



Contrasting patterns of morphology, fluctuating asymmetry and leaf herbivory in three plant species of different successional stages of a tropical dry forest

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Abstract

Key message This paper shows the intraspecific plant responses in leaf morphology, herbivory and FA between mature and successional tropical dry forests, as well as the difficulty associated with understanding the relationship between FA and herbivory

Abstract Understanding the processes that affect biotic interactions during secondary succession has implications for the maintenance of species diversity in the tropics. We studied the changes in leaf morphology, fluctuating asymmetry (FA) and herbivory in three dominant tropical dry forest plant species that occur in mature and secondary forests. We selected eight study sites: four in mature and four in successional forests. At each site, a plot of 20 × 50 m was established to characterize the vegetation structure and soil fertility. Subsequently, leaf morphology, FA and herbivory were measured in all individuals of *Cordia elaeagnoides*, *C. alliodora* and *Achatocarpus gracilis* with DBH ≥ 2.5 cm found in the plots. Plant abundance and plant height decreased in secondary forest, while the content of nitrates and phosphates increased in soil of secondary forests. Differences in leaf morphology between forest conditions were found for the three species. Total leaf area was higher in mature than in secondary forests for *C. elaeagnoides* and *A. gracilis*. An opposite pattern was found for *C. alliodora*. In both *Cordia* species, herbivory was higher in secondary than in mature forests. The opposite pattern was found for *A. gracilis* in secondary forests. For all the cases, FA was higher in mature forests than in secondary forests. Herbivory was positively correlated with FA in secondary forests in *C. elaeagnoides* and in *A. gracilis*, whereas in *C. alliodora* herbivory was positively related with FA in mature forests. Overall, we detected changes in foliar morphology, fluctuating asymmetry and herbivory between mature and secondary forests, with a general pattern of higher FA levels in mature forests. Our findings illustrate the difficulty associated with understanding the relationship between FA and herbivory throughout the regeneration process in tropical dry forests due to the complexity of abiotic and biotic factors that can affect plant–herbivore interactions.

Keywords Environmental stress · Secondary succession · Tropical dry forests · Fluctuating asymmetry · Herbivory

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Introduction

The most threatened tropical ecosystems are the tropical dry forests (TDFs), which represent 42% of the tropical forests of the world (Trejo and Dirzo 2000). During the last decades, anthropogenic activities such as deforestation, tourism, urbanization and the conversion of mature forests to agricultural fields and livestock soils have negatively affected the TDF, generating a mosaic of fragments with natural vegetation and patches of abandoned lands under different stages of natural regeneration. Therefore, it is common to observe a high occurrence of secondary forests under different successional stages (Chazdon and Guariguata 2016).

The secondary succession process represents a feedback phenomenon between abiotic factors and vegetation characteristics. In fact, abiotic factors such as precipitation, relative humidity, temperature and soil fertility change during the successional process and can influence plant performance, population structure, biotic interactions, food webs and, therefore, the community structure (Loik and Holl 2001; Lebrija-Trejos et al. 2011). In turn, vegetation structure aspects such as plant species dominance, diversity, density and life forms may affect the abiotic factors, which in turn influence the forest regeneration process (Madeira et al. 2009; Lebrija-Trejos et al. 2010). In this way, some studies have shown that secondary forests of TDFs are characterized by a higher incidence of solar radiation and temperature and lower relative humidity and water availability in comparison with mature forests (Lohbeck et al. 2015; Pineda-García et al. 2013). Because secondary forests are hotter and drier environments that can represent situations of environmental stress for plants, it is possible to expect changes or adjustments in physiological and morphological attributes according to the ability of plant species to acclimate to such stress (Alvarez-Añorve et al. 2012; Schöb et al. 2013).

Fluctuating asymmetry (FA) describes the random differences in size or shape between two sides of a bilateral character in organisms and reflects the developmental instability caused by stressors such as pollution, extremes of temperature, solar radiation and humidity, habitat disturbance and global climate change (Cuevas-Reyes et al. 2013, 2018a; Tucić et al. 2018). Consequently, it has been considered a reliable indicator of environmental stress in different organisms such as birds (Cuervo and Restrepo 2007), mammals (Marchand et al. 2003) and amphibians (Niemeier et al. 2019). Particularly, in plants, high levels of FA have been correlated with the reduction of survival and reproduction (Díaz et al. 2004), increased urbanization (Cuevas-Reyes et al. 2013), extreme climatic conditions (Valkama and Kozlov 2001) and excesses or deficits

of solar radiation, temperature and soil nutrients (Hagen et al. 2008; Cornelissen and Stiling 2011). In addition, herbivory can also be a plant stressor (Cuevas-Reyes et al. 2018b). In some cases, FA levels are positively related with herbivory, indicating more susceptibility to herbivores (plant stress hypothesis) (Cornelissen and Stiling 2005; Cuevas-Reyes et al. 2011a). However, other studies have not found this relationship (Bañuelos et al. 2004). Differences in nutritional quality and chemical defenses of plants have been suggested to explain these contrasting results (Cornelissen and Stiling 2011). On the other hand, plants can be directly stressed by herbivorous insects producing changes in leaf symmetry and increasing FA levels (herbivory-induced stress hypothesis) (Alves-Silva and Del-Claro 2016). In this way, FA in plants can increase as a result of herbivory intensity (Cuevas-Reyes et al. 2011b).

