Research

Insect herbivory declines with forest fragmentation and covaries with plant regeneration mode: evidence from a Mexican tropical rain forest

Betsabé Ruiz-Guerra, Roger Guevara, Néstor A. Mariano and Rodolfo Dirzo

B. Ruiz-Guerra (bruiz@ecologia.unam.mx), Depto de Ecología Evolutiva, Inst. de Ecología, UNAM, AP 70-275, 04510 D.F., Mexico. – R. Guevara, Depto de Ecología Evolutiva. Inst. de Ecología AC. Km 2.5 carretera antigua a Coatepec 351, MX–91070 Xalapa, México. – N. A. Mariano, Depto de Ecología, Centro de Educación Ambiental e Investigación Sierra de Huautla, Univ. Autónoma de Morelos, Cuernavaca, MX–62210 Mor, Mexico. – R. Dirzo and present address for RG: Dept of Biology, Stanford Univ., 371 Serra Mall, Stanford, CA 94305, USA.

The consequences of tropical forest fragmentation on herbivory are poorly understood. The limited evidence suggests that forest fragmentation can have positive, negative or neutral effects on herbivory. Inconsistencies may be partly explained by plant interspecific variation and differential responses related to plant life history. In this study we examined the effects of forest fragmentation and plant regeneration mode (shade-tolerant and light-demanding species) on sapling herbivory using a large sample of the community (97 species, representing 25% of the woody flora of the study site), and a subset of species shared by forest fragments and continuous forest. For the latter, we also analyzed the effects of species identity on variation in herbivory. Also, for the shared species we used two techniques to measure herbivory: standing herbivory (i.e. instantaneous, actual damage) and cumulative herbivory (i.e. damage, accumulated over time, on initially intact leaves). Insect herbivory was the predominant type of damage in the two forest types, and standing herbivory at both the community and the shared species level was significantly higher in continuous forest than in fragments. Considering shared species, both standing and cumulative herbivory were significantly higher in light-demanding than in shade-tolerant species. Cumulative herbivory also showed a significant interaction between forest fragmentation and plant regeneration mode, whereby a significant decline in herbivory in fragments was driven by reduced herbivory in shade-tolerant species, whereas for light-demanding species herbivory did not change significantly, due to contrasting species-specific responses. We conclude that tropical forest fragmentation reduces insect herbivory, but this depends on plant regeneration mode and species identity. These changes could have effects on plant regeneration and diversity in forest fragments via long-term demographic consequences.

Although deforestation and habitat fragmentation are recognized as major threats for the maintenance of tropical biodiversity (Dirzo and Raven 2003), we are still far from understanding their ecological consequences, given that most fragmentation studies have focused largely on the loss or changes in abundance of species. It is only recently that studies are beginning to evaluate the consequences of fragmentation on ecological processes, such as species interactions (Terborgh et al. 2001, Tscharntke and Brandl 2004), in part motivated by early calls for attention to be paid to fragmentation effects on plant-insect interactions (Didham et al. 1996). While studies on the consequences of tropical fragmentation for mutualistic interactions are abundant (Ghazoul 2005), there is a dearth of studies on antagonistic interactions such as herbivory (Tscharntke and Brandl 2004). Herbivory influences not only the dynamics and structure of tropical ecosystems, but also individual plant performance and population-level processes (Coley and Barone 1996), thus it has the potential to influence forest regeneration and maintenance of plant diversity (Marquis 2005).

Sixteen studies have examined the consequences of habitat fragmentation on plant-herbivore interactions (excluding studies specifically addressed at examining edge effects), but no general conclusion can be drawn from such studies. Half of them show that herbivory increases in small fragments (positive effect) because of: 1) a decrease in the abundance of the herbivore's natural enemies (Rao et al. 2001, Terborgh et al. 2001, Lienert and Fisher 2003, Christie and Hochuli 2005, Elzinga et al. 2005), 2) an increase in phytophagous insect oviposition rates with consequent increases in larvae abundance (Elzinga et al. 2005), 3) an increase in the nutritional quality of plants due to soil fertilization resulting from fertilizer spill over from surrounding agricultural fields (Lienert et al. 2002, Christie and Hochuli 2005, del-Val et al. 2007), and 4) the predominance of light-demanding plant species (at edges and small fragments), which are more palatable to herbivores than shade-tolerant species (Wirth et al. 2008). In contrast, seven studies show the opposite trend: decreased herbivory in forest fragments as a consequence of: 1) a

decrease in the abundance of herbivores due to fragment isolation (Vásquez et al. 2007, Fáveri et al. 2008), 2) detrimental edge effects (e.g. increased temperature and decreased relative humidity) on insect herbivore survival and performance (Zenteno 2001, Ledergerber et al. 2002, Valladares et al. 2006), 3) a reduction in the abundance of palatable host plants (Groom 2001, Arnold and Asquith 2002), or 4) negative changes in plant palatability (Simo-netti et al. 2007). Finally, one study reports a null effect of forest fragmentation on herbivory (Benitez-Malvido et al. 1999).

The effects of forest fragmentation on herbivory may be masked by multiple sources of variability such as vegetation type (tropical rain forest, grasslands, temperate forest, etc.), levels of biological organization (community, individual species), life forms (trees, herbs, palms), species-specific responses, and different methods used to measure herbivory. We argue that in order to understand the consequences of forest fragmentation on herbivory, those sources of heterogeneity need to be taken into account. In particular, no study has examined the effects of fragmentation on herbivory considering variability across plant regeneration modes (light-demanding and shade-tolerant species), a key life history trait for forest colonization, and known to be a main driver of interspecific variation of herbivory in tropical ecosystems (Coley 1983). Furthermore, while standing levels of herbivory are readily obtained, and used in numerous studies (Coley and Barone 1996), they tend to underestimate damage levels by 38–60% (Lowman 1984, Filip et al. 1995) because entirely eaten leaves and those that abscise after being heavily damaged are overlooked, and there is unaccounted variability in leaf life spans. These limitations are overcome by measurements of cumulative damage (commonly expressed as herbivory rates), in which individually marked leaves are followed over time (see Coley 1982, Lowman 1984 and Filip et al. 1995 for a detailed description of the contrast between both sampling protocols).

