://doi.org/10.1093/aob/mcr193.

Legrand, D., Cote, J., Fronhofer, E.A., Holt, R.D., Ronce, O., Schtickzelle, N., Travis, J.M. J., Clobert, J., 2017. Eco-evolutionary dynamics in fragmented landscapes. Ecography 40, 9–25. https://doi.org/10.1111/ecog.02538.

Lloyd, D.G., Webb, C.J., 1977. Secondary sex characters in seed plants. Bot. Rev. 43, 177–216.

Loiselle, B.A., Sork, V.L., Nason, J., Graham, C., 1995. Spatial genetic structure of a tropical understory shrub, Psychotria officinalis (Rubiaceae). Am. J. Bot. 82, 1420–1425. https://doi.org/10.2307/2445869.

Loveless, M.D., Hamrick, J.L., 1984. Ecological determinants of genetic structure in plant populations. Annu. Rev. Ecol. Syst. 15, 65–95. https://doi.org/10.1146/annurev.es.15.110184.000433.

Lowe, A.J., Boshier, D., Ward, M., Bacles, C.F., Navarro, C., 2005. Genetic resource impacts of habitat loss and degradation; reconciling empirical evidence and predicted theory for neotropical trees. Heredity 95, 255–273. https://doi.org/10.1038/sj.hdy.6800725.

Mandujano, S., Gallina, S., Bullock, S.H., 1994. Frugivory and dispersal of Spondias purpurea (Anacardiaceae) in a tropical deciduous forest in México. Rev. Biol. Trop. 42, 107–114.

Miller, A.J., Knouft, J.H., 2006. GIS-based characterization of the geographic distributions of wild and cultivated populations of the Mesoamerican fruit tree Spondias purpurea (Anacardiaceae). Am. J. Bot. 93, 1757–1767. https://doi.org/10.3732/ajb.93.12.1757.

Miller, A., Schaal, B., 2005. Domestication of a Mesoamerican cultivated fruit tree, Spondias purpurea. Proc. Natl. Acad. Sci. 102, 12801–12806. https://doi.org/10.1073/pnas.0505447102.

Mitchell JD, Daly DC. 2015. A revision of Spondias L. (Anacardiaceae) in the Neotropics. PhytoKeys:1–92. DOI:https://doi.org/10.3897/phytokeys.55.8489.

Nason, J.D., Hamrick, J.L., 1997. Reproductive and genetic consequences of forest fragmentation: two case studies of neotropical canopy trees. J. Hered. 88 (4), 264–276. https://doi.org/10.1093/oxfordjournals.jhered.a023104.

Niesenbaum, R.A., 1992. Sex ratio, components of reproduction, and pollen deposition in Lindera benzoin (Lauraceae). Am. J. Bot. 79, 495–500. https://doi.org/10.2307/2444859.

Obeso, J.R., 2002. The costs of reproduction in plants. New Phytol. 155, 321–348. https://doi.org/10.1046/j.1469-8137.2002.00477.x.

Oostermeijer, J.G., Luijten, S.H., Den Nijs, J.C., 2003. Integrating demographic and genetic approaches in plant conservation. Biol. Conserv. 113 (3), 389–398. https://doi.org/10.1016/S0006-3207(03)00127-7.

Orrock, J.L., Danielson, B.J., Burns, M.J., Levey, D.J., 2003. Spatial ecology of predator–prey interactions: corridors and patch shape influence seed predation. Ecology 84, 2589–2599. https://doi.org/10.1890/02-0439.

Orrock, J.L., Levey, D.J., Danielson, B.J., Damschen, E.I., 2006. Seed predation, not seed dispersal, explains the landscape-level abundance of an early-successional plant. J. Ecol. 94, 838–845. https://doi.org/10.1111/j.1365-2745.2006.01125.x.

Ouborg, N.J., Vergeer, P., Mix, C., 2006. The rough edges of the conservation genetics paradigm for plants. J. Ecol. 94, 1233–1248. https://doi.org/10.1111/j.1365-2745.2006.01167.x.

Peakall, R., Smouse, P.E., 2012. GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research—an update. Bioinformatics 28, 2537–2539. https://doi.org/10.1093/bioinformatics/bts460.

Quesada, M., Fuchs, E.J., Lobo, J.A., 2001. Pollen load size, reproductive success, and progeny kinship of naturally pollinated flowers of the tropical dry forest tree Pachira quinata (Bombacaceae). Am. J. Bot. 88, 2113–2118. https://doi.org/10.2307/3558436.

Quesada, M., Stoner, K.E., Rosas-Guerrero, V., Palacios-Guevara, C., Lobo, J.A., 2003. Effects of habitat disruption on the activity of nectarivorous bats (Chiroptera: Phyllostomidae) in a dry tropical forest: implications for the reproductive success of the neotropical tree Ceiba grandiflora. Oecologia 135, 400–406. https://doi.org/10.1007/s00442-003-1234-3.

Quesada, M., Stoner, K.E., Lobo, J.A., Herrerias-Diego, Y., Palacios-Guevara, C., Munguía-Rosas, M.A., O.-Salazar, K.A., Rosas-Guerrero, V., 2004. Effects of forest fragmentation on pollinator activity and consequences for plant reproductive success and mating patterns in bat-pollinated Bombacaceous trees. Biotropica 36, 131–138. https://doi.org/10.1111/j.1744-7429.2004.tb00305.x.

R Core Team, 2018. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.

Renner, S.S., 2014. The relative and absolute frequencies of angiosperm sexual systems: dioecy, monoecy, gynodioecy, and an updated online database. Am. J. Bot. 101 (10), 1588–1596. https://doi.org/10.3732/ajb.1400196.

Renner, S.S., Ricklefs, R.E., 1995. Dioecy and its correlates in the flowering plants. Am. J. Bot. 82, 596–606. https://doi.org/10.2307/2445418.

Riba-Hernández, P., Segura, J.L., Fuchs, E.J., Moreira, J., 2014. Population and genetic structure of two dioecious timber species Virola surinamensis and Virola koschnyi (Myristicaceae) in southwestern Costa Rica. For. Ecol. Manag. 323, 168–176. https://doi.org/10.1016/j.foreco.2014.03.018.

Ritland, K., 2002. Extensions of models for the estimation of mating systems using n independent loci. Heredity 88, 221–228. https://doi.org/10.1038/sj.hdy.6800029.

Rosas, F., Quesada, M., Lobo, J.A., Sork, V.L., 2011. Effects of habitat fragmentation on pollen flow and genetic diversity of the endangered tropical tree Swietenia humilis (Meliaceae). Biol. Conserv. 144, 3082–3088. https://doi.org/10.1016/j.biocon.2011.10.003.

Sánchez-Azofeifa, G.A., Quesada, M., Cuevas-Reyes, P., Castillo, A., Sánchez-Montoya, G., 2009. Land cover and conservation in the area of influence of the Chamela-Cuixmala Biosphere Reserve, Mexico. For. Ecol. Manag. 258, 907–912. https://doi.org/10.1016/j.foreco.2008.10.030.

SAS Institute Inc, 2008. SAS/STAT® 9.2 User's Guide. SAS Institute Inc., Cary, NC.

Schemske, D.W., Pautler, L.P., 1984. The effects of pollen composition on fitness components in a neotropical herb. Oecologia 62, 31–36. https://doi.org/10.1007/BF00377369.

Sih, A., Baltus, M.-S., 1987. Patch size, pollinator behavior, and pollinator limitation in catnip. Ecology 68, 1679–1690. https://doi.org/10.2307/1939860.

Solís-Hernández, W., Fuchs, E.J., 2019. Effective gene flow patterns across a fragmented landscape in southern Costa Rica for Symphonia globulifera (Clusiaceae); a species with mobile seed and pollen dispersers. Revista de Biología 67 (2), S95–111. https://doi.org/10.15517/RBT.V67I2SUPL.37209.

Sork, V.L., Smouse, P.E., 2006. Genetic analysis of landscape connectivity in tree populations. Landsc. Ecol. 21, 821–836. https://doi.org/10.1007/s10980-005-5415-9.

Vranckx, G.U., Jacquemyn, H., Muys, B., Honnay, O., 2012. Meta-analysis of susceptibility of woody plants to loss of genetic diversity through habitat fragmentation. Conserv. Biol. 26 (2), 228–237. https://doi.org/10.1111/j.1523-1739.2011.01778.x.

White, G.M., Boshier, D.H., Powell, W., 2002. Increased pollen flow counteracts fragmentation in a tropical dry forest: an example from Swietenia humilis Zuccarini. Proc. Natl. Acad. Sci. 99, 2038–2042. https://doi.org/10.1073/pnas.042649999.

Wilson, K., Hardy, I.C.W., 2002. Statistical analysis of sex ratios: an introduction. In: Hardy, I.C.W. (Ed.), Sex Ratios. Cambridge University Press, Cambridge, pp. 48–92.

Winfree, R., Aguilar, R., Vázquez, D.P., LeBuhn, G., Aizen, M.A., 2009. A meta-analysis of bees' responses to anthropogenic disturbance. Ecology 90, 2068–2076. https://doi.org/10.1890/08-1245.1.

Winfree, R., Bartomeus, I., Cariveau, D.P., 2011. Native pollinators in anthropogenic habitats. Annual Review of Ecology, Evolution, and Systematics 42, 1–22. https://doi.org/10.1146/annurev-ecolsys-102710-145042.

Winsor, J.A., Davis, L.E., Stephenson, A.G., 1987. The relationship between pollen load and fruit maturation and the effect of pollen load on offspring vigor in Cucurbita pepo. Am. Nat. 129, 643–656. https://doi.org/10.1086/284664.

Xiao, Y., Li, X., Cao, Y., Dong, M., 2016. The diverse effects of habitat fragmentation on plant–pollinator interactions. Plant Ecol. 217 (7), 857–868. https://doi.org/10.1007/s11258-016-0608-7.

SUPPLEMENTAL MATERIAL

Appendix A. Number of males, females and sex ratio for each locality of *Spondias purpurea* in two habitat conditions in Chamela, Jalisco Mexico. ^{NS}: Nonsignificant deviations from 1:1 sex ratio ($p>0.05$)

Habitat type	Population	Males	Females	Sex ratio
Continuous habitat	Chamela	103	107	0.47 ^{NS}
	Careyes	47	51	0.49 ^{NS}
Fragmented habitat	Ranchitos	20	31	0.39 ^{NS}
	Mesa	31	30	0.50 ^{NS}
	Nacastillo	28	34	0.45 ^{NS}
Grand Total		229	253	0.47 ^{NS}

Appendix B. Genetic diversity estimates for each habitat condition and developmental stage of *Spondias purpurea* in two habitat conditions in Chamela, Jalisco Mexico. N: Number of individuals; N_a: mean allele number per locus (± SD); H_O: observed heterozygosity (± SD); H_e: expected heterozygosity (± SD); F: fixation index (± SD).