The levels of herbivory can vary between plant species and individuals (del-Val and Armesto 2010) as a result of differences in the intrinsic characteristics (e.g., genetic composition, type of growth, longevity, and leaf functional traits) (Coley 1982), temporal and spatial variation in richness and abundance of herbivores and changes in local environmental conditions, which in turn can influence the nutritional quality and chemical defenses of plants (Silva et al. 2012; Sousa-Souto et al. 2014; González-Esquivel et al. 2019). According to this idea, it is possible to expect a decrease in resource availability for plants (e.g., soil nitrogen and phosphorous) during the forest succession process, where plants that occur in early successional stages invest more resources in vegetative growth and replacement of leaf tissues lost by herbivory than in chemical defense as a result of higher photosynthetic rates and faster vegetative growth (Coley et al. 1985; González-Esquivel et al. 2019). Conversely, plants of mature forests allocate more resources for carbon-based chemical defenses because producing new leaves results in being more expensive (Silva et al. 2012).

Considering these ideas, it is possible to test the resource availability hypothesis in plants of the same species that occur in contrasting successional habitats because they might exhibit differences in their contents of chemical defenses, nutritional quality and herbivory rates (Silva et al. 2012; González-Esquivel et al. 2019). Therefore, we hypothesized that plants immersed in secondary forests will have higher levels of herbivory because they are rich in nutrients with fewer compounds associated with defense in comparison with plants of mature forests (Silva et al. 2012; González-Esquivel et al. 2019).

Finally, the relationship between FA and herbivory suggests that FA may also serve as an indicator of plant susceptibility to herbivory, where more asymmetric leaves have higher nutritional quality than symmetric leaves, making them more susceptible to herbivory and implying that leaf morphology and FA can also be used as an indicator of plant

quality (Lempa et al. 2000; Cornelissen et al. 2003, Cornelissen and Stiling 2005). However, herbivory itself can also act as a stressor and directly contribute to increase the level of FA (Zvereva et al. 1997; Møller and Shykoff 1999; Maldonado-López et al. 2019). Consequently, environmental factors such as temperature, humidity and incidence of light can affect both leaf morphology and plant quality, which in turn, might influence the herbivory levels (Cunningham et al. 1999; Givnish 1987; Cuevas-Reyes et al. 2011a, b). Thus, based on these ideas and considering that secondary forests are hotter and drier habitats, we hypothesized that secondary forests present stressful conditions to plants that will be expressed in changes in leaf morphology, higher levels of foliar FA, and a higher preference of herbivores for asymmetric leaves that could be reflected in higher levels of herbivory.

In the present study, we tested this hypothesis using geometric morphometric techniques to analyze leaf size and shape, fluctuating asymmetry and leaf herbivory in three dominant plant species in mature and secondary forests of a tropical dry forest in Chamela-Cuixmala, Mexico. Specifically, we addressed the following questions: (1) do leaf morphology and size of *C. elaeagnoides*, *C. alliodora* and *A. gracilis* change in function of successional TDFs stages (i.e., mature and successional TDFs); (2) do levels of FA differ between the two forest conditions and between plant species? (3) Are herbivory levels different between mature and secondary forests in the three plant species? (4) Is FA related with total leaf area and levels of herbivory in both forest conditions?

Materials and methods

Study area

This study was conducted at the Chamela-Cuixmala Biosphere Reserve on the Pacific coast of Jalisco, Mexico (19° 22'–19° 35' N, 104° 56'–105° 03' W) that covers an extension of 16,000 ha, and in outside areas of abandoned agricultural fields, hereafter called secondary forests (García-Oliva et al. 2002; Sánchez-Azofeifa et al. 2009).

We selected eight study sites using Google Earth high-resolution imagery (<http://earth.google.com>) and classified satellite images (Avila-Cabadilla et al. 2012); four sites of mature forests localized within the Chamela-Cuixmala Biosphere Reserve and four secondary forests outside the Biosphere reserve. Mature forests have remained intact in the last 60 years, while secondary forests were cattle pastures and have between 3 and 8 years of abandonment (Avila-Cabadilla et al. 2012). In general, the land use history of the secondary forests in the study sites was the following: (1) most of the original vegetation was removed through

slash and burn; (2) lands were used for agriculture between 2 and 5 years and then converted to cattle ranching by burning the vegetation periodically; and (3) secondary succession occurred because of the land abandonment by farmers (Jimenez-Rodríguez et al. 2018).

Because topographic variables can affect the composition of the plant community, our study sites were located at an average elevation of 143 m with slopes averaging 10° (range 15°), mainly oriented to the south and southeast (Balvanera et al. 2002). We characterized each forest condition by measuring soil properties and vegetation structure (Jimenez-Rodríguez et al. 2018).

Vegetation measurements

To characterize the vegetation structure of mature and secondary forests, in each study site, all woody plants with diameter at breast height (DBH) ≥ 2.5 cm were marked and identified at the species level and then the following parameters were measured: (1) number of primary and secondary branches up to 1.30 m, (2) DBH, (3) plant height, (4) total number of individuals, (5) plant species richness and (6) basal area (Jimenez-Rodríguez et al. 2018).

Soil properties

Fifteen soil samples (0–10 cm depth) were randomly collected from each study site using a bucket auger and mixed into a single soil sample. This sample was divided into three subsamples that were used to obtain the following measurements: (1) total content of C, N and P, (2) soil humidity, pH, inorganic N and P, and (3) enzymatic activity of three enzymes, phosphatase, beta-glucosidase and *N*-acetylglucosaminidase. Before soil collection, all the aboveground materials were carefully removed. All soil samples were stored in sealed plastic bags and transported to the laboratory in a cooler (Jimenez-Rodríguez et al. 2018).

