

UNIVERSITY OF CALIFORNIA

Los Angeles

Impacts of hunting on seed dispersal in a Central African tropical forest

A dissertation submitted in partial satisfaction of the  
requirements for the degree Doctor of Philosophy  
in Biology

by

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2008

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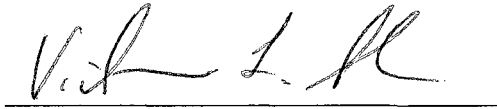
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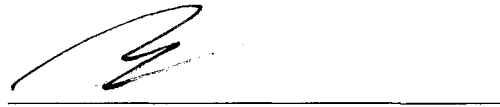
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To my parents,  
Show-Chi and Shirley Wang

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## ABSTRACT OF THE DISSERTATION

Impacts of hunting on seed dispersal in a Central African tropical forest

by

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Throughout the tropics, mammalian seed dispersers are being hunted to local extinction, generating concern not only about the loss of these species, but also about the consequences for plants they disperse. In this dissertation, I compare two rainforest sites in Cameroon -- one with heavy hunting pressure and one protected from hunting -- to appraise the loss of mammalian seed dispersers and to assess the impact of this loss on (1) hornbills in genera *Ceratogymna* and *Bycanistes* (which compete with mammals for fruits), and (2) *Antrocaryon klaineianum* (Anacardiaceae), a tree which relies on mammals for seed dispersal.

Surveys of arboreal frugivores indicate that three of the five monkey species, as well as chimpanzee and gorilla, have been extirpated from the hunted forest. However, hornbills

seem to be thriving, with higher diversity, relative abundance, and diet species richness than in protected forest. I found evidence that they consume fruits of 50 species of tree and liana, disperse 26 species to their nest sites, and seedling plot surveys at nests confirmed that hornbill activity influences seedling composition in both protected and disturbed forests.

Although hornbill-diet species are receiving dispersal services, mammal-dispersed species (such as *A. klaineianum*) may be in peril. Diaspore counts underneath *A. klaineianum* adults indicate that seed removal is severely reduced in the hunted forest. Furthermore, genetic exclusion analysis of maternally-inherited endocarp tissue from diaspores collected under the canopies revealed that seed dispersal in the hunted forest is also greatly reduced. Far fewer seeds had an origin other than the putative “mother” above in the hunted than the protected forest (2% vs 48%) and far fewer seeds were dispersed away from conspecific canopies (4% vs. 88%). This results in an effective genetic neighborhood ( $N_e$ ) that 55% smaller in the hunted forest (3.49 vs. 7.83) and an effective neighborhood area that is less than one-sixth that in the protected forest (0.42 vs 3.09 km<sup>2</sup>).

This study provides strong evidence that loss of dispersal agents can lead to reduced seed dispersal and drastically reduced genetic neighborhoods, disrupting the dispersal loop and creating an acute risk of loss of genetic variability.

## CHAPTER 1

### **Hornbills provide effective seed dispersal in hunted and protected Afrotropical forest**

#### **Introduction**

Tropical forests around the world are threatened by commercial logging and hunting, slash-and-burn agriculture, and fuelwood exploitation (FAO 1993; Laurance 1999). These negative forces sometimes act synergistically (Laurance et al. 2002): in Africa, once logging roads penetrate the forest, hunting of wildlife increases dramatically because the roads provide a means of transporting the “bushmeat” to urban markets (East et al. 2005; Robinson et al. 1999; Wilkie et al. 2000). In African tropical forests, the majority of animals sold as bushmeat are mammalian frugivores (Fa et al. 2005), and in many areas, important seed-dispersing mammals are being hunted to local extinction (Wang et al. 2007), with potentially negative consequences for the approximately 80% of tree species with seeds that are adapted for vertebrate dispersal (Jordano 1992). Nonetheless, some frugivorous animals (notably large birds, such as hornbills and turacos) can persist in disturbed African forests, and with the decline of the other animals in their guild, it is increasingly important to understand the dispersal services they provide in disturbed habitats.