Habitat type	Population and developmental stage	N	Na	Ne	Ho	He	F
Continuous habitat	Chamela - Adults	210	7.111 (1.16)	2.439 (0.38)	0.436 (0.07)	0.501 (0.07)	0.083 (0.115)
	Chamela - Juveniles	144	6.33 (1.09)	2.435 (0.38)	0.418 (0.07)	0.498 (0.07)	0.113 (0.11)
	Chamela - Seeds	127	4.667 (1.01)	2.014 (0.34)	0.347 (0.09)	0.376 (0.09)	0.059 (0.08)
	Careyes - Adults	98	5.444 (0.85)	2.868 (0.49)	0.433 (0.06)	0.571 (0.06)	0.194 (0.102)
	Careyes - Juveniles	28	4.889 (0.71)	2.567 (0.4)	0.512 (0.08)	0.533 (0.06)	0.007 (0.124)
	Careyes - Seeds	150	5 (0.72)	3.29 (0.49)	0.577 (0.06)	0.622 (0.06)	0.06 (0.041)
	Mean	757	5.574 (0.95)	2.603 (0.43)	0.454 (0.07)	0.517 (0.08)	0.086 (0.06)
Fragmented habitat	Ranchitos - Adults	51	5.556 (1.01)	3.126 (0.56)	0.432 (0.05)	0.583 (0.07)	0.23 (0.06)
	Ranchitos - Juveniles	22	4.111 (0.71)	2.385 (0.31)	0.293 (0.07)	0.514 (0.07)	0.382 (0.13)
	Ranchitos - Seeds	150	5.444 (0.83)	3.14 (0.539)	0.269 (0.026)	0.604 (0.06)	0.474 (0.1)
	Nacastillo - Adults	62	5.778 (1.03)	2.45 (0.39)	0.371 (0.06)	0.503 (0.07)	0.251 (0.08)

	Nacastillo - Juveniles	25	3.556	2.53	0.281	0.547	0.446
			(0.47)	(0.29)	(0.08)	(0.06)	(0.15)
	Nacastillo - Seeds	150	4.889	2.758	0.328	0.562	0.343
			(0.82)	(0.46)	(0.02)	(0.06)	(0.09)
	Mesa - Adults	61	5.111 (0.8)	2.867	0.315	0.579	0.446
				(0.4)	(0.04)	(0.06)	(0.05)
	Mesa - Juveniles	23	3.778	2.583	0.242	0.538	0.556
			(0.59)	(0.41)	(0.06)	(0.06)	(0.12)
	Mesa - Seeds	150	4.222	2.778	0.335	0.578	0.322
			(0.57)	(0.35)	(0.01)	(0.06)	(0.12)
	Mean	694	4.716	2.736	0.318	0.556	0.338
			(0.82)	(0.27)	(0.05)	(0.03)	(0.1)
Grand mean		1451	5.059	2.683	0.373	0.541	0.266
			(0.22)	(0.1)	(0.01)	(0.01)	(0.03)

CAPITULO III. Genetic diversity and structure in cultivated and wild populations of Mesoamerican tree *Spondias purpurea* (Anacardiaceae)

Genetic diversity and structure in cultivated and wild populations of Mesoamerican tree Spondias purpurea (ANACARDIACEAE)

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ABSTRACT

Understanding the levels and distribution of genetic diversity within and among cultivated and wild relatives of a crop constitutes a key element in conservation of genetic resources. In order to characterize the genetic variation and mating patterns of cultivated and wild populations of *Spondias purpurea*, we analyzed the genetic diversity and structure, and correlated paternity of adult individuals and seeds respectively of three cultivated and wild stands in northwestern Costa Rica region. By using seven microsatellite polymorphic loci, we genotyped 201 adult and 648 seeds from cultivated and wild stands in three localities. Overall, we found an average of 3.85 alleles per locus and expected heterozygosity of 0.49. We found no differences in genetic diversity between cultivated and wild stands and genetic structure analysis revealed genetic admixture within and among localities. Clonal diversity was high and similar between cultivated and wild trees (D> 0.9). Evenness measures were also similar between cultivated and wild trees (E >0.62). Correlated paternity analysis revealed high paternity correlation (r_p= 0.9) as result of a low number of pollen donors by progeny array (N_{ep}= 1.01). We found some progeny arrays with a multilocus genotype identical to the maternal tree indicative of asexual seed formation. Our results indicate that in dioecious clonally propagated crops high genetic diversity levels are maintained as a result of outcrossing and vegetative propagation of heterozygous genotypes. Gene flow by pollinators maintain genetic admixture between wild and cultivated stands. Fruit formation is ensured by sexual reproduction and reproductive assurance mechanisms as apomixis.

Keywords: Dioecious crops, Anacardiaceae, tropical fruit tree, wild and cultivated species.

INTRODUCTION

Plant domestication is a process of artificial selection by which humans have selected and fixed desired traits to satisfy their needs (food, utilitarian, and aesthetics) (Casas et al. 2007; Vaughan et al. 2007; Casas et al. 2019; Aguirre-Dugua and González-Rodríguez 2016). As a result of artificial selection processes, cultivated plants share suites of traits known as the 'domestication syndrome', that distinguishes them phenotypically from wild relatives (Darwin, 1868; Pickersgill, 2007; Meyer et al., 2012; Stetter et al., 2017). For example, plant cultivation often involves larger and more sugary fruits, larger seeds and inflorescences, reduction in seed dormancy and defenses against herbivory in the parts used by humans (Larson et al., 2014). In addition, domestication involves humans acting as dispersers and modifiers of crop's biotic and abiotic environment (Larson et al., 2014).

Domesticated perennial plants are characterized by long juvenile phases, obligate outcrossing (regulated by self-incompatibility or dioecy), high rates of gene flow and capacity to produce fruits by sexual and asexual via (Zohary and Spiegel-Roy, 1975; Zohary, 2004; Miller and Gross, 2011).

A high number of cultivated perennial plants were domesticated for their edible fruits and propagated vegetatively under cultivation (Van Tassel et al., 2010; Miller and Gross, 2011). In dioecious species, where fruits are produced only by females, vegetative propagation of clones introduces some limitations on fruit production. Under cultivation fruit production in dioecious trees may be maintained by a) sexual reproduction if male or hermaphrodite individuals are added into a plantation to act as pollen donors of female clones (e.g. pistachio, papaya) (Barghchi and Alderson, 1989; Moore, 2014), b) genetic shift from dioecy to hermaphrodite condition (e.g. grapevine) (Picq et al., 2014) or c) replacement of pollination by parthenocarpy (e.g. figs) (Zohary, 2004; Kislev et al., 2006).

In domestication centers where domesticated plants regularly coexist with their wild relatives, fruit production may occur by cross pollination between wild relatives and domesticated plants. The Mesoamerican region, which extends from central Mexico to northwestern Costa Rica (Purugganan and Fuller, 2009), is one of the most important domestication centers, where important perennial fruit trees as avocado (*Persea americana*), cacao (*Theobroma cacao*) and guava (*Psidium guajava*) (Miller and Gross, 2011) were domesticated. Currently, the Mesoamerican landscape consists of small remnants of original forest surrounded by an agricultural matrix (Jauker et al., 2009). Forest remnants can harbor a high species richness and thereby provide ecosystem services as pollination to agricultural landscapes (Kremen et al., 2004; Klein et al., 2007).

In landscapes where domesticated species coexist with their wild relatives, analyses of crop to wild gene flow and consequent hybridization and introgression are of particular interest (Ellstrand et al., 1999; O'Connor et al., 2015). Low levels of genetic diversity are expected in crop cultivars due to domestication-enforced bottlenecks (Delplancke et al., 2012). Thus, gene flow both via pollen and seeds from crop cultivars to wild relatives may result in an influx of offspring with low levels of genetic diversity into wild populations (Ellstrand et al., 1999). The extent of crop to wild gene flow is expected to vary among species and will depend on pollinators mobility and landscape traits, for example distance between the crop and wild population (Arriola and Ellstrand, 1996; Ellstrand et al., 1999). Such information is not only critical to understand the genetic vulnerability of these species, but also could be used to implement management and conservation strategies, particularly in domestication centers where wild and cultivated populations coexist.

Spondias purpurea is a dioecious fruit tree domesticated in Mesoamerican region (Miller and Schaal, 2005, 2006). In Mesoamerica perennial domesticated species are generally cultivated in traditional agricultural habitats, as backyard gardens and living fences where they

frequently coexist with natural wild populations (Miller and Schaal, 2005). *Spondias purpurea* is propagated vegetatively but only females are planted for fruit consumption. Thus, fruit production can be reached via sexual reproduction, if male trees from nearby wild populations act as sources of pollen for female cultivated trees, or by asexual fruit production (i.e. apomixis). In addition, genetic diversity and gene flow of cultivated trees can be maintained by cross pollination in the wild-cultivated landscape. Therefore, *S. purpurea* provides a model to understand mating patterns, genetic diversity and structure in auto-incompatible trees that coexist with wild relatives.

Our study took place in the northwest pacific region of Costa Rica, where wild individuals of *S. purpurea* occur in early regeneration forest and in small forest fragments. In the region farmers cultivated *S. purpurea* under traditional practices as backyard gardens and living fences. The objectives of our study were (1) to determine whether wild and cultivated *S. purpurea* differ genetically, (2) to estimate mating patterns of wild and cultivated trees and (3) to quantify sexual and asexual seed production.

METHODS

Study species

Spondias purpurea L. (Anacardiaceae) is a small (3-10 m) tree, known locally as "jocote", "ciruelo", "abal" or "jobo", native to the tropical dry forests of Mexico and Central America. Natural populations of *S. purpurea* are dioecious that depend on insects, mainly stingless bees, for cross-pollination. Jocote was domesticated for their plumlike fruits in the Mesoamerican region (Miller and Schaal, 2006). These fruits are sold fresh in local markets or made into jams and beverages (Miller and Knouft, 2006; Miller and Schaal, 2006). Cultivated mature fruits vary widely in color, size, texture and taste (Cuevas, 1994). Female cultivated Jocote trees are propagated exclusively vegetatively for fruit consumption (Miller, 2011). Cultivated trees are regularly planted in backyard gardens, living fences, and small multicrop farms, although

formal cultivation exists in orchards (Cuevas, 1994; Miller and Schaal, 2006). Wild jocote trees occur in remnants of tropical dry forest regularly in close spatial proximity to their cultivated relatives. Wild jocote fruits are usually bright red, smaller, more acidic and less fleshy than cultivated fruits (Cristobal-Perez et al 2020) (Miller and Schaal, 2006).

Study area and sampling

Spondias purpurea trees were located in Agua Caliente (AC, 10º 55' 55.16''N, 85º 39' 56.56'' W) (Fig. 1), Murcielago (MU, 10º 54' 41.97''N, 85º 41' 25.25'' W) (Fig. 1) and Estación Experimental Forestal Horizontes (EEFH, 10º 42' 47.05''N, 85º 35' 43.63'' W) (Fig. 2) in the Área de Conservación Guanacaste (ACG); these populations are located in Guanacaste Province, Northwest Costa Rica. The three sites are tropical dry forests that differ in land use. AC and MU are disturbed habitats mainly composed by small remnant forest patches and isolated trees surrounded by an agricultural matrix. Along both sites cultivated trees are planted in backyard gardens and living fences, wild trees are growing within small forest remnants and in pasture lands as isolated trees. EEFH is a 7 317 ha managed secondary forest. In the past, large portions of EEFH were used for rice, cotton, and sorghum production as well as cattle grazing, however three decades ago when EEFH was created the forest began to regenerate (Waring et al., 2019). At EEFH cultivated trees are planted in backyard gardens nearby to station facilities, wild trees are growing in secondary forest as part of the regeneration process.

We selected two groups of individuals (cultivated and wild trees) classified by fruit traits described above. Male trees are not propagated under cultivation; thus, all male individuals were considered into the wild group. To estimate mating patterns and pollen gene flow we collected fruits from cultivated and wild female trees (303 from cultivated trees and 343 from wild trees). Fruits were collected directly from the canopy of each individual to guarantee that we sampled progeny from a single maternal tree.