For soil moisture determination (H), a subsample (100 g) was oven-dried at 75 °C to constant weight, using the gravimetric method to adjust for water content when expressing nutrient concentration on the basis of dry soil mass. Carbon forms analyzed in all samples were determined in a total carbon analyzer (UIC model CM5012, Chicago, USA), while the N and P forms analyzed were determined colorimetrically in a Bran-Luebbe AutoAnalyzer 3 (Norderstedt, Germany). Prior to the total soil nutrient analyses, soil samples were dried and ground with a pestle and mortar. Total carbon (Ct) was determined by combustion and colorimetric detection (Huffman 1977). Total organic carbon (Cti) was calculated as the difference between TC and inorganic carbon. For total N (Nt) and total P (Pt) determination, samples were acid digested at 360 °C. Soil N (N) was determined by the macro-Kjeldahl method (Bremner 1996), while soil P

(P) was determined by the molybdate colorimetric method following ascorbic acid reduction (Murphy and Riley 1962).

Available, dissolved and microbial nutrient forms were extracted from field moist soil samples. Available inorganic N (NH_4^+ and NO_3^-) was extracted from 10 g of fresh soil subsamples with 2 M KCl, followed by filtration through a Whatman No. 1 paper filter and determined colorimetrically by the phenol-hypochlorite method. The PO_4 content was determined by extraction with 0.5 M NaHCO_3 at pH 8.5 according to Hedley sequential P fractionation (Tiessen and Moir 1993). Enzyme activity was analyzed colorimetrically and expressed as micromoles of product formed per gram dry weight of soil per hour.

Study species

Cordia elaeagnoides A. DC. It is a deciduous tree that can grow up to 20 m, distributed exclusively on the Pacific coast of Mexico, including the states of Sinaloa, Jalisco and Oaxaca. It has been shown that herbivores such as *Coptocycla leprosa* (Chrysomelidae) are associated with *C. elaeagnoides* (Trager and Bruna 2006).

Cordia alliodora (Ruíz and Pav.) Oken. It is a deciduous tree growing up to 25 m. In Mexico, it is distributed along the Gulf of Mexico and Pacific coast from Sinaloa to Chiapas (Pennington and Sarukhán 2005). Herbivorous insects attacking *C. alliodora* belong to Saturniidae, Chrysomelidae and Tingidae (Trager and Bruna 2006).

Achatocarpus gracilis H. Walter. It is a deciduous shrub typical of mature forests with a low frequency in secondary forests (Boege et al. 2019). To our knowledge, the herbivore community associated with *A. gracilis* has not been documented, but during the study, we observed at least four species of Lepidoptera of different families responsible for most of the apparent herbivory.

Sampling design

Sampling was conducted at the end of the rainy season, after the peak of herbivore activity and the leaves were completely expanded. In each study site, a transect of 20×50 m (0.1 ha) was established. On each transect, all individuals of the three species with diameter at breast height (DBH) \geq to 2.5 cm were selected, and then marked and georeferenced to avoid sampling the same individual twice (*C. elaeagnoides*: $N = 67$, 33 individuals of mature and 34 of secondary forests; *C. alliodora*: $N = 68$, 34 in mature and 34 for secondary forests; and *A. gracilis*: $N = 106$, 55 in mature and 51 in secondary forest). Plants sampled from mature forest ranged in DBH as follows: *C. elaeagnoides* varied from 7.8 to 10.2 cm, *C. alliodora* from 8.5 to 11.4 cm and *A. gracilis* from 3.8 to 6.4 cm, while in secondary forests the DBH ranged in *C. elaeagnoides* from 7.6 to 11.6 cm,

C. alliodora from 8.1 to 12.2 cm and *A. gracilis* from 3.7 to 6.9 cm. For each individual, three branches were randomly chosen of each tree canopy strata (i.e., high, middle and low) (Cuevas-Reyes et al. 2011b), and then we selected 50 leaves in total: 25 undamaged leaves to determine the patterns of leaf morphology and fluctuating asymmetry using geometric morphometric techniques and 25 randomly selected leaves to evaluate herbivory levels using ImageJ software (Cuevas-Reyes et al. 2018a).

Morphometric analysis of leaves

To evaluate the differences in leaf morphology and size of the three plant species between forest conditions (mature forest vs secondary forest), a digital image was obtained of each undamaged leaf (25 leaves in total per individual) and then 32 anatomical marks (i.e., landmarks) were placed on each image. These landmarks represent the leaf shape and correspond to homologous loci, which are unambiguous and repeatable in all the leaves (Cuevas-Reyes et al. 2018a). For each leaf image, the coordinates (x and y) of the 32 landmarks were recorded using a TpsDig program (Rohlf 2015). We performed a Procrustes superimposition analysis using the Integrated Morphometric Package (IMP series: <http://www.canisius.edu/~sheets/morphsoft.html>) to configure the landmark coordinates. After that, the average configuration of all leaves was considered as reference to eliminate the leaf size effect (Bookstein 1991). The shape variables (Procrustes distances) were calculated by a superimposition coordinates analysis (Cuevas-Reyes et al. 2011b). Finally, for each plant species separately, a principal components analysis was applied to determine the differences in leaf morphology between mature and secondary forests (Cuevas-Reyes et al. 2018a).