Throughout Central Africa, the trade of bushmeat has emerged as a driving force of local economies (Fa et al. 2006). In the Congo Basin alone, over 4.9 million tons of bushmeat are harvested annually (Fa et al. 2002), and logging truck drivers routinely earn

extra income by carrying bushmeat, including that of endangered species such as chimpanzee and gorilla, to urban markets (Amman & Pierce 1995). In Cameroon, approximately 75% of the forests are currently in logging concessions (Bikie et al. 2000), and for many families, the sale of game meat is the second largest source of income after cocoa farming (Bikie et al. 2000; Muchaal & Ngandjui 1999). This hunting has pronounced effects on wildlife populations: in many of the selectively logged forests of Central Africa, many important seed dispersers, including elephant – *Loxodonta africana*, gorilla – *Gorilla gorilla*, chimpanzee – *Pan troglodytes*, monkeys – *Cercopithecus sp.*, *Lophocephus sp.*, duikers – *Cephalophus sp.*, and red river hogs – *Potamochoerus porcus* have been severely reduced or extirpated from the system, creating “half-empty” (Redford & Feinsinger 2001) or “empty” (Redford 1992) forests with relatively intact vegetation, but reduced animal populations (Wang et al. 2007).

In the heavily-hunted forests of Central Africa, frugivorous hornbills may be the most important remaining group of seed dispersers. They are occasionally taken for subsistence purposes, but generally these large birds are not commercially hunted in that region. In 1999, the selling price for a hornbill carcass in rural Cameroonian villages was less than the cost of the rifle cartridge needed to shoot the bird (B. Wang *pers. obs.*; M. Dethier *pers. comm.*). Previous research on hornbills in the protected forests inside the Dja Biosphere Reserve in southern Cameroon has shown that three species (*Ceratogymna atrata*, *Bycanistes cylindricus albotibialis*, and *B. fistulator sharpii*) disperse seeds of over 22% of the tree species, and that seeds passed by hornbills are still viable for germination (Whitney et al. 1998). Hornbill movement patterns and seed-passage times indicate that they can create extensive seed shadows, with an estimated 80% of consumed seeds moved more than 500 m from the parent plant (Holbrook & Smith 2000). Furthermore, hornbills can make long distance



movements of up to 290 km or more, suggesting that they sometimes move seeds vast distances (Holbrook et al. 2002). However, the effects of hornbills on vegetation structure of African forests has yet to be shown empirically, and little is known about hornbill ecology and their seed dispersal role in disturbed forests that have been impacted by human activities.

Here we compare seed dispersal dynamics of large forest hornbills in a human-disturbed and a protected Central African forest. First, we use a between-site comparison of hornbill species diversity, relative abundance, and biomass to establish whether the disturbed forest supports hornbill populations. Second, we present diet profiles of the four primarily frugivorous hornbill species (the three species listed above plus *Tockus fasciatus*) at the disturbed forest site and make between-site comparisons of diet species richness. We also make between-site comparisons of fruit availability to determine if differences in diet species richness can be explained by differences in fruit availability. Third, we examine hornbill seed dispersal by quantifying passed seeds collected from traps underneath hornbill nests – these are seeds that have been dispersed away from their parent plants to the sites where they were collected. Fourth, we assess the impact of hornbill seed dispersal on vegetation composition at their nesting sites by evaluating whether seedling plots in front of hornbill nests (that receive the input of hornbill-dispersed seeds) have higher abundance and diversity of hornbill diet species than control plots located behind those nests. Finally, we discuss conservation implications of these findings.

## Methods

### *Study areas*

The data for this study were collected in 1997, 1999, and 2003 in the vicinity of the Dja Biosphere Reserve in Southern Cameroon. The 526,000 ha Dja Reserve is the largest protected area in Cameroon (Sayer et al. 1992); it is bounded on three sides by the Dja River, a tributary of the Congo (Fig 1.1). The vegetation is semi-deciduous lowland forest, and elevations range from 400-800m (Letouzey 1968). Average annual rainfall is 1600 mm, and the climate features two wet seasons and two dry seasons, with major and minor rainfall peaks in October and May, respectively (Laclavère 1980).

Our human-disturbed forest site was the 16.3 km<sup>2</sup> Kompia Community Forest, centered around the village of Kompia, pop. 317 (Dethier 1998), located at (3°32'N, 12°52'E). Situated 23 km north of the Dja Reserve, Kompia's Community Forest abuts the less disturbed forests at the periphery of the Reserve. It received its official community forest designation from the Cameroonian government in 2000. Small-scale commercial selective logging operations were active there until 1995, small-scale slash-and-burn agriculture continues to be practiced, and the hunting pressure is so intense that most of the large-bodied mammal species (including elephants, gorillas, chimpanzees, and all but the smallest of the monkey species) have been extirpated (Wang et al. 2007). The habitat at Kompia is a mosaic of relatively mature forests that have never been under cultivation (44%), abandoned fields/secondary forests (20%), swamps (26%) and active plots - mostly manioc, peanuts, coffee, and cocoa (10%) (Tchatchou 1997).