Microsatellite analysis

We collected fresh leaf tissue from adult trees and stored them at -20 ºC until DNA extraction. For mating patterns and pollen dispersal analyses we dissected fruits and sampled embryos for DNA extraction. DNA from both tissues was extracted using a modified Cetylmethylammonium Bromide (CTAB) protocol (Doyle and Doyle, 1987). Nine microsatellites previously developed for *S. purpurea* (Cristóbal-Pérez et al., 2019), were amplified via multiplex PCR using QIAGEN Multiplex kit (QIAGEN, Hilden, Germany) in 12 µL reaction volumes. The multiplex mix primers were amplified as follows: Mix 1: *SPUR44*, *SPUR40, SPUR41*, *SPUR28*; Mix 2: *SPUR57, SPUR29* and *SPUR33*; and Mix 3: *SPUR42*, *SPUR35, SPUR39;* in all cases primers were at a 0.2 µM concentration. The PCR amplification profile included an initial activation step of 95 ºC for 15 min, followed by a touchdown PCR consisting of 32 cycles with denaturation at 95 ºC for 90 s; annealing for 60 s with temperature decreasing 1 ºC every two cycles from 64 ºC (12 cycles), then 10 cycles at 58 ºC and 10 cycles at 57 ºC; elongation at 72 ºC for 60 s; and a final extension at 72ºC for 5 min (Cristóbal-Pérez et al., 2019). Fragments were analyzed on an automatic ABI-PRISM 3100-Avant sequencer (APPLIED BIOSYSTEMS, Carlsbad, California, USA) using GeneScan LIZ 600 (APPLIED BIOSYSTEMS, Carlsbad, California, USA) to determine fragment sizes. Alleles were scored manually using *GeneMarker Software* version 2.6.4 (SOFTGENETICS). To reduce genotyping error, all samples were independently genotyped by two different researchers to reach consensus in the final data set. Monomorphic microsatellites for these populations (SPUR41, SPUR35 and SPUR39) were excluded from further analysis.

Data analysis

Genetic diversity was quantified for each group using the following parameters: allele number averaged across loci (*Na*), allelic richness (*Ar*), observed (*Ho*) and expected (*He*) heterozygosities and fixation indexes (*F*). These parameters were calculated using the library

poppr (Kamvar et al., 2014) implemented in R (R Core Team, 2019). Differences in genetic diversity between groups were tested using FSTAT 2.9.3.2 (Goudet 2005), assuming Hardy-Weinberg equilibrium and using 10 000 permutations to test for the significance of inbreeding coefficients. In order to identify unique multilocus genotypes (genets) we used the software GENODIVE v.3.04 (Meirmans and Tienderen, 2004). Genets assignment was performed as suggested by Meirmans and Tienderen (2004), calculating a genetic distance matrix and a clonal threshold. When the genetic distance between two individuals falls below the threshold, which was set to 0 for our study as suggested by Meirmans and Tienderen (2004), the two individuals are assigned to the same genet. We used the stepwise mutation model option and genotypes with missing data were ignored. After, we calculated the number of unique multilocus genotypes, Simpson`s diversity and evenness index.

To explore overall genetic structure of wild and cultivated trees, we first performed a principal coordinate analysis (PCoA) using GeneAlEx v 6.5 (Peakall and Smouse, 2006, 2012). Second, we applied a Bayesian approach to make inferences and estimate genetic clusters (number of genetically distinct populations, K) using STRUCTURE v 2.3.4 (Pritchard et al., 2000) and STRUCTURE HARVESTER (Earl and vonHoldt, 2012). STRUCURE v 2.3.4 software was executed by using an admixture model, an initial burn-in of 25 000 iterations, with 250 000 subsequent Markov chain Monte Carlo repeats to determine the subdivision of samples. Ten independent replicas (runs) were performed for each K value tested: from K= 2 to K= 10. The optimal K value was selected by using the *delta K* parameter (Evanno et al., 2005).

Multilocus correlation of paternity (r_{pm}) was estimated using MLTR (Ritland, 2002). Correlated paternity is a measure of the proportion of pairs of outcrossed siblings that are full siblings. The standard error of the estimates was calculated by bootstrapping with 1000 repetitions. We estimated the average effective number of pollen donors per maternal plant

(N_{ep}) using multilocus-correlated paternity (r_{pm}). The reciprocal of the r_{pm} corresponds to the effective number of pollen donors (N_{ep}) (Ritland, 2002).

RESULTS

A total of 201 adult individuals (46 cultivated and 155 wild) and 648 seeds (411 cultivated and 237 wild) were sampled and genotyped in the three study sites (Table 1). All loci analyzed were polymorphic. All genetic parameters did not statistically differ between cultivated and wild individuals ($p > 0.56$) (Table 1).

The proportion of distinguishable ramets based on multilocus genotypes in cultivars (G/N= 0.73) was similar to wild jocotes (G/N= 0.77) (Table 2). Clonal diversity was high and similar between cultivated (D= 0.9) and wild trees (D= 0.96) (Table 2). Evenness measures were also similar between cultivated (E= 0.7) and wild trees (E= 0.62) (Table 2).

PCoA analysis of the matrix of genetic distances between individuals generated two axes of variation: the first axis explained 35.87% and the second axis 9.72% of the variation (Fig. 3). This analysis detected three main groups: group1, with high values on axis 1 and low values on axis 2, comprising most of the samples from Horizontes wild group; group 2, with low values on axis 1 and high values on axis 2 is composed principally of samples from wild group of Horizontes and Murcielago; group 3, with low values for axis 1 and axis 2, comprising most of the samples from the three cultivated groups and most of the samples from wild group of Agua Caliente and Murcielago. Bayesian clustering analysis with STRUCTURE software revealed similar patterns. Structure Harvester suggested that K = 3 was the most likely genetic cluster number (Fig. 4). The three cultivated and two wild groups (Agua Caliente and Murcielago) are mostly composed by clusters 1 and 2 (Fig. 4). Horizontes wild group is mostly composed by clusters 2 and 3 (Fig. 4).

Table 3 shows paternity correlations (r_p) and mean effective number of parents (N_{ep}) within seed groups for wild and cultivated groups. A paternity correlation value of 1 indicates that the progeny are full-sibs and a value of 0 indicates that the progeny do not share sires. Paternity correlations were similar for both cultivated and wild groups ($r_{p\ CULTIVATED}$ = 0.99; $r_{p\ WILD}$ = 0.91) (Table 3). Therefore, the effective number of pollen donors were similar for cultivated and wild groups (N_{ep} =1.01; 1.09 respectively). We identified seeds with multilocus identical genotypes to maternal tree (MGM), which suggest asexual production of seeds (Table 4). Seeds with MGM were more frequent in cultivated trees (13 % of total cultivated seeds) in comparison to seeds produced in wild trees (2% of total wild seeds) (x^2 = 28.98, df = 1, $p < 0.001$).

DISCUSSION

The analysis of microsatellite loci in *S. purpurea* revealed intermediate levels of genetic diversity in both cultivated and wild stands. In all localities we observed intermediate levels of genetic diversity which is similar to other tropical trees (Hamrick, 2004; Dick et al., 2008). Our results show that genetic diversity in wild and cultivated *S. purpurea* populations in northwestern Costa Rica, was similar in terms of number of alleles, expected and observed heterozygosity and inbreeding coefficient. These results are similar to other perennial fruit crops (e.g. pecan, apple, olive, pistachio, sweet cherry chestnut, grape, *Leucaena esculenta, Polaskia chichipe*) (Miller and Gross 2011), where domesticated populations maintain as much genetic diversity levels as their wild relatives (Miller and Gross 2011). In outcrossing perennial crops, clonal propagation can help to maintain genetic diversity through propagation of individuals with heterozygous genotypes that show heterosis hybrid vigour (Elias et al. 2004; McKey et al., 2010).

Clonal diversity was similar and high in both cultivated and wild stands. High clonal diversity in cultivated stands can be explained as result of propagation of several genotypes per

locality as in other vegetatively propagated plants (e.g., *Ficus carica, Dioscorea rotundata, Olea europaea, Theobroma cacao)* (Martins-Lopes et al. 2009; Achtak et al 2010; Scarcelli et al. 2013; Chumacero et al 2013). In *S. purpurea* wild clonal trees are young individuals scattered distributed in secondary forests, probably established as a result of dispersion of seeds produced asexually. Our results show a small proportion of progeny arrays with multilocus identical genotypes to their maternal trees, which is evidence of apomictic seed formation (Nassar et al. 2007; Anand et al. 2019). In other tropical genera of the Anacardiaceae family, as *Astronium*, *Mangifera*, *Myracroduon* and *Tapirira,* there is evidence of the occurrence of asexual production of seeds (apomictic seeds) (Firetti 2017).

Genetic structure analyses show that cultivated and wild stands did not represent different genetic groups. Small differences were observed only in EEFH where two genetic groups in the wild are overrepresented in comparison with cultivated individuals (Fig. 4). Genetic admixture observed in this study can be associated with pollen and seed gene flow between cultivated and wild individuals within all localities. In our study site cultivated stands consist of small orchards and live fences surrounded by secondary forests where wild relatives occur. There is evidence that gene flow can be maintained between cultivated plants and their wild relatives by pollinators inhabiting forest remnants (Ellstrand et al. 1999; Chumacero et al. 2013).

Paternity correlation measures indicated that seeds in both cultivated and wild trees are sired by a reduced number of effective pollen donors. In our study *S. purpurea* populations are distributed in disturbed habitats with low forest cover, that includes agricultural landscapes (AC and MU) and secondary forest (EEFH). In *S. purpurea*, a previous study showed that in disturbed habitats floral displays are large but pollinator activity is negatively affected (Cristobal-Perez et al in prep). Larger floral displays reduced pollinator movement between reproductive individuals reducing the diversity of sires and increasing the relatedness of

maternal progeny arrays (Karron et al. 2004; Karron & Mitchell 2012; Cristobal-Perez et al in prep).

In previous studies results show that *S. purpurea* depends on pollinators for sexual reproduction and seed production (Cristóbal-Pérez et al 2020; Cristobal-Pérez et al. in prep.). Under domestication, cultivated stands consist exclusively of female trees propagated vegetatively which can result in mate limitation (Mckey et al. 2010). Our results show that a high proportion of seeds are produced by sexual via, indicating that male trees of wild stands serve as pollen source for seed formation. In other dioecious fruit trees (e.g. pistachio, papaya) male trees are added to cultivated stands and serve as pollen donors to ensure fruit production (Zohary 2004). In areas where domesticated and wild relatives coexist, genetic diversity, gene flow and seed production of cultivated trees can be maintained by constant cross pollination in the wild-cultivated landscape. In addition, some cultivated perennial plants have evolved from producing fruit through sexual reproduction in the wild to parthenocarpic fruit under cultivation (e.g., banana, fig, pear, pistachio) (Miller and Gross, 2011). Our results show that this mechanism occurs in *S. purpurea* ensuring fruit formation under conditions of mate limitation.

In conclusion, in dioecious vegetatively propagated plants breeding system and propagation of heterozygous clones maintain moderate levels of genetic diversity. In areas where domesticated and wild individuals coexist, constant gene flow between groups lets genetic admixture and ensure fruit production. Under mate limitation conditions fruit production is provided by reproductive assurance mechanisms such as apomixis.