In the present study we analyze the effects of forest fragmentation on herbivory and their covariation with plant regeneration mode (light-demanding vs shade-tolerant species) at two different levels: 1) a sample of 97 species representative of the community (standing herbivory), and 2) a subset of species shared by fragments and continuous forest (standing herbivory and cumulative herbivory). We restricted our measurements to saplings (>50 cm height and diameter at breast height (1.3 m) <1 cm), to control for size/ontogeny-related changes in herbivory (Boege and Marquis 2005), and because saplings are more likely to be affected in their performance by herbivory than large/older plants (Dirzo 1984, Marquis 1984), thus potentially affecting recruitment, regeneration and species composition in fragments.

The aim of this study was to evaluate the possible consequences of forest fragmentation on herbivory, and the specific questions we addressed were: 1) does the magnitude of herbivory change with habitat fragmentation? 2) Is herbivory on plants of contrasting regeneration mode (shade-tolerant and light-demanding species) equally affected by forest fragmentation? As a preamble to these comparisons, we determined the type of herbivores responsible for leaf damage in continuous forest and forest fragments.

Material and methods

Study site

This study was conducted at the Los Tuxtlas Research Station, Veracruz, Mexico (18°30′–18°40′N and 95°03′–95°10′W) and adjacent areas (Fig. 1). The area of study is located within the Sierra de Los Tuxtlas. This region represents the northernmost distribution limit for rain forest in the neotropics (Dirzo and Miranda 1992). Mean annual temperature and total mean annual rainfall are 27°C and 4900 mm, respectively (Soto and Gama 1997). The region originally comprised extensive tracts of tropical rain forest in the lowlands, with cloud forest, elfin forest and some patches of coniferous forest along the altitudinal range. The flora of the lowland rain forest is composed of plants of neotropical origin, but higher elevation sites include combinations of neotropical and nearctic taxa (Dirzo 1987).

Conversion of previously extensive tracts of rain forest to agricultural land in the region has produced an agroscape (sensu Janzen 1986) in which forest fragments of different size and degree of isolation are imbedded within a human-dominated matrix (Dirzo et al. 1997, Mendoza et al. 2005). In the northern part of Sierra de Los Tuxtlas, the only remaining area of continuous lowland rain forest corresponds to the Los Tuxtlas Research Station (150 to 650 m a.s.l.), and this is connected, through an elevation gradient, with the San Martin Volcano (1600 m a.s.l.), encompassing a total area of ca 9500 ha (Fig. 1).

Selection of fragments and continuous forest sites

Our approach was to compare two habitat types: small forest fragments and continuous forest (hereafter referred to as forest types). Based on direct exploration of the study area, and using aerial photographs as described by Aguirre and Dirzo (2008), we selected three small fragments (<19 ha, the predominant fragment size in the region – Mendoza et al. 2005, Fig. 1) of similar age of separation from the continuous forest (∼20 years), and three sites within the continuous lowland forest located within the 640-ha Los Tuxtlas Research Station (Fig. 1). All six sites were of similar ecological conditions, located within a restricted altitudinal range (15–150 m a.s.l.), and with similar vegetation composition. All sites were considered to be independent as they were located on three separate hilltops at distances >1 km.

Herbivory: damage types

We describe the damage types to determine which herbivores are responsible for the measured damage. Damage types were assessed considering the three major categories present in this and other rain forests: insect, vertebrate (mammal) or pathogen (Dirzo 1987), which were defined by inspection of leaf scars (de la Cruz and
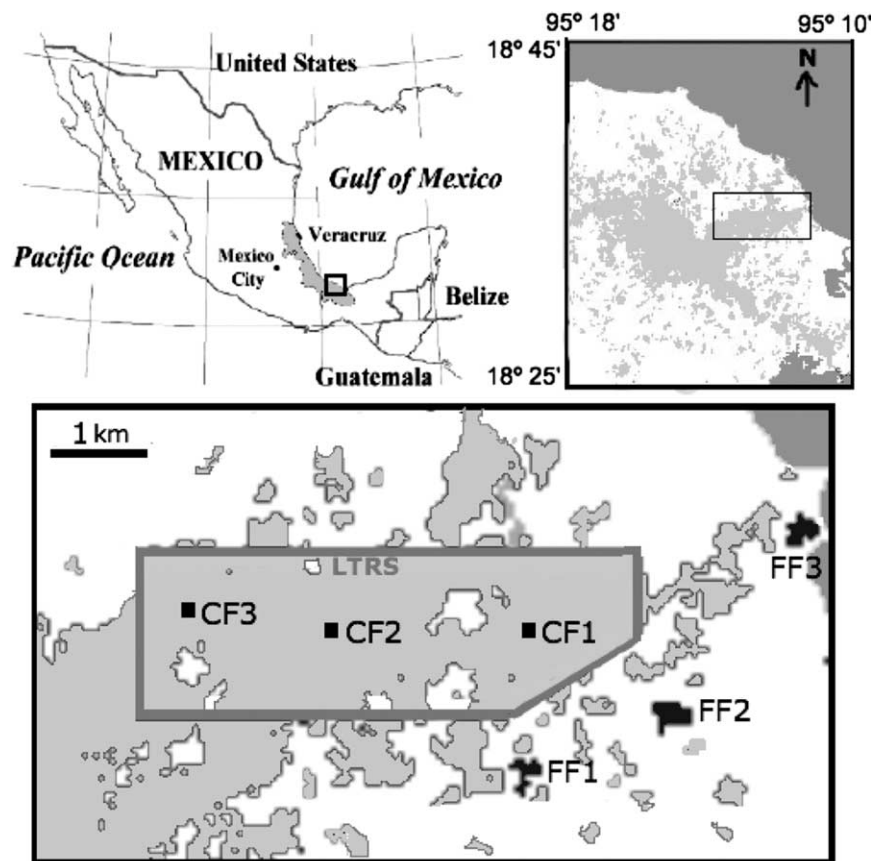
Figure 1. Location of the study site in the State of Veracruz, Mexico, including the specific location of the three forest fragments (FF) and the three sites of continuous forest (CF) located in the Los Tuxtlas Research Station (LTRS). Gray areas correspond to forest; clear areas correspond to cattle grasslands. Modified from Mendoza et al. (2005).