The protected forest site was a 25 km<sup>2</sup> square centered at the Bouamir Research Station (3°11'N, 12°48'E) in the west-central region of the Dja Reserve. The site has never been commercially logged, and there has been no agriculture there for at least 100 years (Whitney & Smith 1998). Although hunting has been documented inside the boundaries of the Dja Reserve (Muchaal & Ngandjui 1995), during most of the study period, Bouamir was relatively well protected from poaching, due to the continuous presence of researchers, and its location 23 km from the nearest road or village. The habitat consists of upland forest interrupted by *Raphia* and *Uapaca* swamps and punctuated by rock inselberg outcroppings that rise up to 400m above the forest floor (Whitney et al. 1998). Bouamir is approximately 22 km south of Kompia (Fig 1.1).

*Hornbill diversity, relative abundance, density and biomass*

Hornbills were surveyed in 1999 using modified line-transects following methods described in Whitney & Smith (1998). At the hunted forest site, frugivores were surveyed on four routes, ranging in distance from 4.4 to 5.8 km, created from a combination of village trails and transects from a prior logging survey. At the protected forest site, surveys were conducted on seven routes, ranging in distance from 6.4 to 7.9 km, created from a network of pre-existing trails. We surveyed the protected forest from January to November, and the hunted forest from February to November. All routes in both forest sites were surveyed 3 times per month, resulting in a total of 640 km and 1,727 km surveyed in the hunted and protected forests, respectively.

All surveys were conducted between 06:00 and 12:00 by one local guide and one researcher working together. To avoid the bias of sampling the same part of the route at the

same time of day, the direction of each route was alternated so that it was never walked in the same direction in two consecutive surveys. Trails were walked at a pace of between 1.5 - 2.5 km/hr, and censuses were suspended or aborted during rain. Observers occasionally left the trail to confirm group size or diet item (see *Hornbill diets* below), but all groups were initially detected from the transect.

To calculate monthly relative abundance estimates, we first normalized for survey length by dividing the number of hornbills encountered by the length of the survey. Following Whitney & Smith (1998), transect width was set at 200m: hornbills estimated to be more than 100m from the trail were not included in our estimates. Estimates for replicate surveys of the same route in a given month were averaged to obtain the best per-kilometer estimate for that survey route for that month. Since each survey route was assumed to be a representative sample of that site, monthly survey route estimates ( $n = 7$  routes at the protected forest site;  $n = 4$  routes at the hunted forest site) were also averaged, yielding monthly relative abundance estimates for each hornbill species at each site. For each month and each species, we performed 10,000 Monte Carlo bootstrap simulations, using per kilometer estimates for each route ( $n = 7$  for protected forest,  $n = 4$  for hunted forest) to generate 95% confidence intervals for the monthly estimates (StataCorp 2003).

We then used the program DISTANCE (Thomas 2005) to estimate hornbill densities for all species that met the minimum statistical requirement of 60-80 sightings at either site (Buckland et al. 2001). DISTANCE calculates density of animal populations by independently calculating group density (using various models applied to the estimates of perpendicular distance from observer to animal groups) and group size (using size-biased regression corrections of estimates of group sizes). After excluding the 10% of the

observations furthest from the transect line to improve model estimation (Buckland et al. 2001), we tested the three available models (hazard rate, half normal, and uniform) using the cosines and simple polynomial adjustments, and for each species selected the density estimates of the model with the lowest Akaike Information Criterion (AIC). Finally, we combined these density estimates with average mass from Kemp (1998) to obtain biomass estimates.

#### *Hornbill diets*

Hornbill feeding observations were recorded during surveys and during walks on census trails with the specific aim of observing feeding frugivores. Based on the assumption that the habitats sampled by the survey routes were roughly representative of habitats of the entire study area, and given the effort to sample each sector of the forest equally, the tabulated feeding observations are assumed to be an accurate reflection of the hornbill diet profiles.