REFERENCES

Achtak H, Ater M, Oukabli A, Santoni S, Kjellberg F, Khadari B. 2010. Traditional agroecosystems as conservatories and incubators of cultivated plant varietal diversity: the

case of fig (Ficus carica L.) in Morocco. BMC Plant Biol. 10:28. doi: 10.1186/1471-2229-10-28

Aguirre-Dugua X, González-Rodríguez A. 2016. Phylogeographical Approaches to the Study of Plant Domestication, with Special Emphasis on Perennial Plants. In: Lira R., Casas A., Blancas J. (eds) Ethnobotany of Mexico. Ethnobiology. Springer, New York, NY. https://doi.org/10.1007/978-1-4614-6669-7_13

Anand A, Kumar V, Sundar S, Singh M, Bhushan S. 2019. Prevalence of apomixis in Jatropha: Is it significant?, Biofuels, DOI: 10.1080/17597269.2019.1706280

Arriola PE, Ellstrand NC. 1996. Crop-to-weed gene flow in the genus *Sorghum* (Poaceae): Spontaneous interspecific hybridization between johnsongrass, *Sorghum halepense*, and crop sorghum, *S. bicolor*. American Journal of Botany. 83: 1153-1159. doi:10.1002/j.1537-2197.1996.tb13895.x

Barghchi M, Alderson PG. 1989. Pistachio (*Pistacia vera* L.). In: Bajaj Y.P.S. (eds) Trees II. Biotechnology in Agriculture and Forestry, vol 5. Springer, Berlin, Heidelberg. https://doi.org/10.1007/978-3-642-61535-1_6

Casas A, Otero-Arnaiz A, Pérez-Negrón E, Valiente-Banuet A. 2007. *In situ* Management and Domestication of Plants in Mesoamerica. *Annals of Botany*: **100 (5):** 1101-1115. https://doi.org/10.1093/aob/mcm126

Casas A, Ladio AH, Clement C. 2019. Editorial: Ecology and Evolution of Plants Under Domestication in the Neotropics. Frontiers in Ecology and Evolution: 7: 231. doi: 10.3389/fevo.2019.00231

Chumacero de Schawe C, Durka W, Tscharntke T, Hensen I, Kessler M. 2013. Gene flow and genetic diversity in cultivated and wild cacao (Theobroma cacao) in Bolivia. American Journal of Botany. 100: 2271-2279. doi:10.3732/ajb.1300025

Cristóbal-Pérez EJ, Fuchs EJ, Harvey N, Quesada M. 2019. Isolation and characterization of microsatellites loci in Spondias purpurea (Anacardiaceae) and cross amplification in congeneric species. Molecular Biology Reports. 46: 5581–5585. https://doi.org/10.1007/s11033-019-04968-4

Cristóbal-Pérez EJ, Fuchs EJ, Olivares-Pinto U, Quesada M. 2020. Janzen-Connell effects shape gene flow patterns and realized fitness in the tropical dioecious tree Spondias purpurea (ANACARDIACEAE). Scientific Reports. 10: 4584. https://doi.org/10.1038/s41598-020-61394-4

Cuevas JA. 1994. in Neglected Crops: 1492 from a Different Perspective. eds. Bermejo JEH, León J. Food Agricultural Organization United Nations. Rome. pp. 111–115.

Darwin CR. 1868. The variation of animals and plants under domestication. London: John Murray. 1st edition, second issue. Volume 1.

Dick C, Hardy O, Jones F, Petit R. 2008. Spatial scales of pollen and seed-mediated gene flow in tropical rain forest trees. Tropical Plant Biology. 1:20– 33. https://doi.org/10.1007/s12042-007-9006-6

Doyle JJ, Doyle JL. 1987. A rapid DNA isolation procedure for small amounts of fresh leaf tissue. Phytochemical Bulletin:11–15.

Earl DA, vonHoldt BM. 2012. STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. Conservation Genet Resour. 4:359–361. https://doi.org/10.1007/s12686-011-9548-7

Elias M, Mühlen GS, McKey D. Roa AC, Tohme J. 2004. Genetic diversity of traditional South American Landraces of Cassava (Manihot esculenta Crantz): an analysis using microsatellites. Economic Botany. 58:242–256. https://doi.org/10.1663/0013-0001(2004)058[0242:GDOTSA]2.0.CO;2

Ellstrand NC, Prentice HC, Hancock JF. 1999. Gene Flow and Introgression from Domesticated Plants into their Wild Relatives. Annual Review of Ecology and Systematics. 30(1): 539-563. https://doi.org/10.1146/annurev.ecolsys.30.1.539

Evanno G, Regnaut S, Goudet J. 2005. Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. Molecular Ecology. 14(8):2611-2620. 10.1111/j.1365-294X.2005.02553.x

Firetti, F. Apomixis in Neotropical Vegetation. Vegetation. 129–148 (2018).

Karron JD, Mitchell RJ. 2012. Effects of floral display size on male and female reproductive success in Mimulus ringens. Annals of Botany 109:563–570. DOI: 10.1093/aob/mcr193

Karron JD, Mitchell RJ, Holmquist KG, Bell JM, Funk B. 2004. The influence of floral display size on selfing rates in Mimulus ringens. Heredity 92:242–248. DOI: 10.1093/aob/mcr193

Hamrick JL. 2004. Response of forest trees to global environmental changes.Forest Ecology and Management. 197(1–3): 323-335. https://doi.org/10.1016/j.foreco.2004.05.023.

Kamvar ZN, Tabima JF, Grünwald NJ. 2014. Poppr: an R package for genetic analysis of populations with clonal, partially clonal, and/or sexual reproduction. PeerJ 2:e281 https://doi.org/10.7717/peerj.281

Kislev M, Hartmann A, Bar-Yosef O. 2006. Early Domesticated Fig in the Jordan Valley. Science 312 (5778): 1372-1374 DOI: 10.1126/science.1125910

Klein AM, Vaissiere BE, Cane JH, Steffan-Dewenter I, Cunningham SA, Kremen C, Tscharntke T. 2007. Importance of pollinators in changing landscapes for world crops. Proceedings of the Royal Society B, Biological Sciences. 274(1608): 303-313. https://doi.org/10.1098/rspb.2006.3721

Larson G, Piperno DR, Allaby RG, Purugganan MD, Andersson L, Arroyo-Kalin M, Barton L, Climer Vigueira C, Denham T, Dobney K, Doust AN, Gepts P, Gilbert MT, Gremillion KJ, Lucas L, Lukens L, Marshall FB, Olsen KM, Pires JC, Richerson PJ, Rubio

de Casas R, Sanjur OI, Thomas MG, Fuller DQ. 2014. Current perspectives and the future of domestication studies. Proceedings of National Academy of Science USA. 111(17):6139-6146. doi: 10.1073/pnas.1323964111.

Martins-Lopes P, Gomes S, Lima-Brito J, Lopes J, Guedes-Pinto H. 2009. Assessment of clonal genetic variability in Olea europaea L. 'Cobrançosa' by molecular markers, Scientia Horticulturae. 123 (1):82-89. https://doi.org/10.1016/j.scienta.2009.08.001.

McKey D, Elias M, Pujol B, Duputié A. 2010. The evolutionary ecology of clonally propagated domesticated plants. New Phytologist. 186: 318-332. doi:10.1111/j.1469-8137.2010.03210.x

Meirmans PG, Van Tienderen PH. 2004. genotype and genodive: two programs for the analysis of genetic diversity of asexual organisms. Molecular Ecology Notes. 4: 792-794. doi:10.1111/j.1471-8286.2004.00770.x

Meyer RS, DuVal AE, Jensen HR. 2012. Patterns and processes in crop domestication: an historical review and quantitative analysis of 203 global food crops. New Phytologist, 196: 29-48. doi:10.1111/j.1469-8137.2012.04253.x

Miller A, Schaal B. 2005. Domestication of a Mesoamerican cultivated fruit tree, Spondias purpurea. Proceedings of the National Academy of Sciences. 102(36): 12801-12806. DOI: 10.1073/pnas.0505447102

Miller AJ, Schaal BA. 2006. Domestication and the distribution of genetic variation in wild and cultivated populations of the Mesoamerican fruit tree Spondias purpurea L. (Anacardiaceae). Molecular Ecology. 15(6):1467-1480. doi:10.1111/j.1365-294X.2006.02834.x.

Miller AJ, Gross BL. 2011. From forest to field: perennial fruit crop domestication. Am J Bot. 98(9):1389-1414. doi: 10.3732/ajb.1000522.

Miller AJ, Knouft JH. 2006. GIS-based characterization of the geographic distributions of wild and cultivated populations of the Mesoamerican fruit tree Spondias purpurea (Anacardiaceae). American Journal of Botany. 93: 1757-177. https://doi.org/10.3732/ajb.93.12.1757

Moore PH. 2014. Phenotypic and Genetic Diversity of Papaya. In: Ming R., Moore P. (eds) Genetics and Genomics of Papaya. Plant Genetics and Genomics: Crops and Models, vol 10. Springer, New York, NY. https://doi.org/10.1007/978-1-4614-8087-7_3

Nassar NMA, Ortiz R. 2007. Cassava improvement: Challenges and impacts. The Journal of Agricultural Science, 145(2): 163-171.

O'Connor K, Powell M, Nock C, Shapcott A. 2015. Crop to wild gene flow and genetic diversity in a vulnerable Macadamia (Proteaceae) species in New South Wales, Australia. Biological Conservation. 191: 504-511.https://doi.org/10.1016/j.biocon.2015.08.001.

Peakall R, Smouse PE. 2012. GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research—an update. Bioinformatics 28:2537–2539. DOI:10.1093/bioinformatics/bts460

Pickersgill B. 2007. Domestication of plants in the Americas: insights from Mendelian and molecular genetics. Annals of Botany: 100(5):925-40. doi: 10.1093/aob/mcm193.

Picq S, Santoni S, Lacombe T, Latreille M, Weber A, Ardisson M, et al. 2014. A small XY chromosomal region explains sex determination in wild dioecious *V. vinifera* and the reversal to hermaphroditism in domesticated grapevines. *BMC Plant Biology.* 14:229. doi: 10.1186/s12870-014-0229-z

Pritchard JK, Stephens M, Donnelly P. 2000. Inference of population structure using multilocus genotype data. Genetics 155:945–959.

Purugganan MD, Fuller DQ. 2009. The nature of selection during plant domestication. Nature. 457(7231):843-8. doi: 10.1038/nature07895

Ritland K. 2002. Extensions of models for the estimation of mating systems using n independent loci. Heredity 88:221–228. DOI: 10.1038/sj.hdy.6800029

Scarcelli N, Couderc M, Baco MN, Egah J, Vigouroux Y. 2013. Clonal diversity and estimation of relative clone age: application to agrobiodiversity of yam (Dioscorea rotundata). BMC Plant Biol 13: 178. https://doi.org/10.1186/1471-2229-13-178

Stetter MG, Gates DJ, Mei W, Ross-Ibarra J. 2017. How to make a domesticate, Current Biology. 27(17): 896-900. https://doi.org/10.1016/j.cub.2017.06.048.

Van Tassel DL, DeHaan LR, Cox TS. 2010. Missing domesticated plant forms: can artificial selection fill the gap?. Evolutionary Applications 3: 434-452. doi:10.1111/j.1752-4571.2010.00132.x

Vaughan DA, Balázs E, Heslop-Harrison JS. 2007. From Crop Domestication to Super-domestication. Annals of Botany: **100 (5):** 893–90. https://doi.org/10.1093/aob/mcm224

Waring BG, Perez-Aviles D, Murray JG, Powers JS. 2019. Plant community responses to stand-level nutrient fertilization in a secondary tropical dry forest. Ecology 100: e02691. https://doi.org/10.1002/ecy.2691

Zohary D, Spiegel-Roy P. 1975. Beginnings of fruit growing in the old world. Science. 187(4174):319-327. doi: 10.1126/science.187.4174.319

Zohary D. 2004. Unconscious Selection and the Evolution of Domesticated Plants. *Economic Botany*. *58*(1): 5-10. https://doi.org/10.1663/0013-0001(2004)058[0005:USATEO]2.0.CO;2

TABLES AND FIGURES

TABLE 1. Genetic diversity parameters of wild and cultivated *Spondias purpurea*. N: Number of individuals; N_A: mean allele number per locus; A_R: Allelic richness; H_O: observed heterozygosity; H_E: expected heterozygosity.