Dirzo 1987) from a bulk sample of 450 leaves for fragments and continuous forest sites, collected in March 2009. The bulk samples were obtained from the first six plants (saplings, as defined above) encountered on each of five randomly-positioned transects (50×2 m) in each of three fragments and three continuous forest sites (n = 90 plants from fragments, and 90 from continuous forest). From each sapling we collected the five oldest (most basal) leaves to determine the frequency of damage types.

Herbivory: fragmentation-related changes

We considered three levels of analysis: 1) standing herbivory at the community level, represented by all sampled saplings in each forest type (82 species in continuous forest and 64 species in fragments, for a total of 97 species), 2) standing levels of herbivory in a subset of species shared between forest types (Table 1), and c) cumulative herbivory for the subset of shared species. Plant species shared between forest types were selected on the basis of their importance value (IV), which was calculated as: $IV_i = RF_i + RA_i$, where: RF_i, the relative frequency of the ith species, is the proportion of sites, out of six, where the ith species was present, divided by the sum all species' frequency, multiplied by 100; and RA_i, the relative abundance of the ith species, is the number of individuals of the ith species divided by the total number of individuals from all species in all six sites, multiplied by 100 (Skeen 1973). The range of variation of IV was 0.33 to

Table 1. Species shared between forest fragments and continuous forest. Shared species were defined on the basis of their importance value (IV, see Material and methods). S =standing levels of herbivory and C =cumulative herbivory.

Species	Family	Plant regeneration mode	IV (%)	Herbivory measurement
Acalypha diversifolia	Euphorbiaceae	light-demanding	3.11	S and C
Hampea nutricia	Malvaceae	light-demanding	2.54	S and C
Myriocarpa longipes	Urticaceae	light-demanding	3.10	S and C
Siparuna andina	Monimiaceae	light-demanding	2.94	S and C
Nectandra ambigens	Lauraceae	shade-tolerant	2.60	S
Pseudolmedia oxyphyllaria	Moraceae	shade-tolerant	5.39	S and C
Rheedia edulis	Guttiferae	shade-tolerant	2.38	S and C
Cymbopetalum baillonii	Annonaceae	shade-tolerant	2.31	C
Faramea occidentalis	Rubiaceae	shade-tolerant	2.05	C

8.5, and we selected the shared species that: 1) were among the twenty species with highest IV, 2) belong to different families (to increase phylogenetic variation), 3) are representative of the two regeneration modes, and 4) were present in all six sampling sites. In total nine species met these criteria (Table 1).

Sampling design and estimation of leaf damage per plant

To evaluate herbivory, we established 0.1-ha permanent observation plots in the center of each fragment, and therefore as far from edges as fragment size would permit. In continuous forest the observation plots were located more than 500 m from forest edges. Each permanent observation plot consisted of ten 50×2 m randomly selected transects. For the community-wide survey, in July 2002, we sampled herbivore damage on leaves of saplings from all woody plants rooted in each transect. We identified each individual plant to the species level, and randomly collected 10 leaves (or the maximum number possible if the plant had less than 10). Leaves were collected by assigning a number to all leaves on the plant, and a random sample of 10 of them was selected using a random numbers table. In total, we sampled 1054 individuals from 97 species, excluding singletons. In August 2002, we collected ten randomly selected leaves (as described before) from ten individuals of each of seven shared species in the permanent observation plots (Table 1) to measure standing levels of herbivory.

We classified plants by regeneration mode as either shade-tolerant or light-demanding species, based on our own knowledge, information provided by the station's resident botanist (Alvaro Campos), and from published literature on plant regeneration modes in the study site (Martínez-Ramos 1994).

From all collected leaves we quantified standing herbivory damage as the percent leaf area eaten by herbivores. We considered leaf damage when parts of the lamina were missing, including holes, scraped-off areas, or incomplete leaf margins. Most of this damage corresponds to chewing insects (alone or combined with pathogen). Upon collection, leaves were flattened out and oven-dried. We then obtained a digital image of each leaf using a lamp-box attached to a photographic camera. These images were imported to WinDias software (Delta-T Devices Ltd, ver. 2), which measures the actual leaf area (ALA), and we estimated the potential leaf area (PLA) by drawing out the contours and filling in the spaces of damaged area. When damage was very extensive we estimated PLA by matching the remaining area of leaves with a comparable, intact leaf of the same species. When a leaf only had part of its central vein left, we considered it to have 100% damage. Leaf herbivory (LH), defined as the percentage of leaf area damaged, was calculated as: $LH = \dfrac{PLA - ALA}{PLA} \times 100$. We estimated herbivory per plant using the average herbivory of all leaves sampled from the plant.

From an additional sample of plants, we also determined cumulative herbivory on marked leaves from shared species in forest fragments and in continuous forest (Table 1),

following the same criteria of location of sampled plants away from edges, as described above. In April 2007, we selected two undamaged leaves (position 1 and 2 of the phyllotaxis) from 10 individuals of eight species in each site. All selected plants were located in the permanent observation plots referred to above, thus n = 240 plants per forest type, or 480 in total. The selected leaves were marked with plastic rings placed at the base of the petioles, and were harvested after six months. We then measured the leaf damage accumulated over 180 days using the same method as for standing herbivory. Only those leaves which still maintained the plastic ring were considered in the analysis, and we excluded marked leaves that died from unknown causes.