Fluctuating asymmetry measurements

Fluctuating asymmetry was measured in 25 fully expanded and intact mature leaves of each tree sampled in the two forest conditions. For this, we obtained a digital image of each leaf and measured the distance from the right side (RW) and the left side (LW) from the leaf border to the midrib at the midpoint of the leaf corresponding to its widest part, using the program ImageJ 1.51j87 (<https://imagej.nih.gov/ij/>) (Cuevas-Reyes et al. 2018b). FA was calculated as the absolute value of the difference between the distances from the midrib to the left and right borders of the leaf ($|A_i - B_i|$), divided by the average distance $(A_i + B_i/2)$, to correct for the fact that asymmetry may be size dependent (Cornelissen and Stiling 2005). A value of FA was obtained for each tree from the average of the 25 leaves measured. Besides, a subsample of 30 leaves was measured again to control the measurement error in FA, and then we calculated the

significance of FA relative to measurement error using a two-way mixed-model ANOVA (Palmer and Strobeck 2003; Cuevas-Reyes et al. 2018b). The significance of the interaction (individual \times leaf \times side) showed that the variation in FA between sides was greater than expected by measurement error ($F = 102.4 = 26$; $P < 0.0001$).

Because there are three types of asymmetry (i.e., fluctuating asymmetry, directional asymmetry and antisymmetry) (Palmer and Strobeck 2003), it is important to discriminate between them (Cuevas-Reyes et al. 2018b). FA calculates the variance of random differences between the two sides of a bilateral trait distributed with a mean value of zero. Directional asymmetry is defined as the differences between the two sides that are distributed about a mean that is significantly greater or less than zero. Finally, antisymmetry is the absence of symmetry and is characterized by presenting a bimodal or platykurtic distribution of the differences between two sides about a mean of zero (Palmer and Strobeck 1992). Therefore, FA determines small random variation from the optimal development of a trait with bilateral symmetry, whereas directional asymmetry and antisymmetry are unsuitable descriptors of developmental instability, since they are developmentally controlled and are probably adaptive (Palmer and Strobeck 1986).

To test if our data presented only FA and no other type of symmetry, a Student's t test was performed to discard directional asymmetry, considering if the average value of the differences of the right side minus the left (L–R) differed from zero. Additionally, to discard antisymmetry, a Lilliefors' normality test was used, considering the distribution of the differences of L–R (Alves-Silva and Del-Claro 2016). Since we did not find a significant deviation from zero ($t = 1.6$; $P > 0.05$) in the average value of the L–R differences, and the distribution of L–R differences showed a normal distribution ($P > 0.05$), the values were considered a reliable measure of FA.

Measurement of herbivory

The herbivory by insects was assessed in 25 leaves of each tree sampled in the two forest conditions, from digital images that were taken for each leaf previously. To calculate the percentage of herbivory, we first estimated the total leaf area and leaf area removed by herbivores using the program ImageJ 1.51j87 (<https://imagej.nih.gov/ij/>). Then, herbivory data were transformed as square root arcsine and then the normal distribution was confirmed in all cases (Cuevas-Reyes et al. 2018b).

Statistics analyses

We performed a one-way permutation test based on Monte Carlo resampling separately to determine the differences

between mature and secondary forests in vegetation structure (species richness, plant abundance, basal area, plant height) and soil properties (humidity, pH, C, N, P, C:N, C:P, N:P, NH_4 , NO_3 , PO_4).

To determine the effects of forest condition (mature forest vs secondary forest) on total leaf area of each plant species, we conducted a one-way ANOVA test for each plant species separately, where forest condition was considered as the explanatory variable and the total leaf area as the response variable (SAS 2000). We conducted a Student's t test to compare the mean of foliar FA levels between mature and secondary forests for each plant species, respectively. We used a two-way ANOVA test to determine the effects of forest condition and plant species on herbivory. The model considered forest condition and plant species as explanatory variables, and herbivory was used as response variable. A posteriori tests of LSMeans were performed to compare means when ANOVA tests presented significant differences ($P < 0.05$) (SAS 2000). Finally, to assess the relationship between total leaf area, FA levels and herbivory, a Spearman's rank correlation was used within each forest condition for each plant species separately. Because these tests involved multiple comparisons, a Bonferroni correction was applied to adjust P values.

Results

We found that plant abundance and plant height were higher in mature than in secondary forests. We did not find differences in the other vegetation measurements analyzed (Table 1). In the same way, only nitrates and phosphate content of the soil was different between forest conditions (Table 1).

Based on coordinate superimposition analysis, we found differences between mature forests and secondary forests in the leaf shape of *C. elaeagnoides*. Leaves of mature forests were more elongated and thinner than leaves of secondary forests (Fig. 1a). This result is supported by the principal components analysis, where the PC1 and PC2 accounted for 57.61 and 14.37% of the total variation, respectively, and separated two different groups (Fig. 2a). Similarly, in *C. alliodora*, leaves of mature forests were more elongated and wider than leaves of secondary forests (Fig. 1b). This result was confirmed by the principal components analysis, where the PC1 accounted for 49.76% and PC2 explained the 17.57% of variation, separating two distinct groups of leaves (Fig. 2b). Finally, in the case of *A. gracilis*, leaves of mature forests were wider at the base in comparison with leaves of secondary forests (Fig. 1c). The principal components analysis separated leaves into two groups: leaves from mature forest and leaves of secondary forest, where PC1 explained 62.76% and PC2 13.13% of variation (Fig. 2c).