Group	Locality and size class	N	N_a	A_r	H_o	H_e	F
Cultivated	Agua Caliente - Adults	22	3.86	2.98	0.642	0.50	-0.208
			(0.705)	(0.307)	(0.139)	(0.215)	(0.19)
	Agua Caliente - Seeds	141	4.86	1.932	0.47	0.437	-0.063
			(0.829)	(0.220)	(0.108)	(0.069)	(0.15)
	Murcielago - Adults	16	3	2.20	0.49	0.46	0.04
			(0.436)	(0.401)	(0.130)	(0.089)	(0.21)
	Murcielago - Seeds	154	3.58	1.93	0.75	0.46	-0.55
			(0.429)	(0.149)	(0.124)	(0.048)	(0.154)
	EEFH - Adults	8	3.15	2.37	0.50	0.57	0.08
			(0.34)	(0.292)	(0.093)	(0.063)	(0.132)
	EEFH - Seeds	116	3.45	2.36	0.52	0.53	-0.04
			(0.481)	(0.268)	(0.121)	(0.077)	(0.206)
	Mean	**76**	**23.3**	**2.92**	**0.54**	**0.51**	**0.13**
Wild	Agua Caliente - Adults	29	4	2.3	0.65	0.51	-0.192
			(0.535)	(0.238)	(0.141)	(0.074)	(0.198)
	Agua Caliente - Seeds	84	4.86	1.94	0.47	0.44	-0.063
			(0.829)	(0.22)	(0.108)	(0.069)	(0.15)
	Murcielago - Adults	25	3.6	3.58	0.55	0.55	-0.01
			(0.459)	(0.369)	(0.071)	(0.046)	(0.122)
	Murcielago - Seeds	47	3.72	2.32	0.66	0.53	-0.22
			(0.522)	(0.298)	(0.106)	(0.053)	(0.159)
	EEFH - Adults	101	5	1.87	0.34	0.40	0.24
			(0.787)	(0.22)	(0.127)	(0.084)	(0.217)
	EEFH- Seeds	106	3.8	2.32	0.66	(0.53)	-0.22
			(0.522)	(0.298)	(0.106)	(0.053)	(0.159)
	Mean	**65**	**29.3**	**2.85**	**0.50**	**0.49**	**0.14**
	Grand mean	**849**	**3.85**	**2.17**	**0.55**	**0.49**	**-0.01**
			(0.18)	**(0.077)**	**(0.034)**	**(0.02)**	**(0.052)**

TABLE 2. Clonal diversity parameters of wild and cultivated *Spondias purpurea*. N: Number of individuals; G: number of unique multilocus genotypes; D: Simpson's Diversity; E: evenness.

Group	Locality	N	***G***	***G/N***	***D***	E
Cultivated	Agua Caliente	19	12	0.63	0.9	0.56
	Murcielago	11	7	0.58	0.81	0.55
	EEFH	7	7	1	1	1
	Mean	**37**	**8.66**	**0.73**	**0.9**	**0.7**
Wild	Agua Caliente	28	21	0.75	0.94	0.51
	Murcielago	20	19	0.95	0.99	0.95
	EEFH	84	53	0.63	0.96	0.41
	Mean	**132**	**31**	**0.77**	**0.96**	**0.62**

Table 3. Paternity correlation of wild and cultivated Spondias purpurea. n: offspring; r_p: multilocus correlation of outcrossed paternity; *Nep*: effective number of parents.

Jocote type	Locality	n	r_p	*Nep*
Cultivated	Agua Caliente	141	0.99 (0.03)	1.01
	Murcielago	154	0.99 (0.01)	1.01
	EEFH	116	0.99 (0.09)	1.01
	Mean	**411**	**0.99**	**1.01**
Wild	Agua Caliente	84	0.95 (0.06)	1.05
	Murcielago	47	0.83 (0.07)	1.2
	EEFH	107	0.95 (0.04)	1.05
	Mean	**238**	**0.91**	**1.09**

Table 4. Progeny arrays with multilocus identical genotypes to maternal trees, from wild and cultivated trees of *Spondias purpurea*. n: number of seeds analysed per tree; *Mig*: number of seeds with multilocus identical genotypes to maternal tree.

Jocote type	Locality	Maternal tree	*n*	*Mig*
Cultivated	Agua Caliente	ACF-16	10	5
		ACF-38	16	5
	Murcielago	MUF-41	10	5
		MUF-3	16	5
	Horizontes	HF-1	13	1
Wild	Murcielago	MUF-24	17	7
		MUF-49	12	2

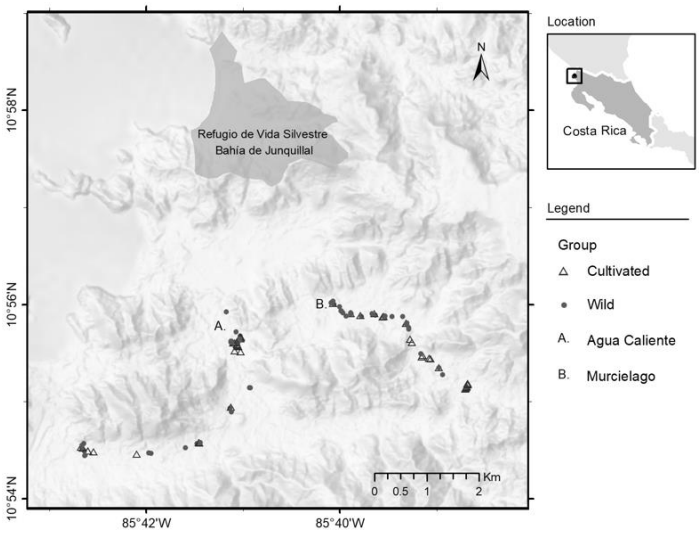

Figure 1. Spatial distribution of cultivated and wild *Spondias purpurea* individuals in A) Agua Caliente y B) Murcielago, Guanacaste, Costa Rica. This map was created in Quantum GIS v.3.4 (Quantum GIS Development Team (2018). Quantum GIS Geographic Information System. Open Source Geospatial Foundation Project. http://qgis.osgeo.org).

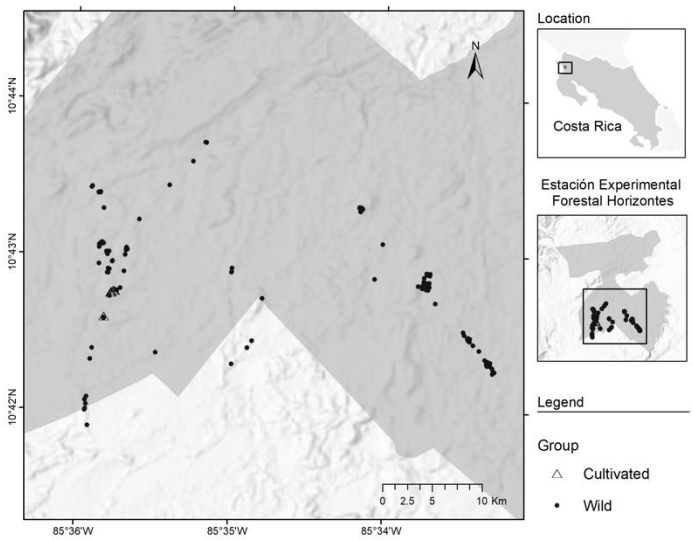

Figure 2. Spatial distribution of cultivated and wild *Spondias purpurea* individuals in Estación Experimental Forestal Horizontes, Guanacaste, Costa Rica. This map was created in Quantum GIS v.3.4 (Quantum GIS Development Team (2018). Quantum GIS Geographic Information System. Open Source Geospatial Foundation Project. http://qgis.osgeo.org).

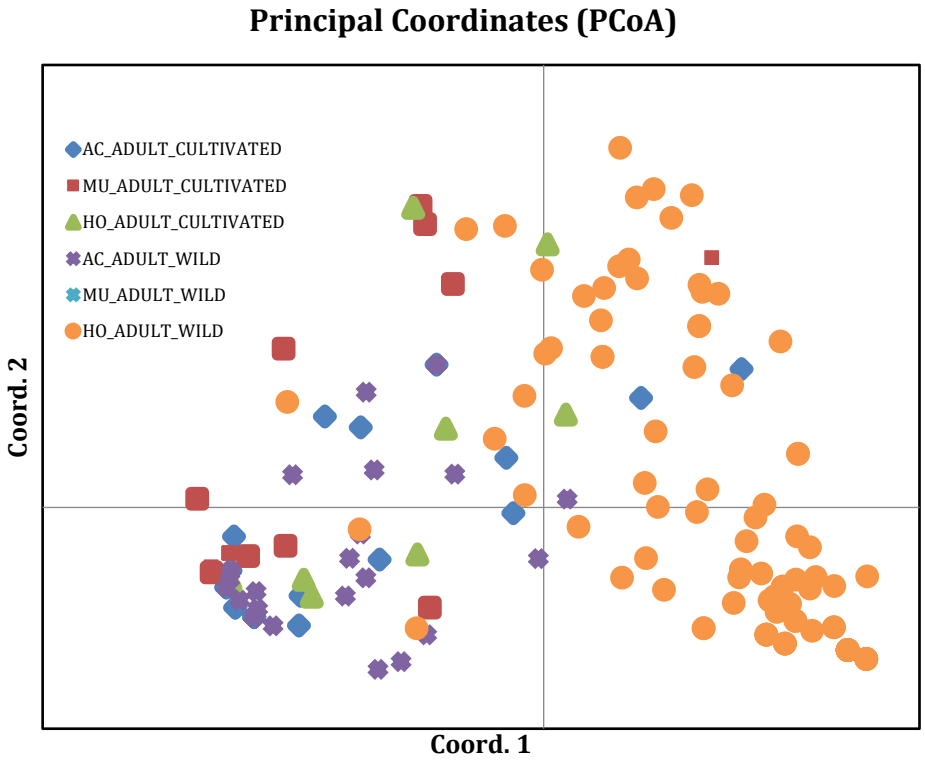

Figure 3. Representation of first two axes of principal coordinate analysis (variation explained: axis 35.87%; axis 2, 9.72%) made from the matrix of genetic distances between individuals of Spondias purpurea obtained from 7 microsatellite genotypes. Symbols indicate the group and locality of the sample.

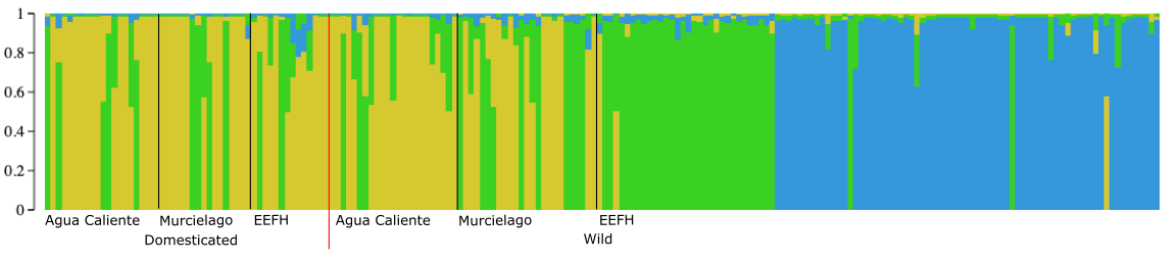

Figure 4. Mean results of 10 runs of Structure at *K*=3 for 201 Spondias purpurea individuals in 6 wild or cultivated groups.

CAPÍTULO IV. Isolation and characterization of microsatellites loci in *Spondias purpurea* (Anacardiaceae) and cross amplification in congeneric species

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SHORT COMMUNICATION

Isolation and characterization of microsatellites loci in *Spondias purpurea* (Anacardiaceae) and cross amplification in congeneric species

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Abstract
Microsatellite markers provide high polymorphism levels, useful to study genetic diversity and gene flow patterns in plant populations. Here we develop and characterize microsatellite primers to evaluate patterns of genetic structure and diversity, and gene flow levels in the dioecious tropical tree *Spondias purpurea* (Anacardiaceae). Twenty-four microsatellite primers were developed for *Spondias purpurea*. Polymorphism was evaluated in 139 individuals from three localities in Mexico. Ten loci were polymorphic. The number of alleles ranged between two and 21, the average number of alleles was 5.88. Cross-amplification trials on *S. mombin*, *S. radlkoferi*, *Astronium graveolens* and *Amphipterygium adstringens* achieved successful amplification for only six microsatellites in *S. mombin* and *S. radlkoferi*. Microsatellites developed for *S. purpurea* will be a useful tool to estimate genetic diversity within and among populations, as well as to assess the consequences of habitat fragmentation on gene flow patterns of this species.