To compare hornbill diet species richness between sites we used the EcoSim software package (Gotelli & Entsminger 2001) to perform a rarefaction analysis which allows a comparison of diversity when number of observations are different between sites (Gotelli & Colwell 2001; Hurlbert 1971). This procedure randomly draws observations from the larger pool of observations until the number of randomly drawn observations reaches the “ $n$ ” of observations of the less numerous pool, and then calculates the species richness of the drawn observations. We performed 10,000 repetitions of this procedure, and report the average species richness of the draws and the 95% confidence interval.

### *Fruit availability*

We measured fruit availability using the raked-trail survey method (Whitney & Smith 1998; Zhang & Wang 1995). At each site, we made twice-monthly surveys of fallen fruits on a 1-m wide route (4.38 km in length in protected forest, and 4.67 km in disturbed forest) that was designed to sample the habitats roughly in proportion to their occurrence. Surveys at the protected forest site began in January; surveys at the disturbed forest site began in March. For each fruit patch encountered, we recorded the species and number of ripe and unripe fruits, and then cleared the fruits off the trail so they would not be recorded at the next sampling date. All fruit surveys were conducted in collaboration with experienced local guides.

Although we collected data on all fleshy fruits, in this analysis, we only included the hornbill diet species listed in Whitney et al. (1998) and/or Table 1.2 of this paper. For each sampling period in each site we first calculated: (1) the number of fruits, (2) the number of fruiting trees, and (3) the number of fruiting species, and then divided those totals by the length of the survey route to normalize for the difference in survey length. Since the fruits were removed from the trail after being counted, the fruits found on the trail were statistically independent from one month to the next, allowing us to use *t*-tests for between-site comparisons of the number of fruits. However, since the same fruiting trees and species might be fruiting from one sample period to the next, these measures are *not* independent, so we conducted repeated measures 1-factor ANOVA using 1<sup>st</sup> order auto-regressive covariance matrix AR1 to make between-site comparisons of the number of fruiting trees and fruiting species. Use of this covariance matrix adjusts for potential non-independence of the individual fruiting trees and the fruiting species from one sampling period to the next

(SPSS 2001). Dates when we missed surveys from either site were not used in these tests; the sampling date following a missed survey was also not used (because that sample would be biased towards more fruit).

*Seed dispersal to hornbill nest sites*

During the nesting season, each breeding hornbill female walls herself into a tree cavity with mud and her own feces, leaving a slit just wide enough for her bill to fit through. She lays her eggs and remains in this nest hole until her chicks are ready to fledge or her nest is disturbed. All food for the female and the developing chicks is provided by the male and all of the food waste (mostly seeds and insect carcasses) is ejected from the nest cavity by the female (Kemp 1995; Stauffer & Smith 2004). This material falls in a plume in front of the nest tree, and samples of this material lend insight into hornbill diets as well as provide direct evidence of seed dispersal. At the protected forest site, once we observed signs of nesting activity in mid-May, we erected 1m<sup>2</sup> elevated seed traps in front of 37 known and suspected nest cavities. At the disturbed forest site, seed traps were not installed until July, after an agreement was reached with local hunters that no hornbills would be shot at their nests. While hornbills were not commercially hunted in the region, on rare occasions, hornbills were shot for personal consumption (M. Dethier *pers. comm.*; B. Wang *pers. obs.*). After obtaining assurances that our seed traps would not serve as beacons for would-be hornbill hunters, we erected seed traps in front of 25 potential nest cavities. The material in the traps was collected, counted and identified every 7 to 10 days, and the information was tabulated for each hornbill species.

### *Seedling composition at hornbill nest sites*

Approximately one month after the end of breeding season (in late November and early December), we surveyed seedling plots at all nests that showed over 4 weeks of hornbill activity at Bouamir (1997,  $n=22$  nests; 1999,  $n=10$ ) and Kompia (1999,  $n=7$ ; 2003,  $n=10$ ). Following Kinnaird (1998), at each nest site we located an experimental 5x5 m seedling plot in front of the hornbill nest tree and a 5x5 m control plot behind the hornbill nest, equidistant from the trunk. Using the help of two experienced Baka guides, we identified and recorded all seedlings (< 1 m in height). In this paired-sample design, control plots experienced roughly the same environmental conditions as experimental plots, whilst receiving only ambient seed rain; experimental plots received ambient seed rain, plus the input of thousands of seeds brought to the nest site by the breeding hornbills. To analyze these data, we separated diet and nondiet seedlings according to hornbill diet lists presented in Whitney et al. (1998) and in this paper (Table 1.2), and used paired-samples  $t$ -tests (SPSS 2001) to test for differences in numbers of seedlings found in control and experimental plots.