Table 1 Comparison of vegetation attributes and soil properties between mature and secondary forests

Attributes	Mature forests	Secondary forests	Z	P <
Vegetation				
Species richness	83 ± 12	62 ± 42	0.94	n.s.
Plant abundance	477 ± 83	304 ± 28	1.8	0.04
Basal area	19,641.2 ± 4687	11,003.5 ± 2056.1	2.9	0.04
Plant height	3.8 ± 0.3	3.6 ± 0.5	0.9	n.s.
Soils				
Humidity (%)	16.6 ± 8.8	19.1 ± 9.4	− 0.4	n.s.
pH	6.6 ± 0.5	6.8 ± 0.3	− 0.5	n.s.
Carbon (mg/g)	18.2 ± 6.3	20.3 ± 6.9	− 0.5	n.s.
Nitrogen (mg/g)	1.2 ± 0.6	1.7 ± 0.5	− 0.9	n.s.
Phosphorous (mg/g)	0.27 ± 0.26	0.34 ± 0.21	− 0.4	n.s.
C:N ratio	15.5 ± 2.7	12.9 ± 2.9	1.2	n.s.
C:P ratio	106.2 ± 57.0	76.4 ± 44.4	0.9	n.s.
N:P ratio	6.7 ± 3.3	5.7 ± 1.9	0.6	n.s.
Ammonium (NH ₄) (μg/g)	15.9 ± 8.6	4.1 ± 5.6	1.9	n.s.
Nitrate (NO ₃) (μg/g)	8.0 ± 9.3	11.9 ± 5.6	1.5	0.04
Phosphate (PO ₄) (μg/g)	23.9 ± 8.5	53.4 ± 15.2	1.9	0.03

Comparisons were made using one-way permutation tests based on Monte Carlo resampling. The means and the SE are shown

Total leaf area of *C. elaeagnoides* differed significantly between forest conditions ($F = 7.3$; $d.f. = 1$; $P < 0.007$). In mature forests, the mean of total leaf area was $95.0 \text{ cm}^2 \pm 1.67 \text{ SE}$ and in secondary forests $88.1 \text{ cm}^2 \pm 1.9 \text{ SE}$ (Fig. 3a). We found significant differences in leaf size of *C. alliodora* between the two successional stages ($F = 51.8$; $d.f. = 1$; $P < 0.0001$). For this species, the leaf size was smaller in mature forests ($35.3 \text{ cm}^2 \pm 0.6 \text{ cm}^2 \text{ SE}$) than in secondary forests ($41.9 \text{ cm}^2 \pm 0.7 \text{ cm}^2 \text{ SE}$). For *A. gracilis*, the total leaf area was different between the forest conditions ($F = 98.3$; $d.f. = 1$; $P < 0.0001$), being higher in mature forest ($6.0 \text{ cm}^2 \pm 0.08 \text{ cm}^2 \text{ SE}$) than in secondary forests ($4.9 \text{ cm}^2 \pm 0.06 \text{ cm}^2 \text{ SE}$) (see Fig. 3a).

The herbivory differed significantly between forest conditions ($F = 7.4$; $d.f. = 1$; $P < 0.006$) and between plant species ($F = 12.1$; $d.f. = 2$; $P < 0.0001$). In both *Cordia* species, the herbivory was higher in secondary forests in comparison with mature forests. In contrast, herbivory was higher in mature than in secondary forests for *A. gracilis*. *Cordia alliodora* had higher herbivory levels than *A. gracilis* and *C. elaeagnoides* (Fig. 3b).

For all plant species analyzed, FA was higher in mature forests than in secondary forests: *C. elaeagnoides* ($t_{1402} = 24.5$; $P < 0.01$); *C. alliodora* ($t_{1669} = 30.8$; $P < 0.001$); *A. gracilis* ($t_{2585} = 18.2$; $P < 0.02$) (Fig. 4). In the case of *C. elaeagnoides*, after a Bonferroni correction, Spearman's rank correlation revealed that herbivory was positively correlated with FA in secondary forests (Table 2a), whereas in *C. alliodora* herbivory was positively related with FA in mature forests (Table 2b). A positive relationship between

herbivory and FA in *A. gracilis* was found in secondary forest (Table 2c).

Discussion

The process of ecological succession not only implies sequential changes in species richness, composition and abundance, but also variations in abiotic factors (Lebrija-Trejos et al. 2010). For example, previous studies in TDF have found that the vegetation canopy openness varies from 5.3 to 18.7% in mature forest and from 5.9 to 32% for secondary forests, producing differences in solar radiation, temperature and availability of water between the two conditions, with the secondary forests being hotter and drier (Maza-Villalobos et al. 2011; Alvarez-Añorve et al. 2012). In our study, we found that abundance and basal area of plants were higher in mature than in secondary forests. These results suggest that secondary forests are hotter and drier as a result of differences in solar radiation and temperature associated with the vegetation structure (Jimenez-Rodríguez et al. 2018). Differences in these environmental conditions along forest succession impose physiological demands to plants, resulting in adjustment in some traits such as leaf morphology, symmetry and leaf size according to both the degree of tolerance of individuals to environmental stress and phenotypic plasticity (Cuevas-Reyes et al. 2011b; Alvarez-Añorve et al. 2012). This is in accord with our results, because all plant species studied presented differences in leaf morphology and size between mature and secondary forests. For example, *C.*

