SHORT COMMUNICATION

Possible implications of seed dispersal by the howler monkey for the early recruitment of a legume tree in small rain-forest fragments in Mexico

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Abstract: Primates are important seed dispersers in tropical forests, but they are being lost due to forest fragmentation. We compared post-dispersal seed fate and seedling density for the tree *Dialium guianense*, in forest fragments in which its main seed disperser, the mantled howler monkey (*Alouatta palliata*) is present, and in fragments in which it is absent. We also carried out a germination experiment to assess gut-passage effect. Seeds extracted from fruits had very low germination (2%) compared with defecated seeds (70%). Seeds in monkey faeces had a lower probability of being attacked by predators, but a higher probability of being attacked by fungus, when compared with seeds inside fruits. Seed fate was similar in fragments with *vs.* without monkeys, and in sites under parent trees *vs.* under monkey sleeping sites. Seedling density was highest under sleeping sites in fragments with monkeys, followed by sites under parent trees in fragments with monkeys, and it was lowest in uninhabited fragments, regardless of deposition site. Our study adds to an increasing line of evidence suggesting that loss of frugivores can have strong consequences for plant recruitment.

Key Words: defaunation, fragmentation, germination, seed fate, seedling density

Seed dispersal by frugivorous animals creates heterogeneous seed templates upon which post-dispersal processes act to determine plant recruitment. Dispersal effectiveness depends on characteristics of the primary disperser, but also on many factors affecting post-dispersal seed fate (Schupp *et al.* 2010). Further, the effects of most of these factors are highly context-dependent (Schupp 2007), a characteristic that is particularly evident in scenarios of anthropogenic disturbances, such as forest fragmentation. In fragmented forests many frugivores, granivores and herbivores have declined or disappeared entirely from the remaining forest patches, with cascading effects on plants (Galetti & Dirzo 2013). However, since both plant mutualists and antagonists are often affected by disturbance, net effects are not easily predictable (Kurten 2013).

Primates are recognized as important seed dispersers who often produce aggregated patterns of seed deposition (Russo & Chapman 2011). This pattern has been associated with seedling recruitment foci in primate resting areas (Anzures-Dadda *et al.* 2011, Bravo 2012). Unfortunately, primates are also recognized as suffering large negative impacts due to anthropogenic disturbances (Schwitzer *et al.* 2011), and an increasing number of studies have been documenting the consequences of primate decimation for plant recruitment (Anzures-Dadda *et al.* 2011, Gutiérrez-Granados & Dirzo 2010, Levi & Peres 2013).

The objective of our study was to explore some of the mechanisms that might be related to a previously found result: that seedling density of the tree *Dialium guianense* (Aubl.) Sandwith under sleeping sites of its main seed disperser, the mantled howler monkey (*Alouatta palliata* Gray), in monkey-inhabited fragments, was 33 times higher when compared with seedling density in 'simulated' sleeping sites in fragments without monkeys (Anzures-Dadda *et al.* 2011). We asked the following questions: (1) Does passage through the monkey's gut favour germination? (2) Do seeds deposited in monkey dung survive better than seeds inside fruits? (3) Are the patterns of seed survival different in fragments

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inhabited *vs.* uninhabited by monkeys and/or under monkey sleeping sites *vs.* parent trees? (4) Does the density of *D. guianense* seedlings differ under monkey sleeping sites *vs.* under parent trees? (5) Is the density of seedlings under parent trees similar in fragments with *vs.* without primates? To answer these questions we conducted a laboratory experiment and two field studies in six small (3–10 ha) rain-forest fragments in Chiapas, Mexico: three with and three without the howler monkey (*Alouatta palliata*). Mean annual temperature and precipitation are 25.4°C and 2600 mm, respectively. Monkey-inhabited (9–15 individuals per fragment) and uninhabited (for $\geq$ 17 y) fragments were similar in area, perimeter, shape and vegetation characteristics (Anzures-Dadda & Manson 2007, Anzures-Dadda *et al.* 2011). Density of adult ($\geq$ 20 cm diam) *D. guianense* trees was 62 ha^{-1} and 46 ha^{-1} in fragments inhabited and uninhabited by monkeys, respectively (Anzures-Dadda *et al.* 2011). We did not control for connectivity, or other biotic/abiotic characteristics that may vary among fragments.

Dialium guianense is a deciduous canopy tree (up to 45 m high) widely distributed in the Neotropics. Fruits (1–2 cm) have a soft pericarp and contain one or two large seeds (9–11 mm long) surrounded by a sweet aril. *Dialium guianense* seeds are found in howler monkey defecations (Anzures-Dadda *et al.* 2011), and in our study site these primates seem to be the main or only disperser of this species (Anzures-Dadda 2008). Seeds have physical dormancy and previous evidence suggests that gut-passage may be necessary for germination of this and other *Dialium* species (Beaune *et al.* 2013, Estrada & Coates-Estrada 1984).