## **Results**

### *Hornbill diversity, relative abundance, density and biomass*

Hornbill diversity was actually higher at Kompia, the human-disturbed site, than at Bouamir, the protected forest site. At Bouamir, we observed seven species of hornbills: *Ceratogymna atrata* - Black-casqued hornbill, *Bycanistes cylindricus albotibialis* - White-thighed hornbill, B.



*fistulator sharpii* - Piping hornbill – *Tockus fasciatus fasciatus* - African pied hornbill, *T. albocristatus* - White-crested hornbill, *T. camurus* - Red-billed dwarf hornbill, and *T. hartlaubi* - Black dwarf hornbill (Table 1.1). All of the observed *Ceratogymna* and *Bycanistes* species are primarily frugivorous, and all of the observed *Tockus* species are primarily insectivorous, with the exception of *T. fasciatus*, which has a diet that is split between fruits and insects (Kemp 1995). In the disturbed forest at Kompia, we observed all seven of the hornbill species that we found in the protected forest, plus one additional species: the Black-and-white casqued hornbill - *Bycanistes subcylindricus*. This large, frugivorous hornbill species was never observed at the protected forest site during 6 years of continuous research. Though the sightings at the disturbed forest site were relatively rare, this species has also been observed in other disturbed forests in the region (B. Wang *pers. obs.*; R. Fotso *pers. comm.*). These observations corroborate those of Kemp (1995) and Kalina (1988) who note that this species does particularly well in secondary forests on the periphery of large blocks of more mature forest.

Overall, hornbill relative abundances were also higher in the disturbed forest than in the protected forest. The relative abundances of four species: *B. cylindricus* (Fig 1.2b), *B. fistulator* (Fig 1.2c), *T. fasciatus* (Fig 1.2d) and *T. camurus* (Fig 1.2f) were significantly higher in disturbed forest than protected forest ( $p < 0.0002$  for all species). *C. atrata* (Fig 1.2a) and *T. albocristatus* (Fig 1.2e) abundances were not statistically different between the two forest types ( $p = 0.67$  and  $p = 0.62$ , respectively). *B. subcylindricus* and *T. hartlaubi* occurred too rarely to be included in these calculations. None of the hornbill species had significantly higher abundances in the protected forest.

Density estimations from DISTANCE software corroborate these findings (Table 1.1). With the exception of *T. fasciatus* densities – which showed no statistical difference

between the sites - the density measures show the same statistical trends as the relative abundance calculations. Three of the hornbill species (*B. cylindricus*, *B. fistulator*, and *T. camurus*), were more dense in the disturbed forest, and three of the species (*C. atrata*, *T. albocristatus*, and *T. fasciatus*) did not have statistically different densities between the two sites. As with relative abundance estimations, none of the species were significantly more dense in the protected forest than in disturbed forest. Using DISTANCE, we estimated the density of hornbills to be 70.9 birds/km<sup>2</sup> in the disturbed forest and 47.2 birds/km<sup>2</sup> in the protected forest, resulting in total biomasses of 62.4 and 43.5 kg/km<sup>2</sup> in the two forests, respectively.

#### *Hornbill diets and fruit availability*

A diet list of a fruit species taken by *C. atrata*, *B. cylindricus*, *B. fistulator*, and *T. fasciatus* in Kompia, the disturbed forest (compiled from 629 feeding observations and 3928 seeds collected at hornbill nests) reveals that hornbills consumed a wide variety of fruits in disturbed forest (Table 1.2). For purposes of comparison of diet species richness, we also summarize the 898 feeding observations and 6145 seeds collected at hornbill nests at Bouamir, the protected forest, during the same study period (Table 1.3). Both of the predominately frugivorous species, *C. atrata* and *B. cylindricus*, were observed to feed on about 40 species of fruits from just over 20 plant families. *B. fistulator* had a slightly higher insect intake - but it still was observed to take 25 fruit species from 14 plant families. Even *T. fasciatus*, whose diet is more evenly split between insectivory and frugivory, was observed taking fruits of 18 species from 12 plant families. Plant families whose fruits were particularly well represented in the diets of hornbills in disturbed forest were Annonaceae, Meliaceae, Moraceae, and Myristicaceae.