Statistical analyses

To evaluate the effect of forest type (fragments vs continuous forest) and plant regeneration mode (light-demanding vs shade-tolerant) on herbivory levels, we used mixed-effects models based on restricted maximum likelihood estimation of parameters. Forest type (FT), plant regeneration mode (PRM), and their interaction were defined as fixed factors, whereas the random component of the model was defined as the overall mean of herbivory given the identity of the species nested within sites. Further, we modeled the variance in the hierarchical nesting structure with the varPower command of R. We also applied a mixed effect model to test the effect of species identity on herbivory. In this model, forest type, species identity, and their interaction were defined as fixed factors while the random component was defined as the overall mean of herbivory given the plant regeneration mode. Contrasts in cumulative herbivory for each species in the two forest types were performed with a t-test based on the estimated standard error of the differences. In the case of standing damage analyses, average percent leaf herbivory per plant was angular-transformed to meet model assumptions for the distribution of residuals. In the case of cumulative herbivory we used the rank-transformation (Conover and Iman 1981), which is robust for additive factorial designs (Seaman et al. 1994). In addition, a simulation analysis showed that type I error of the models described above, fitted to rank-transformed data, was unbiased compared to untransformed data (unpubl.). In all cases we report mean and standard error values for untransformed data. All statistical analyses were carried out using R 2.5.1 GUI 1.2 (R Development Core Team 2007). We fitted linear mixed-effects models using the lme function in R (Pinheiro et al. 2007).

Results

Herbivory: damage types

Overall distributions of damage types on leaves were similar in both forest types (Table 2). The most evident feature is the prevalence of insect herbivory. Damage by pathogens alone was present in a proportion of only 2.9% of the leaves in continuous forest and 1.3% in forest fragments, but it occurred at higher frequencies when combined with insect

Table 2. Types of damage present in a sample of leaves from forest fragments and continuous forest (n = 90 plants and 450 leaves in each forest type). Numbers correspond to the number (and percentage) of leaves. Types of damage were defined from the scars left by herbivores on the leaves (see text for details).

Type of damage	Forest types	
	Continuous forest	Forest fragments
Insects	99 (22%)	80 (17.8%)
Pathogenic agents	13 (2.9%)	6 (1.3%)
Insects and pathogens	213 (47.3%)	199 (44.2%)
Mammals	0	0
Intact	125 (27.8%)	165 (36.7%)

Table 3. Summary of mixed-effects models comparing the fixed factors of forest type (fragments or continuous forest), plant regeneration mode (light-demanding or shade-tolerant), and their interaction term on: (a) standing levels of herbivory in the plant community (n =1054 individuals), (b) standing herbivory levels for shared species (n =411 individuals), (c) cumulative herbivory for shared species (n =374 surviving individuals, out of the initial 480), and (d) cumulative herbivory for shared species considering species identity as fixed factor.

Effect	DF	F	p
(a) Community level			
forest type	1,4	7.6	0.051
plant regeneration mode	1,181	3.1	0.079
forest type ×regeneration mode	1,181	1.8	0.185
(b) Shared species (standing herbivory)			
forest type	1,4	7.7	0.050
plant regeneration mode	1,34	8.23	0.007
forest type ×regeneration mode	1,34	0.5	0.474
(c) Shared species (cumulative herbivory)			
forest type	1,4	0.17	0.700
plant regeneration mode	1,40	32.0	<0.001
forest type ×regeneration mode	1,40	10.2	0.003
(d) Shared species (cumulative herbivory)			
forest type	1,357	5.02	0.026
species identity	7,357	2.89	0.006
forest type ×species identity	7,357	4.97	<0.001

damage. Considering the incidence of insect damage, alone and combined with pathogens, the overall predominance of insect herbivory becomes even more marked in both forest types. Nonetheless, the frequency distribution of damage types was contrasting between forest types. Leaves with insect damage, pathogen damage, and the combination thereof, were more frequent in continuous than in fragmented forests, while intact leaves were less frequent in continuous than in fragmented forests. In most of the insect-damaged leaves we further identified the general type of insect damage. Damage by lepidopteran caterpillars, beetles and orthopterans, was predominant in both forest types. No evidence of damage by vertebrates was evident in either forest types.

Herbivory: fragmentation-related changes

Considering all levels of analysis, we measured herbivory on 13,163 leaves collected from 1839 saplings of 97 plant species. This represents 25.5% of the total richness of woody plant species reported for Los Tuxtlas, and includes the most common as well as several rare species (Ibarra-Manríquez and Sinaca-Colín 1997). Overall, 93% of the sampled plants showed signs of damage, with an average standing herbivory of 10.7%±0.6% per plant.

Plant community: standing herbivory
At the community level, standing herbivory was significantly different between forest fragments and continuous forest (Table 3). Herbivory was 1.3-times greater in continuous forests (11.7±0.53%) than in forest fragments (9.04±0.28%, Fig. 2a). In addition, we found that light-demanding species had on average 1.2 times more herbivory (11.6%±0.58%) than shade-tolerant species (9.5%± 0.30%), although this was not significantly different. The interaction term of forest fragmentation and regeneration mode was not significant either.

Shared species: standing damage
Standing levels of herbivory for seven shared species also differed significantly between fragmented and continuous forest (Table 3). Shared plant species had 1.9-times more herbivory in continuous forests (16.5%±0.84%) than in forest fragments (8.9%±0.64%, Fig. 2b). There was a significant effect of plant regeneration mode on standing

herbivory (Table 3), with herbivory levels 1.7-times greater in light-demanding species (15%±0.75%) than in shade-tolerant species (8.9%±0.71%, Fig. 2c). We did not detect a significant interaction between forest type and plant regeneration mode (Table 3).

Shared species: cumulative herbivory
Cumulative herbivory over 180 days varied significantly between plant regeneration modes (Table 3). Cumulative herbivory was 2.7-times greater in light-demanding species (12.5%±1.19%) than shade-tolerant species (4.6%± 0.79%). The interaction term forest type x plant regeneration mode was statistically significant (Table 3), as cumulative herbivory among shade-tolerant species was four-fold greater in continuous forest (6.6%±1.28%) than in forest fragments (1.7%±0.49%, t=3.5, DF=4, p<0.024), while light-demanding species did not differ in cumulative herbivory between fragmented and continuous forest (t = 0.5, DF=4, p=0.696). When considering the identity of the shared species as a factor, we found that differences in herbivory between forest fragments and continuous forest were consistent among shade-tolerant species, showing significantly higher damage in continuous forest (t >2.1, p ≤ 0.03 in all cases), while light-demanding plant species varied in their herbivory response between forest types (Table 3, Fig. 3): *Hampea nutricia* and *Myriocarpa longipes* were less damaged in forest fragments than in continuous forest (t =2.3, DF =357, p=0.023; and t=2.5, DF = 357, p=0.013, respectively), while *Acalypha diversifolia* and *Siparuna andina* experienced higher herbivory in forest fragments than in continuous forest (t =2.2, DF =357, p=0.025 and t=1.97, DF =357, p<0.049, respectively).