Keywords Anacardiaceae · Habitat fragmentation · Microsatellites · *Spondias*

Introduction

Spondias purpurea L. is a small (3–10 m) tree known locally as Ciruelo, Jobo, or Jocote. *S. purpurea* is a dioecious tree in the Anacardiaceae family, native to the tropical dry forests of Mexico and Central America [1, 2]. This species depends on insects, mainly stingless bees, for cross-pollination [3]. Female trees produce small, bright red, juicy and sweet-acidic fruits that are consumed by a large number of mammals and birds and are an important source of water during the dry season when weather conditions are critical [4, 5]. Throughout the Ciruelo distribution both wild and domesticated populations are commonly found [6]. Ciruelos are cultivated for their fruits, which are sold fresh in local markets or made into jams and beverages [7]. Like other domesticated fruit trees, individuals are propagated vegetatively but only female trees are planted for fruit consumption [6]. Under domestication, cultivated perennials could have evolved from producing fruit through sexual reproduction in the wild to parthenocarpic fruit production in cultivation [8]; however, female trees could also produce fruit via sexual reproduction, if male trees from nearby wild populations act as sources of pollen for female cultivated trees. Therefore, genetic diversity and gene flow of cultivated trees is maintained by constant cross pollination in the wild-cultivated landscape.

Here we describe twenty-four new SSR markers for *S. purpurea* and tested them for cross-amplification with related species within the genus and other species of the Anacardiaceae family. We also describe genetic diversity in three populations of the tropical dry forests of Mexico. These markers will be used to (1) describe and evaluate the factors that cause fine-scale spatial genetic structure in a population of this dioecious species, (2) determine the consequences of anthropogenic fragmentation on gene flow

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patterns and (3) evaluate the levels of gene flow among cultivated and wild populations. A previous study developed microsatellite markers for a monoecious sister species in the genus (*Spondias radlkoferi* Donn. Sm.) [9] and these markers were used to study paternity of seeds in latrines of spider monkeys [10]; however our study is the first to develop SSR markers for *Spondias purpurea*.

Materials and methods

Microsatellite isolation was performed by the simple sequence repeat (SSR) development company Genetic Marker Services (Brighton, United Kingdom; www.geneticmarkerservices.com). We extracted Genomic DNA using the DNeasy Plant Mini Kit (QIAGEN, Valencia, California, USA) from a single *S. purpurea* individual collected at Chamela Biological Station-UNAM (Table 1). The microsatellite isolation process was based on the production of an enriched library using a hybridization capture protocol. Enrichment involved incubating adaptor-ligated, restricted DNA, with filter-bonded synthetic repeat motifs: [AG]17, [AC]17, [AAC]10, [CCG]10, [CTG]10, and [AAT]10. The filters were then washed at a range of temperatures (50–60 °C) and SSC stringencies (0.5X–2X) to optimize enrichment. Forty-seven motif-positive *Escherichia coli* JM109 clones were detected and sequenced, of which 24 contained exploitable repeat motifs with sufficient flanking regions to design forward/reverse primer pairs. Primers were designed for the most promising loci using the online primer design software PRIMER 3.0 [11]. We selected primer pairs that amplified products ranging from 100 to 250 bp to help minimize later multi-loading overlap ambiguities during sequencer genotyping. Primers were then tested on 7 individuals from different localities (Table 1), using a touchdown PCR protocol. PCR amplifications were performed in a 25 µL final volume containing 7 pmol of each primer, 1.5 mM of $MgCl_2$, 0.2 mM of each dNTP, 1 X PCR buffer, 0.8 µg/µL BSA, 0.5 U Taq Polymerase, and 1.5 µL of DNA diluted 20-fold. Touchdown PCR consisted of 32 cycles of denaturation at 95 °C for 60 s, annealing for 60 s as 2 cycles, each 64–59 °C; 10 cycles at 58 °C; 10 cycles at 57 °C, elongation at 72 °C for 60 s, and a final extension at 72 °C for 5 min.

Products were checked for specificity, polymorphism and null alleles on cooled high-resolution agarose gels consisting of 4% MetaPhor (Lonza, Basel, Switzerland) agarose gels in TAE, run in a cold room at 10 °C. Twenty-four loci showed clear and specific bands among the seven individuals assayed (Table 2).

To evaluate the polymorphisms at each locus, we used genomic DNA extracted from 139 individuals of three localities situated in our study region (Table 1). PCR amplifications were performed in a 12-µL final volume containing

Table 1 Voucher information, geographic location and number of individuals sampled for each species used in this study

Species	Voucher information[a]	Locality	Geographic coordinates	N
S. purpurea L.	257123—EJCP	Chamela, La Jalisco, Mexico.	19°29′59.40″N 105°2′35.43″W	69
	257122—EJCP	La Mesa, Jalisco, Mexico.	19°37′2.09″N 104°53′17.81″W	26
	257128—EJCP	Ranchitos, Jalisco, Mexico	19°35′42.45″N 105°0′34.87″W	44
S. mombin L.	244062—AGZ	Marqués de Comillas, Chiapas, Mexico	16°16′56″N 90°50′21″W	2
	207907—AGZ	Ocosingo, Chiapas, Mexico	17°0′6″N 91°16′47″W	3
S. radlkoferi Donn. Sm.	244065—AGZ	Marqués de Comillas, Chiapas, Mexico	16°16′60″N 90°50′2″W	2
	239171—GIB	Montepío, Veracruz, Mexico	18°38′36″N 95°05′46″W	1
A. graveolens Jacq.	257133—EJCP	Careyes, Jalisco, Mexico	19°26′56.00″N 105°0′46.00″W	5
A. adstringens (Schltdl.) Standl.	257118—FJBO	Chinameca, Morelos, Mexico	18°37′90.5″N 95°59′36.9″W	5

Vouchers are deposited at the IEB Herbarium (Instituto de Ecología, A.C., Pátzcuaro, Mexico)

EJCP Edson Jacob Cristóbal-Pérez, *AGZ* Arturo González-Zamora, *GIB* Guillermo Ibarra Manríquez, *FJBO* Francisco Javier Balvino Olvera

[a]Letters at the end of the voucher number identify the collector

Table 2 Primer sequences (F, forward an R, reverse), repeat motifs and allele sizes for 24 microsatellite loci isolated for *Spondias purpurea*

Locus	Primer sequences (5′–3′)	Repeat motif	Allele size range (bp)	GenBank accession no.
SPUR44	F: AGCTGGTTCCACCCTTTGTT R: AGAAAACAGATGGGGCCTTT	$(CT)_9$	126–140	MF084856
SPUR40	F: TTCAGGACTTTGCCAAGAGAA R: CGCGTGTGGTCTAACTCTCA	$(AG)_9$	133–175	MF084852
SPUR28	F: TGAACATTGCAGTGGAAGAGA R: ACGGCTGGAAATTTTAGTTTT	$(GA)_{13}$	133–159	MF084845
SPUR35	F: ATCTGAAGCCACCCTGTGAC R: CTCTCTCTCCCCAAAGATGC	$(TG)_9$	129	MF084842
SPUR33	F: TCAATCTAAACACCCCACAC R: GCTGTCTAAGCATATTAAGTTTTCA	$(AC)_9$	123–131	MF084841
SPUR39	F: TCTTCCGAGATGAAAAGAAA R: TTCACCTCAAACAGTTGAAAA	$(TTG)_7$	97–115	MF084851
SPUR42	F: TACAAAATTCGCTGCAAAAA R: CGGTGCCAAGAGGAGAGTAA	$(GT)_8(GA)_8$	136–160	MF084854
SPUR41	F: CCACCACAACAAAACCCTTC R: AAGTTGAGGGATGATTTGCTG	$(AAC)_6$	98–104	MF084853
SPUR29	F: GCCCAAATTCTATGGTTCTC R: CTGTAGATTTCACTGTTCACAAG	$(AG)_{11}$	187	MF084846
SPUR57	F: GATGATTATTGCTTTGATATCCTT R: TCACATCTTAGCTTTTCTGTTTC	$(CT)_{11}$	127	MF084864
SPUR21	F: ACCCGTGAAAACAGCACATT R: AACACGCCATCCTCGTCTT	$(TTC)_8$	164	MF084844
SPUR33b	F: AATCGTCGAACACGAAGGTC R: TCGAAAATCGGAAACTTTGA	$(CT)_{14}$	192	MF084847
SPUR36	F: CGATCCCGAGTTATTTGCAC R: GCCTTCAACCTCTTTTTCTCC	$(TC)_{15}$	221	MF084848
SPUR37	F: GCGTAGCAATGAATAGTCTCAA R: TAATAACACCACCAGCAGCA	$(TTG)_6$	123	MF084849
SPUR38	F: GAGGGGGAGAGAGAGAGGAG R: ACTCTGCGCACCAAGTCTCT	$(AG)_{18}$	225	MF084850
SPUR43	F: GACGAAACAAAGTTCCAGAG R: AAAGGAGTTGCCAAGGTATC	$(GT)_{13}$ G-$(AG)_{11}$	127	MF084855
SPUR48	F: AGGCATGGGAGTGTAAGACA R: TGCAATCTCATCTGTGAAAAA	$(GT)_9$	174	MF084859
SPUR50	F: GCAAAACAACAAGTCCGAAG R: CAATGTCATAATGTTTTTGTATTGTTT	$(AG)_{11}$	105	MF084860
SPO53	F: GCTTGAAGGTTTTCATCAGCA R: TGGAACATGCTTTTTGCTTC	$(GT)_{10}$	127	MF084861
SPUR55	F: CGGGGCTACACATATTCCAC R: TGTTGTTGGCTTTTGTTGGA	$(GT)_{25}$	215	MF084862
SPUR56	F: TTTGGAATGCACACACAACA R: CCTTGCTGGACCTCTTTGAT	$(GT)_{19}$	155	MF084863
SPUR44b	F: TGTAGACGTTGAAAACGAAAA R: TCTTCAATCGCTATCACAAACT	$(AG)_{10}$	85–103	MF084857
SPUR57	F: GATGATTATTGCTTTGATATCCTT R: TCACATCTTAGCTTTTCTGTTTC	$(CT)_{11}$	120–138	MF084864
SPUR47	F: CCCCCTTTCTCCATTGTTTT R: ATGGTGGCCGTCAAATAAGT	$(TC)_{11}$	116–120	MF084858

Table 2 (continued)

Locus	Primer sequences (5′–3′)	Repeat motif	Allele size range (bp)	GenBank accession no.
SPUR19	F: TACCCGCACTCTGGAGAGAA R: AATAAGAGCCCAACCCCTGT	$(AG)_{12}$	118	MF084843

final concentrations of 1 × QIAGEN Multiplex PCR (QIAGEN, Hilden, Germany), 0.10 µM of primer mix and 20 ng of template DNA. PCR amplification was performed in a Veriti thermal cycler (Applied Biosystems, Carlsbad, California, USA) using the following conditions: first an initial activation step of 95 °C at 15 min, followed by a touchdown PCR consisting of 32 cycles with denaturation at 95 °C for 90 s; annealing for 60 s with temperature decreasing 1 °C every two cycles from 64 °C (12 cycles), then 10 cycles at 58 °C and 10 cycles at 57 °C; elongation at 72 °C for 60 s; and a final extension at 72 °C for 5 min. Loci that were successfully amplified were then tested with a fluorescent forward primer. These fragments were analysed on an automatic ABI-PRISM 3100-Avant sequencer (Applied Biosystems, Carlsbad, California, USA) using GeneScan LIZ 600 (Applied Biosystems, Carlsbad, California, USA) to determine fragment sizes. Alleles were scored manually using GeneMarker Software version 2.6.4 (SoftGenetics).