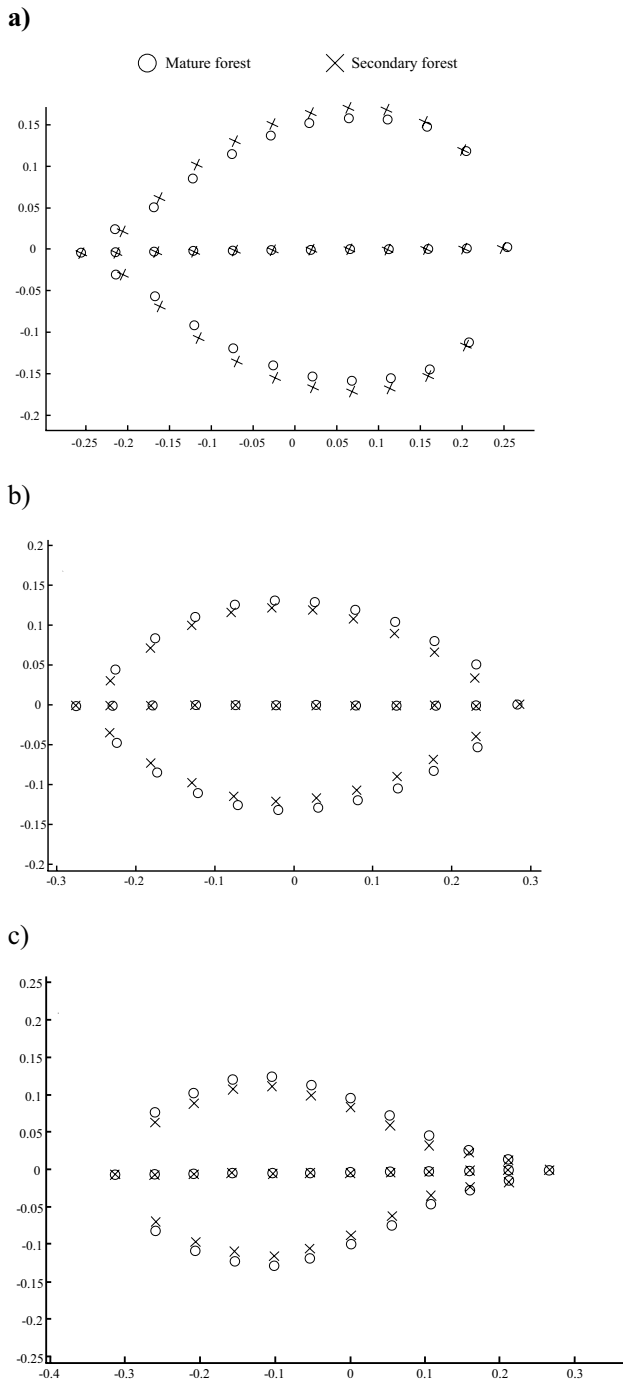


Fig. 1 Leaf morphological variation between mature (black circles) and secondary forests (black crosses) for each plant species: mean of coordinates of landmark configuration of leaves. *C. elaeagnoides* (a), *C. alliodora* (b) and *A. gracilis* (c)

elaegnoides and *A. gracilis* had smaller leaves in secondary than in mature forests, which can be the result of morphological and physiological adjustment to avoid water loss in hotter and drier sites such as secondary forests (Alvarez-Añorve et al. 2012; Pineda-García et al. 2013). In contrast,