For germination trials we used 150 seeds in each of four treatments: (1) monkey-defecated seeds, (2) mechanical scarification, by making a small incision on the seed coat; (3) chemical scarification, by submerging seeds in concentrated sulphuric acid for 6 min; (4) no scarification (control). Seeds for treatments 2–4 were obtained from ripe fruit. Seeds were placed in Petri dishes (15 seeds per dish, 10 dishes per treatment) in a germination chamber; germination (radicle emergence) was recorded over 30 d. We did not test for viability of ungerminated seeds. We analysed data with generalized linear models (GLMs), with % germination as response variable and treatment as a fixed factor.

To assess post-dispersal seed fate and seedling densities, in each of the six forest fragments we selected two types of site in which high densities of seeds of *D. guianense* are deposited naturally: below parent trees and below monkey sleeping sites (real and simulated). Simulated sleeping sites had similar structural characteristics as real sleeping sites (canopy height > 20 m, canopy cover > 60%, and diameter at breast height of the central tree > 50 cm; Anzures-Dadda *et al.* 2011). For each type of seed-deposition site we had two replicates per fragment (6 fragments $\times$ 2 deposition sites $\times$ 2 replicates

= 24 sites). In the centre of each site we placed four groups of 24 experimental seeds (2 m between groups). Seeds of each group were exposed to two deposition conditions, mimicking natural densities (Anzures-Dadda unpubl. data): (1) 18 seeds inside mature fruits and (2) six seeds surrounded by monkey faeces (3–5 g). Seeds in the latter treatment were obtained from monkey defecations, cleaned, stored, and then covered with fresh monkey dung at the moment of experimental setup. Additionally, to identify potential post-dispersal seed predators we applied three treatments in each group: rodent exclusion (wire mesh cage), rodent and ant exclusion (cage and tape with Tanglefoot®), and control (no exclusion). Each treatment was applied to six fruits and two seeds in faeces. A total of 1152 seeds were used in this field experiment (192 seeds in each of the 6 fragments). We recorded seed fate for 16 wk. To analyse survival time we used time-to-failure analyses fitting data to a Cox proportional-hazard model. Densities (individuals m^{-2}) of naturally occurring *D. guianense* seedlings were quantified for the same 24 sites. In each site we set up a 20 $\times$ 20-m plot divided into 100 subplots of 2 $\times$ 2 m. We counted all *D. guianense* seedlings < 50 cm tall in 20 randomly selected sub-plots and used these data to estimate the mean seedling density for each site. Data were analysed with GLMs, with seedling density as the response variable and two nested fixed factors: deposition site nested within fragment type.

Passage through the monkey's gut favoured germination. Percentage germination was very low for control seeds (2%), but high ($\geq$ 70%) for the other treatments ($F_{2,27}$ = 206, P < 0.001). All observed germination occurred within the first 10 d. Post hoc tests indicated that germination was lower for defecated seeds (70%) when compared with both methods of artificial scarification (86%). No germination was observed in the field. Survival time was much higher for seeds in faeces *vs.* seeds in fruits (χ^2 = 6983, df = 1, P < 0.001), but at the end of the study, all seeds in both treatments had been killed or removed (Figure 1). Seeds in faeces suffered very little removal (< 1%), and no attack by bruchids, but fungus attack was very high (> 98%). Predator exclusion also had a significant effect on survival time (χ^2 = 1594, df = 2, P < 0.001), but only for seeds in fruit. Seeds in fruits were removed by both rodents and ants. Seed survival patterns were not affected by fragment type (with *vs.* without monkeys) or seed deposition site (under sleeping site *vs.* under parent tree). Finally, *D. guianense* seedling density was affected by seed deposition site ($F_{2,22}$ = 14.2, P < 0.001), but only in fragments with monkeys, where density was significantly higher under sleeping sites (1 seedling m^{-2}) than under parent trees (0.22 seedlings m^{-2}). In fragments without monkeys, mean seedling density was equally low in both simulated sleeping sites and under parental trees (0.03 seedlings m^{-2}). Type of fragment also had a significant effect ($F_{1,5}$
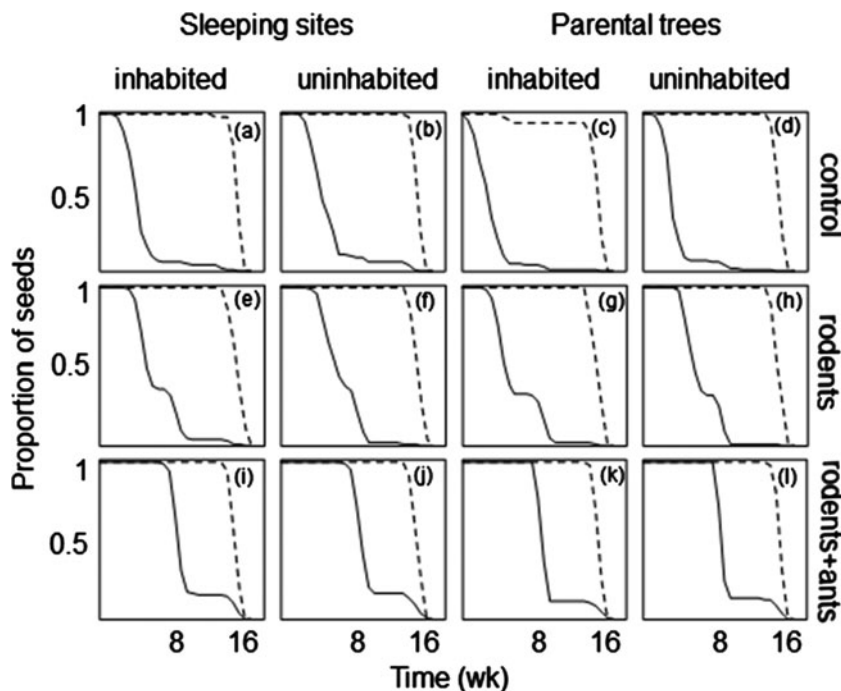
Figure 1. Proportion of *Dialium guianense* seeds surviving over time in six rain-forest fragments in Chiapas, Mexico. Fragments were of two types: inhabited (a, e, f, c, g, k) and uninhabited (b, f, j, d, h, l) by howler monkeys. Seeds were placed in two deposition sites: below monkey sleeping sites (real and simulated) and under conspecific parental trees. In each deposition site seeds were also placed in two deposition conditions: inside fruits (continuous lines) and surrounded by faecal material (dashed lines). Three treatments of predator exclusion were applied: no exclusion (control), rodent exclusion (rodents) and total exclusion (rodents + ants). Each of the 12 panels represents the fate of 72 seeds inside fruits and 24 seeds surrounded by faecal material.