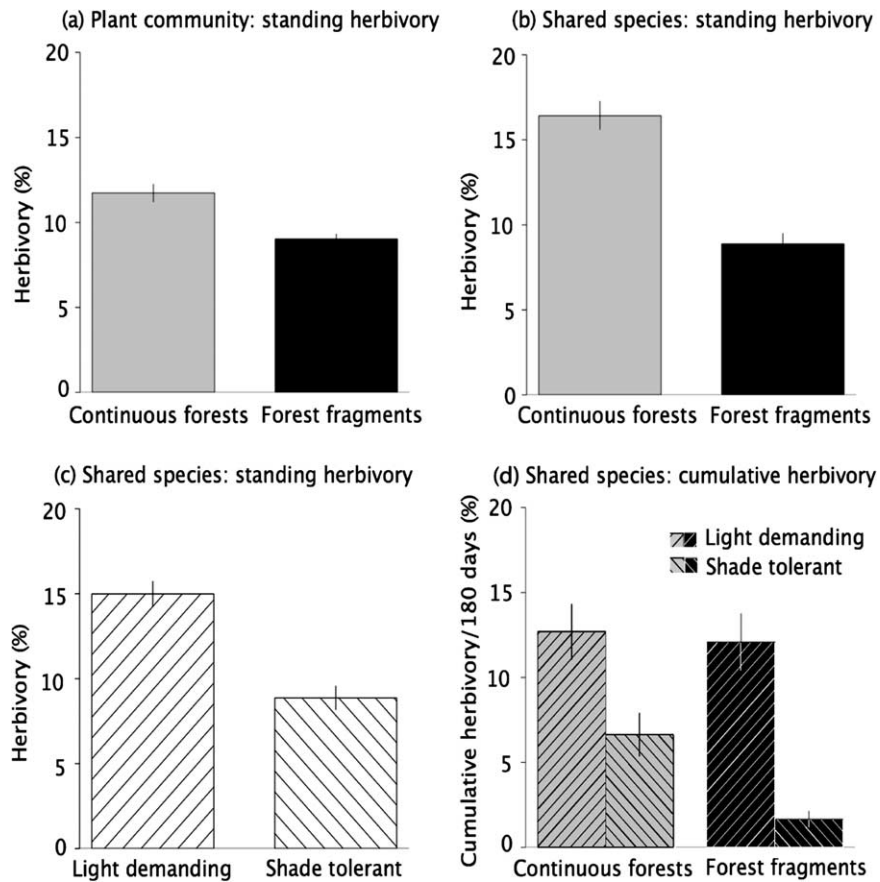Figure 2. Percent herbivory (mean±SE) of plants in two types of forest (continuous forest and forest fragments), in the tropical rain forest of Los Tuxtlas. (a) Standing herbivory in a representative sample of the community of saplings of woody species (97 species), (b) standing herbivory in a subset of seven species shared between continuous forest and forest fragments, (c) standing herbivory in a subset of seven shared species by regeneration modes: light-demanding and shade-tolerant, (d) cumulative herbivory over 180 days in a subset of eight shared species, by forest type and regeneration mode.

Discussion

No other study seems to have addressed the consequences of forest fragmentation on sapling herbivory, considering such a large and representative sample of the plant community in a tropical rain forest. Another similar study in Amazonia (Fáveri et al. 2008) considered a very large sample also, but it focused on juvenile plants. Our results, based on a survey of 97 woody species (1822 reliably identified saplings) show that fragmentation significantly affects herbivory, and underscore the fact that changes in herbivory due to habitat fragmentation are dependent on plant regeneration mode, species identity, and the technique used to estimate herbivory. A salient finding of this study is that herbivory declined in forest fragments, as compared to continuous forest. This finding was consistent when we considered herbivory for the entire community and the shared species (measured both as standing damage or cumulative herbivory). Furthermore, when we measured leaf damage as cumulative herbivory our study uncovered that this effect covaries with plant regeneration mode and species identity: while fragmentation effects are strong and consistent in shade-tolerant species, light-demanding species showed a species-specific response, yielding the overall result that the species of this regeneration mode did not show
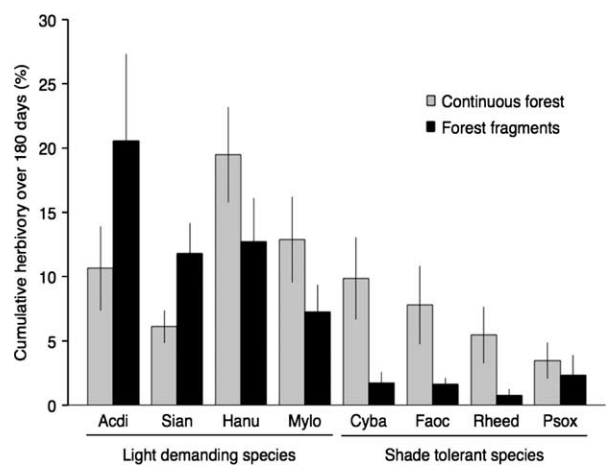

Figure 3. Cumulative percent herbivory over 180 days (mean± SE per plant) in four light-demanding and four shade-tolerant species shared between continuous forest and forest fragments. Light demanding species: *Acalypha diversifolia* (Acdi), *Hampea nutricia* (Hanu), *Myriocarpa longipes* (Mylo), and *Siparuna andina* (Sian); shade-tolerant species: *Cymbopetalum baillonii* (Cyba), *Faramea occidentalis* (Faoc), *Rheedia edulis* (Rheed) and *Pseudolmedia oxyphyllaria* (Psox). All comparisons were statistically significant when tested with rank-transformed data (see text for details).

fragmentation-related effects. Given that shade-tolerant species represent the most abundant regeneration mode in tropical rain forests in general, and in this study site in particular (Dirzo and Boege 2008), we posit that studies attempting to assess the effects of habitat fragmentation on herbivory need to take into account plant regeneration mode, particularly the response of shade-tolerant species.