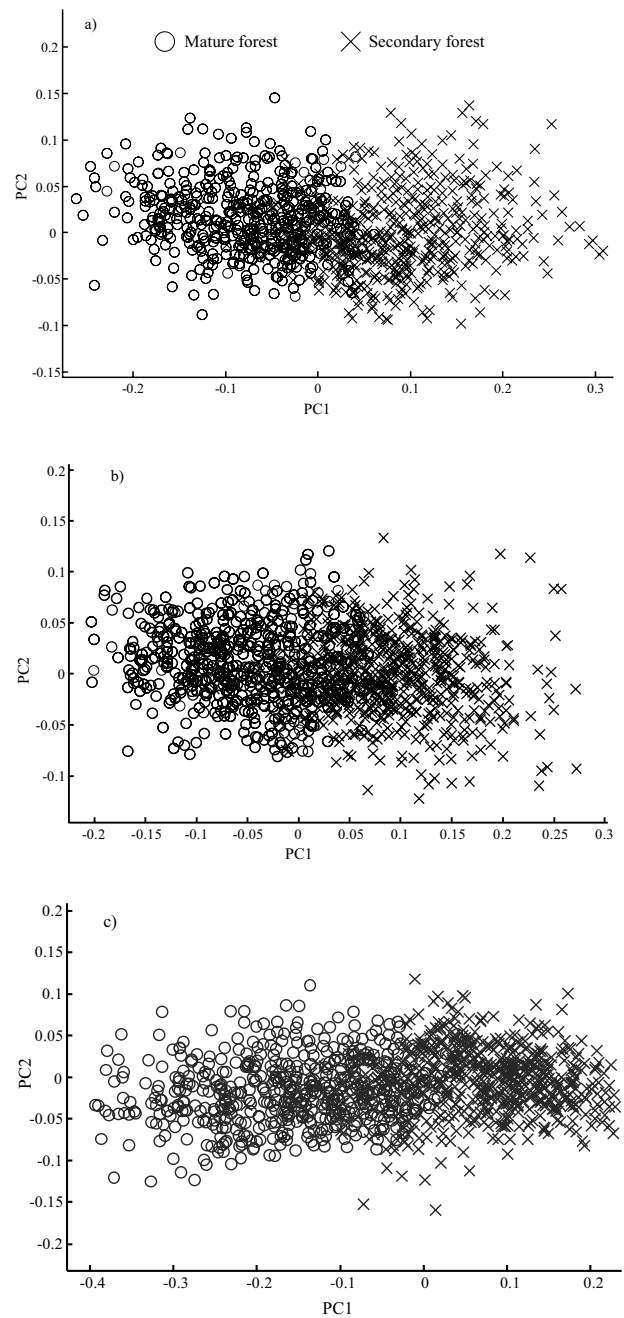


Fig. 2 Differences in leaf shape morphology between mature and secondary forests for each plant species according to canonical variate analysis. Black circles: mature forest. Black crosses: secondary forest. *Cordia elaeagnoides* (a), *Cordia alliodora* (b) and *A. gracilis* (c)

C. alliodora had larger leaves in secondary than in mature forest, supporting the hypothesis of resources availability (Coley et al. 1985), which predicts that fast-growing plant species such as *C. alliodora* develop larger leaves in sites with more availability of resources (e.g., light incidence, N and P) (Cole and Ewel 2006). Therefore, an increase of light

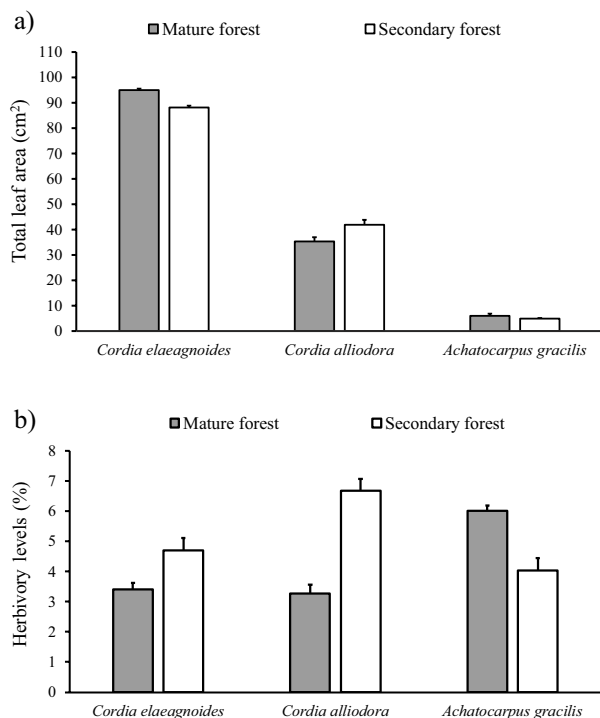


Fig. 3 Comparison of total leaf area between mature and intermediate successional forests for each plant species (a), differences in herbivory by insects between mature and secondary forests and between plant species (b)

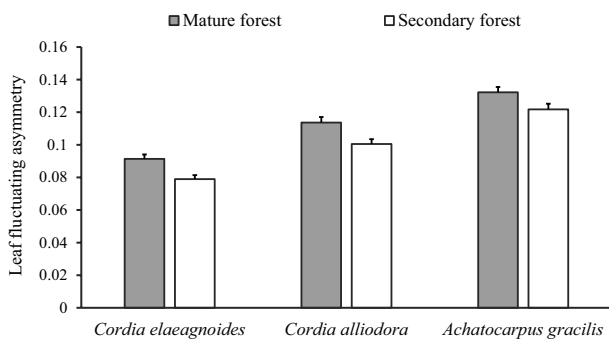


Fig. 4 Differences in leaf fluctuating asymmetry between mature and secondary forests for each plant species

incidence in secondary forests can be associated with high photosynthetic rates to absorb nutrients quickly, grow fast and produce large leaves (Lohbeck et al. 2015).

Fluctuating asymmetry is one of the main indicators used to evaluate environmental stress in plants (Cuevas-Reyes et al. 2018a). However, contrary to our prediction, based on the idea that secondary forests are hotter and drier sites and therefore present stressful conditions to plants, our results showed that in the three plant species analyzed, the FA levels were highest in mature forest in comparison with

secondary forests. Thus, it is possible that the environmental conditions such as high radiation incidence and extremes of temperature and humidity of secondary forests do not act as stressor agents for the plant species studied. In fact, plant species of TDFs have optimal strategies for dealing with drought (e.g., a better water use efficiency) and are adapted to high temperatures (Pineda-García et al. 2013; Lohbeck et al. 2015). On the other hand, the vegetation of mature forest has higher density and larger sized trees that can promote greater dispute for light and soil nutrients. Thus, it is probable that resource limitations, especially light and soil nutrients, may be the stressor agents responsible for the highest FA observed in plant species of mature TDF (Alvarez-Añorve et al. 2012).

Moreover, another important result in this study is the fact that herbivory levels were higher in the mature forests, and in both forest conditions there was a significant relationship between herbivory and FA levels. A similar correlation has been shown in other ecological studies (Zvereva et al. 1997; Cornelissen et al. 2003), suggesting that FA also may serve as an indicator of plant susceptibility to herbivory (plant stress hypothesis) (Cornelissen and Stiling 2011), but also can indicate that herbivory itself acts as a plant stressor and directly increases the level of FA (herbivory-induced stress hypothesis) (Zvereva et al. 1997; Møller and Shykoff 1999).