= 31.3, P < 0.001). Seedling density under parent trees was significantly higher in fragments with monkeys, compared with fragments in which the monkeys are not longer present.

Our results support the generally accepted contention that the loss of frugivorous animals can affect the regeneration of plant species dispersed by them. Our results also emphasize that the regeneration process is complex and affected by multiple biotic and abiotic factors. Yet, given that none of our experimental seeds recruited seedlings in the field, that we lack long-term data on seedling performance, and that fragments might differ in relevant aspects besides howler monkey presence, we must consider our results preliminary and any conclusion ought to be drawn with caution.

Increased seedling establishment under monkey sleeping sites could be a consequence of increased seed rain and/or increased seed germination/survival in these sites. In terms of seed rain, a positive correlation has been reported to occur between seed density and seedling density for *D. guianense* (Anzures-Dadda *et al.* 2011). Although in the present study we did not measure seed density under parent trees, it can be expected to be quite high. In terms of increased germination, seed deposition after passage through the gut, which can be expected to

occur more often under monkey sleeping sites *vs.* parent trees, seems to be crucial for *D. guianense*, as has been shown for several *Dialium* species swallowed by bonobos in Africa (Beaune *et al.* 2013). It is important to mention that mechanical scarification (which was as effective as chemical scarification) can also occur when seeds are manipulated by granivorous animals (Elmouttie & Mather 2012). We must note that *c.* 35% of seeds in fruit were removed by rodents and/or ants and that a proportion of such removal may not represent predation. Future studies ought to determine the possible role of granivorous animals in the dispersal of *D. guianense* seeds.

How seeds are deposited also affects seed fate. Seeds deposited in monkey faeces suffered very little removal/predation by ants, rodents and bruchids. It has been reported that faeces may protect seeds against predators (Rios & Pacheco 2006). However, seeds in dung suffered high mortality due to fungal attack. We must stress the fact that no experimental seed, either surrounded by dung or by fruit pulp, germinated in the field. Fruit pulp has been shown to promote pathogen attack and also to prevent germination through inhibition (Traveset *et al.* 2007). Whether germination inhibition due to pulp and faeces caused attack by fungus, or whether fungus attack prevented germination, remains to

be elucidated. Also, altered microclimatic conditions may negatively affect germination in small forest fragments (González-Di Pierro *et al.* 2011). Whether this is the case in our study system also remains to be assessed by future studies.

In summary, primary dispersal of *D. guianense* seeds by the howler monkey, seems to be necessary for seed germination. This positive effect on germination, together with the aggregated seed deposition patterns under the monkey's sleeping sites, is associated with high seedling densities of *D. guianense* in forest fragments still inhabited by this monkey. The howler monkey inhabits less than 20% of the forest remnants in fragmented landscapes of Mexico (Anzures-Dadda *et al.* 2011), meaning that the uninhabited fragments have lost one of their main seed dispersers. This could represent a serious challenge due to cascading effects in the long term, which eventually might lead to the necessity of costly actions to restore the carrying capacity of habitat fragments for animals.