Herbivory: damage types

Our data showed a predominance of insect damage in the continuous forest, as has been shown by previous studies in this and other tropical forests (Dirzo 1987, Coley and Barone 1996). The incidence of pathogen damage mostly co-occurred with insect damage in continuous forests, confirming previous patterns detected by Dirzo (1987) and García-Guzmán and Dirzo (2001) in the same study site. In addition, we detected a total absence of mammalian damage, consistent with a previous finding by Dirzo and Miranda (1991). In this study we found that the same trends occur in forest fragments: a predominance of insect damage (alone and combined with pathogen damage), low incidence of pathogen damage alone, and absence of mammalian herbivory. Therefore, it was appropriate to focus this study on insect herbivory. Nevertheless, we detected an overall contrast in the frequency of damage across forest types: damage of all types was more frequent in continuous forest than in forest fragments, while the frequency of undamaged leaves showed the opposite trend. This suggests that insect herbivory is lower in fragments than in continuous forest, a result that was confirmed by the analysis of leaf area damaged across forest types.

Herbivory: fragmentation-related changes

We found consistently lower levels of herbivory in forest fragments than in continuous forest at the level of the plant community and when comparing the shared species. This finding is concordant with other studies demonstrating similar effects of fragmentation (Groom 2001, Zenteno 2001, Arnold and Asquith 2002, Ledergerber et al. 2002, Valladares et al. 2006, Vásquez et al. 2007, Fáveri et al. 2008). However, other studies have reported results in the opposite direction: herbivory increases in fragments (Rao et al. 2001, Terborgh et al. 2001, Lienert et al. 2002, Christie and Hochuli 2005, Elzinga et al. 2005, Stoll et al. 2006, del-Val et al. 2007). This discrepancy among studies could be related to site-specific differences and a host of other sources of potential variation in herbivory, including plant regeneration mode, plant life form, species identity, and even measurement technique.

In general terms, changes in herbivory resulting from fragmentation can be driven by changes in top–down or bottom–up controls. For instance, there is evidence that top–down controls of herbivores, such as parasitism and predation, are negatively affected by habitat fragmentation (Didham et al. 1996, Kruess 2003), and this would increase herbivory in fragments, a situation contrary to our findings. On the other hand, several studies have shown that fragmentation increases predation (González-Gómez et al.

2006, Koh and Menge 2006, Fáveri et al. 2008) or parasitoid (Roland and Taylor 1997, Doak 2000) attack rates, potentially or actually leading to a reduction in herbivory in fragmented sites, consistent with our findings. In summary, these studies show that forest fragmentation can either increase or decrease top–down controls of herbivores, and this may be responsible, at least in part, for the inconsistency of results of studies addressing forest fragmentation effects on herbivory.

Independent of alterations in biotic top–down controls of herbivores due to fragmentation, changes in the abundance of phytophagous insects in forest fragments leading to reduced herbivory could be explained by habitat isolation, patch size and quality, and microclimate changes due to edge effects. For example, it has been speculated that warmer microclimatic conditions and exposure to strong winds at fragment edges could negatively affect herbivore survival or performance (Barone and Coley 2002), potentially leading to results similar to ours. Alternatively, foliage quality, a bottom–up control, may be altered by the prevailing conditions at the edge of forest fragments and in small fragments (Coley 1998, Fortín and Mauffette 2001, Yamasaki and Kikuzawa 2003, Wirth et al. 2008). For example, it has been suggested that high solar radiation can induce an increase of secondary metabolites such as tannins, terpenes and phenolic compounds (Coley and Barone 1996, Waterman and Mole 1994). Such changes would produce results similar to the ones we uncovered. However, we have evidence that a variety of leaf defensive traits (including secondary metabolites, water and nutrient content and, in general, plant palatability) remain unchanged in a set of species shared in fragments and continuous forest at Los Tuxtlas (Ruiz-Guerra 2009), suggesting that such plant trait-related changes in bottom-up controls may not be responsible for the observed decline in herbivory with fragmentation at Los Tuxtlas. This, and other related bottom–up controls, such as changes in plant community composition, is an aspect that warrants further work in this and other sites.

The decline in herbivory with fragmentation, considering all species, included both light-demanding and shade-tolerant species. Likewise, the comparison using a set of shared species, which included species of both types of plant regeneration mode, also showed a decline in herbivory in forest fragments. These results showed a consistency of response in both groups of plants. However, we also observed fragmentation-related differences in herbivory between shade-tolerant and light-demanding species in 2007 (using cumulative damage). While herbivory in all shade tolerant species consistently declined with fragmentation, herbivory in light-demanding species, as a group, did not, due to the fact that species responses were idiosyncratic: herbivory in some species declined while in others it increased with fragmentation, an aspect that merits subsequent examination with a larger pool of species. Still, we emphasize that this group of species represents only a lower proportion of the woody flora of this and other tropical sites, compared to the mature-forest, shade-tolerant species (Dirzo and Boege 2008).

It is puzzling that we detected a significant interaction between plant regeneration mode and forest type in 2007,

using cumulative herbivory measurements, and not in 2002, when we measured standing damage of shared species. Standing damage underestimates herbivory (Lowman 1984, Filip et al. 1995), particularly when damage is high and entirely eaten or heavily damaged leaves are abscised and, therefore, overlooked in the standing damage estimates (Bundell and Peart 2000). Since herbivory was greater in light-demanding species and leaf turnover is faster in these species (Coley and Barone 1996), standing measurements might have underestimated herbivory to a larger extent in these species, compared to shade-tolerant species. This suggests that the contrast between light-demanding and shade-tolerant species using standing measurements (a 1.7-fold difference) might have been even greater. Indeed, our cumulative measurements showed that to be the case (a 2.7-fold difference). Nonetheless, absolute levels of damage were, in general, greater when we used standing measurements compared to cumulative damage (12.4% vs 8.1%, respectively). However a comparison of the results using the two methods is not warranted, given that measurements were conducted in different years and temporal variation in a host of variables can lead to temporal variations in herbivory (Benitez-Malvido et al. 1999).