Ten loci were polymorphic (first 10 on Table 2), the rest were monomorphic in these populations or produced multiple peaks and could not be genotyped (Table 2). All microsatellite sequences were deposited in GenBank® (www.ncbi.nlm.nih.gov/genbank/) (Table 2). Number of alleles, observed and expected heterozygosities and deviations from Hardy–Weinberg equilibrium were determined using Arlequin Software version 3.5.1.2 [12]. Cross-species amplifications of *S. purpurea* microsatellites were tested in three to five individuals of *Astronium graveolens* Jacq., *Amphipterygium adstringens* (Schltdl.) Standl. and two other species of the genus *Spondias* L., *S. mombin* L. and *S. radlkoferi* Donn. Sm. PCR conditions for cross-species amplifications were the same as described above for *S. purpurea*.

Results and discussion

We scored a total of 95 alleles. The number of alleles per locus ranged between two (SPUR41 and SPUR 39) and 21 (SPUR40) (Table 3), with an average of 5.37. Observed heterozygosity varied across loci ranging between 0 and 0.808, and we found significant deviations from Hardy–Weinberg Equilibrium for some loci in some localities (Table 3), probably due to the small sample sizes or to the presence of null alleles, particularly in the case of loci SPUR35, SPUR42 and SPUR57 with heterozygote deficit observed at three localities. Six primers successfully cross-amplified both in *S. mombin* as in *S. radlkoferi* (SPUR44, SPUR40, SPUR29, SPUR35, SPUR33 and SPUR42).

The microsatellite markers described here for *S. purpurea* are the first for this species. Cross-amplification of these microsatellites in *S. mombin* and *S. radlkoferi* suggest that they may be used to study genetic diversity in other species within the genus. Previous work have reported the development of microsatellites for *S. radlkoferi* and the cross-amplification of these markers to *S. mombin* [9], thus the microsatellites presented here can increase the loci number for future microsatellite-based studies of the genus. These microsatellites will be a useful tool to analyse the genetic

Table 3 Number of alleles (A), observed and expected heterozygosity (Ho and He) and PHWE P value for Hardy–Weinberg equilibrium (HWE) test for 10 polymorphic microsatellites in *Spondias purpurea* at three localities

Locus	Chamela ($n=69$)				La Mesa ($n=26$)				Ranchitos ($n=44$)			
	A	Ho	He	PHWE	A	Ho	He	PHWE	A	Ho	He	PHWE
SPUR44	11	0.739	0.765	0.160	6	0.615	0.649	0.394	10	0.651	0.655	0.352
SPUR40	8	0.441	0.463	0.504	4	0.269	0.303	0.106	11	0.432	0.765	<0.001*
SPUR28	8	0.565	0.694	0.008*	5	0.846	0.740	0.185	6	0.682	0.675	0.190
SPUR29	5	0.412	0.505	0.008*	4	0.231	0.331	0.084	3	0.452	0.568	0.018*
SPUR35	3	0.087	0.515	<0.001*	5	0.577	0.683	<0.001*	4	0.341	0.614	<0.001*
SPUR33	6	0.391	0.348	0.325	2	0.040	0.039	1	6	0.136	0.131	1
SPUR42	9	0.449	0.807	<0.001*	9	0.462	0.8	<0.001*	10	0.636	0.848	<0.001*
SPUR41	2	0.044	0.043	1	2	0.038	0.038	1	2	0.136	0.127	1
SPUR39	2	0.783	0.482	<0.001*	2	0.808	0.482	<0.001*	2	0.744	0.467	<0.001*
SPUR57	6	0.169	0.427	<0.001*	3	0	0.403	<0.001*	3	0	0.135	<0.001*

*Locus with significant deviations from Hardy–Weinberg Equilibrium ($P<0.05$)

diversity, population genetic structure and gene flow patterns of this tree species in the tropical dry forest. In particular, we will use these genetic markers to evaluate spatial genetic structure on *S. purpurea* at the Chamela-Cuixmala Biosphere Reserve, Jalisco, Mexico and to assess the consequences of habitat fragmentation on genetic structure and gene flow patterns in this dioecious species; thus contributing to the design of appropriate strategies for the conservation of *S. purpurea* and the Tropical Dry Forest.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

1. Miller A, Schaal B (2005) Domestication of a Mesoamerican cultivated fruit tree, Spondias purpurea. Proc Natl Acad Sci USA 102:12801–12806. https://doi.org/10.1073/pnas.0505447102
2. Miller A, Knouft J (2006) GIS-based characterization of the geographic distributions of wild and cultivated populations of the Mesoamerican fruit tree *Spondias purpurea* (Anacardiaceae). Am J Bot 93:1757–1767. https://doi.org/10.3732/ajb.93.12.1757
3. Mitchell JD, Daly DC (2015) A revision of *Spondias* L. (Anacardiaceae) in the Neotropics. PhytoKeys 55:1–92. https://doi.org/10.3897/phytokeys.55.8489
4. Méndez-Toribio M, Pierro A, Quesada M, Benítez-Malvido J (2014) Regeneration beneath a dioecious tree species *Spondias purpurea* in a Mexican tropical dry forest. J Trop Ecol 30:265–268. https://doi.org/10.1017/S0266467414000066
5. Mandujano S, Gallina S, Bullock S (1994) Frugivory and dispersal of Spondias purpurea (Anacardiaceae) in a tropical deciduous forest in México. Rev Biol Trop 42:107–114
6. Miller A (2011) Spondias. In: Kole C (ed) Wild crop relatives: genomic and breeding resources. Springer, Berlin Heidelberg, pp 203–212
7. Macía M, Barfod A (2000) Economic botany of *Spondias purpurea* (Anacardiaceae) in Ecuador. Econ Bot 54:449–458. https://doi.org/10.1007/BF02866544
8. Miller AJ, Gross BL (2011) From forest to field: perennial fruit crop domestication. Am J Bot 98:1389–1414. https://doi.org/10.3732/ajb.1000522
9. Aguilar-Barajas E, Sork VL, González-Zamora A, Rocha-Ramírez V, Arroyo-Rodríguez V, Oyama K (2014) Isolation and characterization of polymorphic microsatellite loci in *Spondias radlkoferi* (Anacardiaceae). Appl Plant Sci 2(11):10. https://doi.org/10.3732/apps.1400079
10. Arroyo-Rodríguez V, Aguilar-Barajas E, González-Zamora A, Rocha-Ramírez V, González-Rodríguez A, Oyama K (2017) Parent-parent and parent-offspring distances in Spondias radlkoferi seeds suggest long-distance pollen and seed dispersal: evidence from latrines of the spider monkey. J Tropical Ecol 33(2):95–106. https://doi.org/10.1017/S0266467417000050
11. Rozen S, Skaletsky H (2000) Primer3 on the WWW for general users and for biologist programmers. Methods Mol Biol 132:365–386
12. Excoffier L, Laval G, Schneider S (2007) Arlequin (version 3.0): an integrated software package for population genetics data analysis. Evol Bioinform 1:47–50

DISCUSIÓN GENERAL Y CONCLUSIONES

Los resultados de esta tesis demuestran el papel de las interacciones bióticas en el mantenimiento de la diversidad genética y los niveles de flujo génico de *S. purpurea.* El efecto de procesos dependientes de la densidad y la distancia, que son claves para explicar la alta riqueza de especies en bosques tropicales, juegan un papel importante sobre los patrones de flujo génico, la formación de estructura genética a escala fina y la adecuación de *S. purpurea*. Nuestros resultados demuestran baja estructuración genética a escala fina a distancias cortas, la cual disminuye en relación inversa con la distancia espacial. Un resultado similar al encontrado en otras especies con semillas dispersadas por vertebrados (Hardesty et al., 2005; Zhou y Chen, 2010). Los dispersores de semillas de *S. purpurea* incluyen aves (*Ortalis poliocephala* e *Icterus pustulatus*) mamiferos (*Odocoileus virginianus, Pecari tajacu, Canis latrans, Nasua narica, Urocyon cinereoargenteus, Didelphis virginiana, Sciurus colliaei* y *Liomys pictus*) y réptiles (*Ctenosaura pectinata*) (Mandujano et al., 1994). A excepción de *P. tajacu,* que depreda las semillas, el resto de estos vertebrados pueden mover las semillas a distancias largas (Mandujano et al., 1994). El planteamiento original de Janzen (1970) establece que como resultado de la interacción entre la dispersión y depredación de semillas, las poblaciones de árboles tropicales presentan una curva óptima de reclutamiento poblacional. En *S. purpurea* esta curva ocurre entre los 150 y 200 metros. Adicionalmente, la comparación entre las curvas de dispersión efectiva y realizada de polen demuestra que las semillas resultado de la polinización a larga distancia, tienen una mayor probabilidad de establecerse que aquellas polinizadas a distancias cortas. Esto indica que la probabilidad de sobrevivir y establecerse en la población está en función de la distancia de los árboles parentales (maternos y paternos) en congruencia con el planteamiento de la hipótesis Janzen-Conell. Entonces factores dependientes de la densidad y la distancia regulan los patrones de flujo génico y la adecuación en *S. purpurea*; árboles que dispersan su polen y semillas a larga distancia tienen mayor fitness masculino y femenino, respectivamente, lo cual resulta en bajos niveles de estructura genética a escala fina (Cristóbal-Pérez et al., 2020).

Sin embargo las interacciones bióticas pueden ser alteradas como consecuencia de la pérdida y fragmentación del hábitat. En nuestro estudio los árboles de los ambientes fragmentados reciben menores tasas de visitas por polinizadores, tienen tasas de fructificación más bajas, menores distancias de dispersión de polen, un menor número de donadores de polen y menor diversidad genética que los árboles de bosques continuos. La alteración de estos elementos puede comprometer la persistencia de las poblaciones y su viabilidad en el largo

plazo (Aguilar et al. 2019). Aún cuando los árboles de *S. purpurea* en ambientes fragmentados produjeron más flores y la composición de la comunidad de polinizadores se mantiene entre hábitats, la tasa de visitas de polinizadores disminuye reflejándose en una baja tasa de fructificación. Además, los resultados obtenidos para el patrón de flujo génico vía polen reflejan que una mayor producción de flores puede afectar el comportamiento de los polinizadores, limitando su movimiento de forrajeo dentro de un mismo árbol o entre individuos espacialmente cercanos (Karron et al. 2004; Karron & Mitchell 2012). Por lo tanto, si como se demuestra en el capítulo 1, semillas producidas por eventos de polinización cercanos tienen una menor probabilidad de establecerse, el fitness masculino estaría afectando negativamente (Cristóbal-Pérez et al., 2020). Adicionalmente, los cambios en los patrones de forrajeo de los polinizadores puede afectar negativamente el número de donadores de polen (Fuchs et al., 2003; Quesada et al., 2001; Quesada et al., 2004). Una reducción en el número de donadores puede afectar negativamente la calidad de las cargas de polen reflejándose en una baja producción de frutos. Nuestros resultados demuestran que alteraciones en la interacción planta-polinizador se reflejan en una disminución en la diversidad genética de *S. purpurea* en ambientes fragmentados lo cual puede comprometer la persistencia de las poblaciones en el largo plazo.

En la actualidad los paisajes tropicales están compuestos por remanentes de bosque rodeados por matrices agrícolas. En centros de origen y domesticación de plantas, como Mesoamérica, las plantas cultivadas generalmente coexisten con sus parientes silvestres en estos paisajes (Miller y Gross., 2011). Nuestros resultados demuestran que las poblaciones silvestres de *S. purpurea* son importantes reservorios de diversidad genética. En plantas perennes, el modo de propagación vegetativa permite la propagación de diferentes genotipos por cultivar (alta diversidad clonal), manteniendo elevados niveles de diversidad genética en las poblaciones cultivadas como los obtenidos en nuestro estudio (Elias et al. 2004; McKey et al., 2010). Nuestros resultados demuestran baja estructuración genética de las poblaciones, lo cual puede ser resultado de flujo génico constante entre las poblaciones cultivadas y sus parientes silvestres (Ellstrand et al. 1999; Chumacero et al. 2013). Además en plantas dioicas domesticadas para el consumo de sus frutos como *S. purpurea,* la producción de frutos es asegurada por vía sexual, a través del flujo génico entre las plantas cultivadas y sus parientes silvestres y por vía asexual a través de la producción de semillas por apomixis (Zohary 2004).