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LITERATURE CITED

ANZURES-DADDA, A. 2008. *Consecuencias de la fragmentación de la selva sobre las poblaciones de monos aulladores* (Alouatta palliata) *y su papel en la dispersión de semillas y el establecimiento de árboles en el norte de Chiapas, México.* Ph.D. thesis, Instituto de Ecología, A.C., Xalapa, Veracruz, Mexico.

ANZURES-DADDA, A. & MANSON, R. H. 2007. Patch- and landscape-scale effects on howler monkey distribution and abundance in rainforest fragments. *Animal Conservation* 10:69–76.

ANZURES-DADDA, A., ANDRESEN, E., MARTÍNEZ, M. L. & MANSON, R. H. 2011. Howler monkey absence influences tree seedling densities in tropical rainforest fragments in southern Mexico. *International Journal of Primatology* 32:634–651.

BEAUNE, D., BRETAGNOLLE, F. O., BOLLACHE, L. C., HOHMANN, G., SURBECK, M., BOURSON, C. & FRUTH, B. 2013. The Bonobo–*Dialium* positive interactions: seed dispersal mutualism. *American Journal of Primatology* 75:394–403.

BRAVO, S. P. 2012. The impact of seed dispersal by black and gold howler monkeys on forest regeneration. *Ecological Research* 27:311–321.

ELMOUTTIE, D. & MATHER, P. B. 2012. Can rodents enhance germination rates in rainforest seeds? *Ecological Management and Restoration* 13:203–207.

ESTRADA, A. & COATES-ESTRADA, R. 1984. Fruit eating and seed dispersal by howling monkeys (*Alouatta palliata*) in the tropical rain forest of Los Tuxtlas, Mexico. *American Journal of Primatology* 6:77–91.

GALETTI, M. & DIRZO, R. 2013. Ecological and evolutionary consequences of living in a defaunated world. *Biological Conservation* 163:1–6.

GONZÁLEZ-DI PIERRO, A. M., BENÍTEZ-MALVIDO, J., MÉNDEZ-TORIBIO, M., ZERMEÑO, I., ARROYO-RODRÍGUEZ, V., STONER, K. E. & ESTRADA, A. 2011. Effects of the physical environment and primate gut passage on the early establishment of *Ampelocera hottlei* Standley in rain forest fragments. *Biotropica* 43:459–466.

GUTIÉRREZ-GRANADOS, G. & DIRZO, R. 2010. Indirect effects of timber extraction on plant recruitment and diversity via reductions in abundance of frugivorous spider monkeys. *Journal of Tropical Ecology* 26:45–52.

KURTEN, E. L. 2013. Cascading effects of contemporaneous defaunation on tropical forest communities. *Biological Conservation* 163:22–32.

LEVI, T. & PERES, C. A. 2013. Dispersal vacuum in the seedling recruitment of a primate-dispersed Amazonian tree. *Biological Conservation* 163:99–106.

RIOS, R. & PACHECO, L. F. 2006. The effect of dung and dispersal on postdispersal seed predation of *Attalea phalerata* (Arecaceae) by bruchid beetles. *Biotropica* 38:778–781.

RUSSO, S. E. & CHAPMAN, C. 2011. Primate seed dispersal. Linking behavioral ecology with forest community structure. Pp. 523–534 in Campbell, C. J., Fuentes, A., MacKinnon, K. C., Bearder, S. & Stumpf, R. M. (eds.). *Primates in perspective.* Oxford University Press, Oxford.

SCHUPP, E. W. 2007. The suitability of a site for seed dispersal is context-dependent. Pp. 445–462 in Dennis, A. J., Schupp, E. W., Green, R. J. & Westcott, D. A. (eds.). *Seed dispersal. Theory and its application in a changing world.* CABI, Wallingford.

SCHUPP, E. W., JORDANO, P. & GÓMEZ, J. M. 2010. Seed dispersal effectiveness revisited: a conceptual review. *New Phytologist* 188:333–353.

SCHWITZER, C., GLATT, L., NEKARIS, K. A.-I. & GANZHORN, J. U. 2011. Responses of animals to habitat alteration: an overview focusing on primates. *Endangered Species Research* 14:31–38.

TRAVESET, A., ROBERTSON, A.W. & RODRÍGUEZ-PÉREZ, J. 2007. A review on the role of endozoochory in seed germination. Pp. 78–103 in Dennis, A. J., Schupp, E. W., Green, R. J. & Westcott, D. A. (eds.). *Seed dispersal. Theory and its application in a changing world.* CABI, Wallingford.