As indicated above, insect herbivory was the predominant type of damage. The frequency of pathogen damage alone was low, but was much greater when it occurred in combination with insect damage. A detailed analysis of pathogen damage in continuous forest at Los Tuxtlas (García-Guzmán and Dirzo 2001) showed that the amount of leaf area infected by pathogens alone was very low, compared to insect damage, and that the incidence of infection depends on insect herbivory. Therefore we expect that pathogen infection in relation to fragmentation will follow a similar trend as that of insect herbivory. Interestingly, an ongoing study on leaf pathogen infection in fragments and continuous forest shows that to be the case (Dirzo unpubl.) but, again, this is an aspect that warrants further work.

This study underscored the complexity of the consequences of fragmentation on herbivory. At the same time, it exposed numerous important aspects that need to be addressed to advance in this field, not only to inform patterns of change in the face of fragmentation, but also to elucidate underlying mechanisms responsible for such patterns. In addition, this study addresses a lacuna in the study of fragmentation effects on saplings, the phenostage in which herbivory can have stronger detrimental effects on plant fitness (Dirzo 1984, Marquis 1984). Since saplings represent the potential future composition of the tropical plant community and this study revealed that sapling herbivory changes with fragmentation and covaries with plant regeneration mode and species identity, further research is needed to understand the long-term consequences of the changes in herbivory regimes driven by habitat fragmentation.

References

Aguirre, A. and Dirzo, R. 2008. Effects of habitat fragmentation on pollinator abundance and fruit set on an abundant understory palm in a Mexican tropical forest. – Biol. Conserv. 141: 375–384.

Arnold, A. E. and Asquith, N. M. 2002. Herbivory in a fragmented tropical forest: patterns from islands at Lago Gatun, Panama. – Biodiv. Conserv. 11: 1663–1680.

Barone, J. A and Coley, P. D. 2002. Herbvorismo y las defensas en plantas. – In: Guariguata, M. R and Catan, G. (eds), Ecología y conservación de bosques neotropicales. Libro Universitario Regional, pp. 466–492.

Benítez-Malvido, J. et al. 1999. Foliar pathogens incidence and herbivory in tropical rain forest fragments: an experimental study. – Biol. Conserv. 91: 143–150.

Boege, K. and Marquis, R. J. 2005. Facing herbivory as you grow up: the ontogeny of resistance in plants. – Trends. Ecol. Evol. 20: 441–448.

Bundell, G. and Peart, D. R. 2000. High abscission rates of damaged expanding leaves: field evidence from seedlings of a Bornean rain forest tree. – Am. J. Bot. 87: 1693–1698.

Conover, W. J. and Iman, R. L. 1981. Rank transformations as a bridge between parametric and non-parametric statistics. – Am. Stat. 35: 124–130.

Christe, J. C. and Hochuli, D. 2005. Elevated levels of herbivory in urban landscapes: are declines in tree health more than an edge effect? – Ecol. Soc. 10: 10.

Coley, P. D. 1982. Rates of herbivory on different tropical trees. – In: Leigh, E. G. Jr. et al. (eds), Ecology of a tropical forest: seasonal rhythms and long-term changes. Smithsonian Institution Press, pp. 123–132.

Coley, P. D. 1983. Herbivory and defensive characteristics of tree species in a lowland tropical forest. – Ecol. Monogr. 53: 209–233.

Coley, P. D. 1998. Possible effects of climate change on plant/herbivore interactions in moist tropical forest. – Climate Change 39: 455–472.

Coley, P. D. and Barone, J. A. 1996. Herbivory and plant defences in tropical forest. – Annu. Rev. Ecol. Syst. 27: 305–335.

del-Val, E. et al. 2007. Effects of herbivory and patch size on tree seedling survivorship in a fog-dependent coastal rain forest in semiarid Chile. – Oecologia 153: 625–632.

de la Cruz, M. and Dirzo, R. 1987. A survey of the standing levels of herbivory in seedlings from a Mexican rain forest. – Biotropica 19: 98–106.

Didham, K. R. et al. 1996. Insects in fragmented forest: a functional approach. – Trends Ecol. Evol. 11: 255–260.

Dirzo, R. 1984. Herbivory: a phytocentric overview. – In: Dirzo, R. and Sarukhán, J. (eds), Perspectives on plant population ecology. Sinauer, pp. 141–165.

Dirzo, R. 1987. Estudios sobre las interacciones planta-herbívoro en Los Tuxtlas, Veracruz. – Rev. Biol. Trop. 35 (Suppl. 1): 119–132.

Dirzo, R. and Miranda, A. 1991. Altered patterns of herbivory and diversity in the forest understory: a case study of the possible consequences of contemporary defaunation. – In: Price, P. W. et al. (eds), Plant–animal interactions: evolutionary ecology in tropical and temperate regions. Wiley, pp. 273–287.

Dirzo, R. and García, M. 1992. Rates of deforestation in Los Tuxtlas, a neotropical area in southeast Mexico. – Conserv. Biol. 6: 84–94.

Dirzo, R. and Miranda, A. 1992. El límite boreal de la selva tropical húmeda en el continente Americano: contracción de la vegetación y solución de una controversia. – Interciencia 16: 240–247.

Dirzo, R. and Raven, P. 2003. Global state of biodiversity and loss. – Annu. Rev. Environ. Resour. 28: 137–167.

Dirzo, R. and Boege, K. 2008. Patterns of herbivory and defence in tropical dry and rain forest. – In: Carson, W. P. and Schnitzer, S. A. (eds), Tropical forest community ecology. Wiley, pp. 63–78.

Dirzo, R. et al. 1997. Introducción general. – In: González-Soriano, E. et al. (eds), Historia Natural de Los Tuxtlas. Univ. Nacional Autónoma de México, pp. 3–6.

Doak, P. 2000. The effects of plant dispersion and prey density on parasitism rates in a naturally patchy habitat. – Oecologia 122: 556–567.

Elzinga, J. A. et al. 2005. Plant population size and isolation affect herbivory of Silene latifolia by the specialist herbivore Hadean bicruris and parasitism of the herbivore by parasitoids. – Oecologia 144: 416–426.