Unfortunately, in our study, the positive correlation between foliar FA and herbivory is not enough to distinguish between both hypotheses, because the FA measurements were made after herbivory had occurred. Hence, for future studies, it is necessary to include analyses of nutritional quality and chemical defense in leaves undamaged by herbivory throughout the successional stages of tropical dry forest. Particularly, it is possible that individuals of *C. elaeagnoides* and *A. gracilis* experience more favorable developmental conditions in mature than in secondary forests considering that they had more elongated, wider and larger leaves in mature forests. As a result of these morphological differences, we hypothesize that in plants from the mature forests defense chemical compounds should be lower and foliar nutritional quality should be higher and, as a consequence, herbivorous insects respond with higher consumption rates in mature forest. This idea is supported by studies that have proposed that the variation in plant chemistry is a possible cause for these differences (Lempa et al. 2000; Bañuelos et al. 2004; Cornelissen et al. 2003; Cuevas-Reyes et al. 2011a, b, 2018a).

Herbivory is an antagonistic interaction that negatively affects the survival, growth and reproduction of plants (López-Carretero et al. 2014). However, plants have evolved physical, nutritional and chemical defenses to prevent the loss of plant tissue by herbivores (Coley et al. 1985). Previous studies have demonstrated that herbivory levels vary along successional gradients due to variations

Table 2 Spearman's correlation coefficients of total leaf area, herbivory and fluctuating asymmetry (** $P < 0.05$)

	Total leaf area	Herbivory	Fluctuating asymmetry	
(a) <i>Cordia elaeagnoides</i>				
Total leaf area (cm ²)	–	– 0.33	0.001	Mature forest
Herbivory (%)	– 0.01	–	– 0.03	
Fluctuating asymmetry	0.14	0.46**	–	
Secondary forest				
(b) <i>Cordia alliodora</i>				
Total leaf area (cm ²)	–	– 0.09	– 0.05	Mature forest
Herbivory (%)	– 0.17	–	0.36**	
Fluctuating asymmetry	– 0.08	0.10	–	
Secondary forest				
(c) <i>Achatocarpus gracilis</i>				
Total leaf area (cm ²)	–	– 0.22	– 0.03	Mature forest
Herbivory (%)	0.06	–	0.02	
Fluctuating asymmetry	0.04	0.35**	–	
Secondary forest				

C. elaeagnoides (a), *C. alliodora* (b) and *A. gracilis* (c) in mature and secondary forests

in herbivory abundance, chemical defenses of plants and high temperatures that interfere with the plant defensive system and physiological processes (Brown et al. 1987; Bach and Tabashnik 1990; Silva et al. 2012; González-Esquivel et al. 2019). For example, plant species of secondary forests tend to be more consumed as a result of higher abundance of herbivore insects and lower chemical defenses. In this way, the resource availability hypothesis proposes that plant chemical defense varies with the successional position of species (Coley et al. 1985). Because plant species of mature forests are characterized by slow growth in low light environments, they produce more chemical defenses because it is less costly to produce them than to replace lost tissues by herbivory. Therefore, these species are well defended against herbivores and other factors that cause plant damage. In contrast, plant species of secondary forests growing in habitats of high-light availability (e.g., gaps) invest more resources in replacing lost tissues by herbivory than in chemical defense. This idea is in agreement with our results, since both species of *Cordia* had higher herbivory levels in secondary than in mature forests. Contrary to this, *A. gracilis* had higher levels of herbivory in mature forest condition. This result could be due to differences in leaf nutritional quality between individuals of mature and secondary forests (bottom-up effects), which can be influenced by differences in soil fertility (Cuevas-Reyes et al. 2004). In our study, the content of nitrates and phosphates was higher in soils of secondary forests. Nitrates are often limited and represent an important structural component of proteins, nucleic acids and many secondary metabolites of plants, while an increase of availability of phosphates is associated

with a high nutritional quality of plants (Patterson et al. 2010). Therefore, to explain our results, we invoke the soil fertility hypothesis that proposes that low soil fertility, indicated by low contents of phosphorous, has been associated with higher herbivore incidence considering that plant species that grow under conditions of infertile soils tend to have lower rates of growth and accumulate higher concentrations of secondary metabolites such as oils, phenols, alkaloids and terpenoids affecting the herbivory levels (Fernandes and Price 1991; Fernandes et al. 1994; Cuevas-Reyes et al. 2004).

In conclusion, our study shows changes in morphological leaf traits along regeneration processes and their effects on herbivory patterns in plant species of tropical dry forests. We detected differences in foliar morphology, fluctuating asymmetry and herbivory between mature and secondary forests. The general pattern showed higher levels of FA in mature forests. Our findings illustrate the difficulty associated with understanding the relationship between FA and herbivory throughout the regeneration process of tropical dry forests due to the complexity of abiotic and biotic factors involved that affect plant–herbivore interaction.

Author contribution statement PCR, JAP and YML proposed the ideas and design of the study. LDAC and MAA provided information of the biotic and abiotic characteristics of the study sites. JAP contributed to the sampling and estimates of total leaf area, herbivory and fluctuating asymmetry. JAP, PCR and YML wrote the manuscript. JAP, MF, MLF and AGR performed the statistical analysis and helped discuss the manuscript. All authors participated in the review of the manuscript.

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Compliance with ethical standards

Conflict of interest The authors mentioned above declare that there is no conflict of interest associated with this manuscript. The work represents an original research carried out by the authors. All authors agree with the contents of the manuscript and its submission to the journal.

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