Este trabajo demuestra el papel de factores dependientes de la densidad y la distancia en la diversidad genética, la estructura genética a escala fina y la adecuación de las poblaciones de plantas. Aporta evidencia de los efectos negativos de la fragmentación del hábitat sobre el

éxito reproductivo, la diversidad genética y el flujo génico y demuestra el papel de las poblaciones silvestres como reservorios de la diversidad genética de poblaciones cultivadas y como fuentes de polen para el aseguramiento de la producción de frutos.

REFERENCIAS BIBLIOGRAFICAS

Aguilar R., Ashworth L., Galetto L., Aizen M.A. 2006. Plant reproductive susceptibility to habitat fragmentation: review and synthesis through a meta-analysis. Ecology Letters 9:968–980.

Aguilar R, Quesada M, Ashworth L, Herrerias-Diego Y, Lobo J. 2008. Genetic consequences of habitat fragmentation in plant populations: susceptible signals in plant traits and methodological approaches. Molecular Ecology 17:5177–5188.

Aguilar R., Cristóbal-Perez E.J., Balvino-Olvera F.J., Aguilar-Aguilar M.J.., Aguirre-Acosta N., Ashworth L, Lobo JA, Martén-Rodríguez S, Fuchs EJ, Sanchez-Montoya G, Bernardello G, Quesada M. 2019. Habitat fragmentation reduces plant progeny quality: a global synthesis. Ecology Letters 22:1163–1173.

Aguirre-Dugua X, González-Rodríguez A. 2016. Phylogeographical Approaches to the Study of Plant Domestication, with Special Emphasis on Perennial Plants. In: Lira R., Casas A., Blancas J. (eds) Ethnobotany of Mexico. Ethnobiology. Springer, New York, NY.

Balvino-Olvera F.J., Sánchez-Gómez K. F., Lobo J. A., Avila-Sakar G., Cruz-Reyes R., Sánchez-Montoya G., Herrerías-Diego Y., González-Rodríguez A., Quesada M. 2017. Latitudinal structured populations of the Mexican wild squash Cucurbita argyrosperma subsp. sororia revealed by microsatellite markers. Crop and Pasture Science. 68: 850-858.

Barghchi M, Alderson PG. 1989. Pistachio (Pistacia vera L.). In: Bajaj Y.P.S. (eds) Trees II. Biotechnology in Agriculture and Forestry, vol 5. Springer, Berlin, Heidelberg.

Bawa K. S., Opler P. A., Rica, D. C., Rica C. 1975. Dioecism in Tropical Forest Trees. Evolution. 29: 167-179.

Bawa, K. S., Perry, D. R. & Beach, J. H. 1985. Reproductive Biology of Tropical Lowland Rain Forest Trees. I. Sexual Systems and Incompatibility Mechanisms. American Journal of Botany. 72: 331.

Breed MF, Ottewell KM, Gardner MG, Marklund MHK, Dormontt EE, Lowe AJ. 2015. Mating patterns and pollinator mobility are critical traits in forest fragmentation genetics. Heredity 115:108–114.

Bullock SH. 1992. Seasonal Differences in Nonstructural Carbohydrates in Two Dioecious Monsoon-Climate Trees. Biotropica 24:140.

Casas A, Ladio AH, Clement C. 2019. Editorial: Ecology and Evolution of Plants Under Domestication in the Neotropics. Frontiers in Ecology and Evolution: 7: 231.

Cascante A, Quesada M, Lobo JJ, Fuchs EA. 2002. Effects of Dry Tropical Forest Fragmentation on the Reproductive Success and Genetic Structure of the Tree Samanea saman. Conservation Biology 16:137–147.

Chumacero de Schawe C, Durka W, Tscharntke T, Hensen I, Kessler M. 2013. Gene flow and genetic diversity in cultivated and wild cacao (Theobroma cacao) in Bolivia. American Journal of Botany. 100: 2271-2279.

Connell J. H. 1971. On the role of natural enemies in preventing competitive exclusion in some marine animals and in rain forest trees in Dynamics of Populations. eds. Den Boer, P. J. & Gradwell, G. R. Centre for Agricultural Publishing and Documentation. 298–312.

Comita, L. S. et al. 2014. Testing predictions of the Janzen–Connell hypothesis: a meta-analysis of experimental evidence for distance- and density-dependent seed and seedling survival. Journal of Ecology. 102: 845–856.

Cristóbal-Pérez EJ, Fuchs EJ, Harvey N, Quesada M. 2019. Isolation and characterization of microsatellites loci in Spondias purpurea (Anacardiaceae) and cross amplification in congeneric species. Molecular Biology Reports. 46: 5581–5585.

Cristóbal-Pérez EJ, Fuchs EJ, Olivares-Pinto U, Quesada M. 2020. Janzen-Connell effects shape gene flow patterns and realized fitness in the tropical dioecious tree Spondias purpurea (ANACARDIACEAE). Scientific Reports. 10: 4584.

Elias M, Mühlen GS, McKey D. Roa AC, Tohme J. 2004. Genetic diversity of traditional South American Landraces of Cassava (Manihot esculenta Crantz): an analysis using microsatellites. Economic Botany. 58:242–256.

Ellstrand NC, Prentice HC, Hancock JF. 1999. Gene Flow and Introgression from Domesticated Plants into their Wild Relatives. Annual Review of Ecology and Systematics. 30(1): 539-563.

Fahrig L. 2003. Effects of Habitat Fragmentation on Biodiversity. Annual Review of Ecology, Evolution, and Systematics 34:487–515.

Fischer J., Lindenmayer D.B. 2007. Landscape modification and habitat fragmentation: a synthesis. Global Ecology and Biogeography 16:265–280.

Fuchs EJ, Lobo JA, Quesada M. 2003. Effects of Forest Fragmentation and Flowering Phenology on the Reproductive Success and Mating Patterns of the Tropical Dry Forest Tree Pachira quinata. Conservation Biology 17:149–157.

Hardesty B. D., Dick C. W., Kremer A., Hubbell S., Bermingham E. 2005. Spatial genetic structure of Simarouba amara Aubl. (Simaroubaceae), a dioecious, animal-dispersed Neotropical tree, on Barro Colorado Island, Panama. Heredity. 95: 290–297.

Hardesty B. D., Hubbell, S. P., Bermingham E. 2006. Genetic evidence of frequent long-distance recruitment in a vertebrate-dispersed tree. Ecology Letters. 9: 516–525.

Hamrick J. L., Godt M. J., W.Sherman-Broyles S. L. 1992. Factors influencing levels of genetic diversity in woody plant species. New Forest. 6: 95–124.

Hamrick J. L. 2002. Response of forest trees to global environmental changes. Forest Ecology and Management. 197: 323–335.

Ibarra-Manriquez G. & Oyama K. 1992. Ecological Correlates of Reproductive Traits of Mexican Rain Forest Trees. American Journal of Botany. 79: 383.

Janzen D. H. 1970. Herbivores and the Number of Tree Species in Tropical Forests. American Naturalist. 104: 501–528.

Kislev M, Hartmann A, Bar-Yosef O. 2006. Early Domesticated Fig in the Jordan Valley. Science 312 (5778): 1372-1374

Karron JD, Mitchell RJ, Holmquist KG, Bell JM, Funk B. 2004. The influence of floral display size on selfing rates in Mimulus ringens. Heredity 92:242–248. DOI: 10.1093/aob/mcr193

Karron JD, Mitchell RJ. 2012. Effects of floral display size on male and female reproductive success in Mimulus ringens. Annals of Botany 109:563–570. DOI: 10.1093/aob/mcr193

Mandujano S, Gallina S, Bullock SH. 1994. Frugivory and dispersal of Spondias purpurea (Anacardiaceae) in a tropical deciduous forest in México. Revista de Biología Tropical 42:107–114.

McKey D, Elias M, Pujol B, Duputié A. 2010. The evolutionary ecology of clonally propagated domesticated plants. New Phytologist. 186: 318-332.

Miller A, Schaal B. 2005. Domestication of a Mesoamerican cultivated fruit tree, Spondias purpurea. Proceedings of the National Academy of Sciences 102:12801-12806.

Miller AJ, Knouft JH. 2006. GIS-based characterization of the geographic distributions of wild and cultivated populations of the Mesoamerican fruit tree Spondias purpurea (Anacardiaceae). American Journal of Botany 93: 1757-1767.

Miller AJ, Gross BL. 2011. From forest to field: perennial fruit crop domestication. Am J Bot. 98(9):1389-1414.

Mitchell JD, Daly DC. 2015. A revision of Spondias L. (Anacardiaceae) in the Neotropics. PhytoKeys:1–92.

Moore PH. 2014. Phenotypic and Genetic Diversity of Papaya. In: Ming R., Moore P. (eds) Genetics and Genomics of Papaya. Plant Genetics and Genomics: Crops and Models, vol 10. Springer, New York, NY.

O'Connor K, Powell M, Nock C, Shapcott A. 2015. Crop to wild gene flow and genetic diversity in a vulnerable Macadamia (Proteaceae) species in New South Wales, Australia. Biological Conservation. 191: 504-511.

Ouborg NJ, Vergeer P, Mix C. 2006. The rough edges of the conservation genetics paradigm for plants. Journal of Ecology 94:1233–1248.

Quesada M, Fuchs EJ, Lobo JA. 2001. Pollen load size, reproductive success, and progeny kinship of naturally pollinated flowers of the tropical dry forest tree Pachira quinata (Bombacaceae). American Journal of Botany 88:2113–2118.

Quesada M, Stoner KE, Lobo JA, Herrerias-Diego Y, Palacios-Guevara C, Munguía-Rosas MA, O.-Salazar KA, Rosas-Guerrero V. 2004. Effects of Forest Fragmentation on Pollinator Activity and Consequences for Plant Reproductive Success and Mating Patterns in Bat-pollinated Bombacaceous Trees. Biotropica 36:131–138.

Quesada M, Stoner KE, Rosas-Guerrero V, Palacios-Guevara C, Lobo JA. 2003. Effects of habitat disruption on the activity of nectarivorous bats (Chiroptera: Phyllostomidae) in a dry tropical forest: implications for the reproductive success of the neotropical tree Ceiba grandiflora. Oecologia 135:400–406.

Renner S. S. & Ricklefs R. E. 1995. Dioecy and its correlates in the flowering plants. American Journal of Botany. 82: 596–606

Riba-Hernández P., Segura J. L., Muñoz-Valverde J. 2016. Female fruit production depends on female flower production and crown size rather than male density in a continuous population of a tropical dioecious tree (Virola surinamensis). American Journal of Botany. 103: 1990–1999.

Sih A, Baltus M-S. 1987. Patch Size, Pollinator Behavior, and Pollinator Limitation in Catnip. Ecology 68:1679–1690.

Sork VL, Smouse PE. 2006. Genetic analysis of landscape connectivity in tree populations. Landscape Ecology 21:821–836.

Van Tassel DL, DeHaan LR, Cox TS. 2010. Missing domesticated plant forms: can artificial selection fill the gap?. Evolutionary Applications 3: 434-452.

Vekemans X. & Hardy O. J. 2004. New insights from fine-scale spatial genetic structure analyses in plant populations. Molecular Ecology. 13: 921–935.

Zhou, H. P. & Chen, J. 2010. Spatial genetic structure in an understorey dioecious fig species: the roles of seed rain, seed and pollen-mediated gene flow, and local selection. Journal of Ecology. 98: 1168–1177.

Zohary D, Spiegel-Roy P. 1975. Beginnings of fruit growing in the old world. Science. 187(4174):319-327.

Zohary D. 2004. Unconscious Selection and the Evolution of Domesticated Plants. Economic Botany. 58(1): 5-10.