Rarefaction analysis revealed that hornbill diets were more species rich in the disturbed forest (Table 1.4). Three of the four hornbill species (*C. atrata*, *B. fistulator*, and *T. fasciatus*) had significantly higher diet species richness at the disturbed forest site. The diet species richness of the fourth species (*B. cylindricus*) in protected forest is on the lower bound of the confidence interval of the disturbed forest; while not quite statistically significant at the 95% confidence level, the trend is also towards higher diet species richness in the disturbed forest.

Higher diet species richness could be a by-product of more available fruits. However, after normalizing for survey length, we found no significant between-site differences in numbers of available hornbill diet fruits, fruit trees, or fruit species (Fig 1.3). Thus, in this case, the between-site differences in diet species richness cannot be explained by differences in fruit availability.

#### *Seed dispersal to hornbill nest sites*

All of the active nests that we found were occupied by either *C. atrata* or *B. cylindricus*. We identified a total of 26 species of seeds in the traps in front of hornbill nests in the disturbed forest (1791 seeds representing 23 species at *C. atrata* nests; 2137 seeds representing 20 species at *B. cylindricus* nests). All of these were passed seeds, devoid of fruit pulp, presumably dispersed by hornbills. Thus, the “N” symbols in Table 1.2 can be regarded as plant species that we observed to be dispersed by hornbills in the breeding season. The diversity of seeds collected at hornbill nests was higher at the protected forest site (29 and 28 species for *C. atrata* and *B. cylindricus*, respectively); this is not surprising considering the longer duration of sampling and the larger number of seeds collected at the primary forest

site. When we considered only the seeds collected in traps at the protected forest site from August to November (a sampling period comparable to that of the disturbed forest), and used EcoSim to rarefy the number of seeds to equal sample sizes, there was no difference in species richness between the two sites (24.8 species [95% confidence interval 24 – 25 species] and 22.7 species [95% confidence interval 20 – 24 species], for *C. atrata* and *B. cylindricus*, respectively).

#### *Seedling composition at hornbill nest sites*

At both sites, in both years surveyed, there were significantly more seedlings of hornbill diet species in front of hornbill nests than behind them (Fig 1.4), supporting our hypothesis that hornbill activity is significantly affecting seedling composition of African forests, at least at their nesting sites. Furthermore, in all cases, the number of non-diet seedlings was not significantly different between front and rear (Fig 1.4), indicating that abiotic conditions in front of and behind nests were similar, and that observed differences in seedlings of hornbill diet species were due to hornbill activity.

## **Discussion**

#### *Effective seed dispersal by hornbills*

This study strongly indicates that seed dispersal by hornbills is affecting seedling composition in both human-disturbed and protected Central African rain forests. It has been notoriously difficult to make direct, empirical links between seed disperser activity and

composition and structure of the resulting vegetation (Schupp & Fuentes 1995; Wang & Smith 2002) and while studies of this type have been emerging for Neotropical monkeys at their roosting sites (Julliot 1997; Russo & Augspurger 2004), and Southeast Asian hornbills at their nesting sites (Kinnaird 1998; Kitamura et al. 2004), ours is the first to make this link in Central African forest and the first to connect disperser activity and vegetation structure in human-disturbed forest.

In a previous three-year study at Bouamir, our protected forest site, Whitney *et. al.* (1998) tabulated feeding observations and seeds collected from traps under nests, finding that the three largest hornbill species collectively consumed fruits of 59 tree and liana species, and dispersed seeds of 25 of those species at their nesting sites. They also found that gut passage times were relatively long and that seeds passed through a hornbill's gut were viable for germination, suggesting that hornbills can be effective dispersers. Here we confirm that suggestion with two years of seedling data from that protected forest – for both years, we found more seedlings of hornbill diet species in front of hornbill nests than behind them, indicating that the seeds dispersed by hornbills are germinating to the seedling stage, and that their diet choices and dispersal activity are affecting vegetation composition.