Fáveri, S. B. et al. 2008. Effects of Amazonian forest fragmentation on the interaction between plants, insect herbivores, and their natural enemies. – J. Trop. Ecol. 24: 57–64.

Filip, V. et al. 1995. Within -and among- year variation in the levels of herbivory on the foliage of trees from a Mexican tropical deciduous forest. – Biotropica 27: 78–86.

Fortín, M. and Maufette, Y. 2001. Forest edge effects on the biological performance of the forest tent caterpillar (Lepidoptera: Lasiocampidae) in sugar maple stands. – Ecoscience 8: 164–172.

Garcia-Guzman, G. and Dirzo, R. 2001. Patterns of leaf-pathogen infection in the understory of a Mexican rain forest: incidence, spatiotemporal variation, and mechanisms of infection. – Am. J. Bot. 88: 634–645.

Ghazoul, J. 2005. The implications of plant spatial distribution for pollination and seed production. – In: Burslem, D. et al. (eds), Biotic interactions in the tropics. Cambridge Univ. Press, pp. 241–266.

González-Gómez, P. et al. 2006. Strengthened insectivory in a temperate fragmented forest. – Oecologia 148: 137–143.

Groom, M. 2001. Consequences of subpopulation isolation for pollination, herbivory, and population growth in Clarkia concinna concinna (Onagraceae). – Biol Conserv. 100: 55–63.

Ibarra-Manríquez, G. and Sinca-Colin, S. 1997. Fanerógamas. – In: González-Soriano, E. et al. (eds), Historia natural de Los Tuxtlas. Univ. Nacional Autónoma de México, pp. 162–174.

Janzen, D. H. 1986. The future of tropical ecology. – Annu. Rev. Ecol. Syst. 17: 305–324.

Kruess, A. 2003. Effects of landscape structure and habitat type on a plant–herbivore parasitoid community. – Ecography 26: 283–290.

Koh, L. P. and Menge, D. N. L. 2006. Rapid assessment of lepidoptera predation rates in neotropical forest fragments. – Biotropica 38: 132–134.

Ledergerber, S. et al. 2002. Effects of experimental small-scale grassland fragmentation on the extent of grazing damage in Trifolium repens seedlings. – Acta. Oecol 23: 329–336.

Lienert, J. and Fischer, M. 2003. Habitat fragmentation affects the common wetland specialist Primula farinosa in northeast Switzerland. – J. Ecol. 91: 587–599.

Lienert, J. et al. 2002. Effects of habitat fragmentation on population structure and fitness components of the wetland specialist Swertia perennis L. (Gentianaceae). – Basic Appl. Ecol. 3: 101–114.

Lowman, M. D. 1984. An assessment of techniques for measuring herbivory: is rain forest defoliation more intense than we thought? – Biotropica 16: 264–268.

Marquis, R. 1984. Leaf herbivores decrease fitness of a tropical plant. – Science 226: 537–539.

Marquis, R. J. 2005. Impacts of herbivores on tropical plant diversity. – In: Burslem, D. et al. (eds), Biotic interactions in the tropics. Cambridge Univ. Press, pp. 328–346.

Martínez-Ramos, M. 1994. Regeneración natural y diversidad de especies arbóreas. – Bol. Soc. Bot. Mex. 54: 179–224.

Mendoza, E. et al. 2005. A quantitative study of forest fragmentation in a neotropical area in southeast México. – Rev. Chil. Hist. Nat. 78: 451–467.

Pinheiro, J. et al. 2007. nlme: linear and nonlinear mixed effects models. R Package ver. 3.1-86.

Rao, M. et al. 2001. Increased herbivory in forest isolates: implications for plant community structure and composition. – Conserv. Biol. 15: 624–633.

Roland, J. and Taylor, P. D. 1997. Insect parasitoid species respond to forest structure at different spatial scales. – Nature 386: 710–713.

Ruiz-Guerra, B. 2009. Consecuencias de la pérdida del hábitat sobre la interacción planta-herbívoros-enemigos naturales en las selva de Los Tuxtlas, Ver. PhD thesis. – Univ. Nacional Autónoma de México.

Seaman, J. W. et al. 1994. Caveat emptor: rank transform methods and interaction. – Trends. Ecol. Evol. 9: 261–263.

Simonetti, J. et al. 2007. Herbivory and seedling performance in a fragmented temperate forest of Chile. – Acta Oecol. 32: 312–318.

Skeen, J. N. 1973. An extension of the concept of importance value in analyzing forest communities. – Ecology 54: 655–656.

Soto, M. and Gama, L. 1997. Climas. – In: González-Soriano, E. et al. (eds), Historia natural de Los Tuxtlas. Univ. Nacional Autónoma de México, pp. 7–23.

Stoll, P. et al. 2006. Experimental habitat fragmentation and invertebrate grazing in a herbaceous grassland species. – Basic Appl. Ecol. 7: 307–319.

Terborgh, J. et al. 2001. Ecological meltdown in predator-free forest fragments. – Science 294: 1923–1926.

Tscharntke, T. and Brandl, R. 2004. Plant–insect interactions in fragmented landscapes. – Annu. Rev. Entomol. 49: 405–30.

Valladares, G. et al. 2006. Habitat fragmentation effects on trophic processes of insect–plant food webs. – Conserv. Biol. 20: 212–217.

Vásquez, P. A. et al. 2007. Herbivory, foliar survival and shoot growth in fragmented populations of Aristotelia chilensis. – Acta. Oecol. 31: 48–53.

Yamasaki, M. and Kikuzawa, K. 2003. Temporal and spatial variations in leaf herbivory within a canopy of Fragus crenata. – Oecologia 137: 226–232.

Waterman, P. G. and Mole, S. 1994. Analysis of phenolic plant metabolites. – Blackwell.

Wirth, R. et al. 2008. Plant herbivore interactions at the forest edge. – In: Luttge, U. et al. (eds). Progress in botany. Springer, pp. 424–448.

Zenteno, R. L. 2001. Efectos de la fragmentación del hábitat sobre la herbivoría en Chamaedorea alternans (Arecaceae) en la selva de Los Tuxtlas, Veracruz. BSc thesis. – Benemérita Univ. Autónoma de Puebla, México.