Our seedling study focuses on seed dispersal to nest sites during the breeding season; however, it is very likely that hornbill dispersal has even more influence on vegetation structure during the rest of the year. Holbrook and Smith (2000) combined hornbill gut passage times and movement patterns during the non-breeding season, finding that 80% of the seeds consumed by hornbills were moved over 500m. Furthermore, these seeds are regurgitated or defecated in much smaller clumps throughout the forest than the dense concentrations of seeds in front of hornbill nests, such that they are much less likely to

suffer from reduced recruitment due to inter and intra-specific density-dependent competition (Connell 1971; Harms et al. 2000; Janzen 1970; Kitamura et al. 2004). As seedlings provide the initial “template” for recruitment of adult forest trees and lianas (Wright et al. 2007), the activities of the hornbills are probably playing a major role in maintaining the populations of the tree species whose seeds they disperse.

*Seed dispersal by hornbills in human-disturbed forests*

The results of this study also allow us to extend those findings to human-disturbed Afrotropical forest. We found that Kompia, our human-disturbed forest supports higher hornbill diversity, relative abundance, and biomass than Bouamir, its protected forest counterpart. Moreover, the hornbills there are also providing extensive seed dispersal services: we provide evidence of them feeding on the fruits of 50 species of trees and lianas and dispersing seeds of 26 species to their nest sites. During the same period (1999) at Bouamir, we observed hornbills feeding on fruits of 54 species of trees and lianas and dispersing 29 species of seeds to their nest sites. When rarefied to equal sample sizes, the hornbill diet species richness is actually significantly higher in the disturbed forest than the protected forest and there is no difference in number of species dispersed to nest sites. Analysis of fruit availability indicates that the higher diet species richness cannot be attributed to a greater abundance or richness of available fruit and may be due dietary release in the absence of the monkeys, with whom they share most of their diet species and by whom they are usually displaced at feeding trees (French & Smith 2005).

As in protected forest, in both years examined, extensive dispersal by hornbills had a significant effect on seedling composition at nesting sites. Furthermore, for the same

reasons as in protected forest, it is very likely that dispersal by hornbills has even more influence on seedlings during the non-breeding season. Indeed, in disturbed forests, hornbill seed dispersal activity probably has even greater influence than in the protected forest, due to the greatly reduced seed disperser assemblage (Wang et al. 2007).

### *Conservation implications*

Our study indicates that human-disturbed forests support hornbill biodiversity, at least in the short term. These findings align with those of other studies of hornbills in disturbed forests of Malaysia (Johns 1987), India (Datta 1998), Indonesia (Anggraini et al. 2000), and Cameroon (Whitney & Smith 1998). As it is becoming increasingly difficult to find and preserve tropical forests that have not been degraded by humans (Boahene 1998; Laurance 1999), we must consider the biodiversity conservation potential of disturbed forests and integrate these forests into our management plans. However, we must be careful not to overestimate the potential of these disturbed and/or secondary forests. It is true that the abundance and biomass of hornbills at our disturbed forest site was actually higher than in the protected forest; however, it is not clear whether this difference is due to some intrinsic property of the disturbed forest, or due to ecological release of the hornbill populations because all but one species of monkey have been ecologically extirpated from the system (Wang et al. 2007).

It is also important to recognize that all human-disturbed forests are not created equal. In this study, we only examine one human-disturbed forest in detail - this forest (the Kompia Community Forest) is heavily hunted, but only lightly impacted by logging and village agriculture. When we compare our results with those from Somalomo (Whitney &

Smith 1998), a site which is subject to similar hunting pressures, but is more impacted by logging and agriculture, we see differences in hornbill abundance -- especially in *C. atrata*. The previous study found lower abundance and biomass of this species in the disturbed forest of Somalomo than in the protected forests at Bouamir, while in the current study, we find no statistical between-site difference in abundance or biomass for this species when summed over the year. *C. atrata* prefers mature forest habitats and is more secretive than the other large hornbill species (Whitney & Smith 1998) - thus, the habitat at Kompia (with more mature forest and more swamps that are difficult for humans to traverse) may be more suitable for *C. atrata* than the habitat in Somalomo.

Of course, responses of disperser populations vary across different levels of disturbance. For example, Chapman *et al.* (2000) report that primate populations in Kibale National Park, Uganda can persist in lightly logged forests, even 28 years after disturbance, but they are sharply depressed in heavily logged forests. The same is probably the case with hornbills: we can hypothesize that there is a threshold of disturbance (Huggett 2005) where habitat becomes unsuitable for hornbills, and that threshold differs across hornbill species. Even if those thresholds are not reached, the effectiveness of hornbills as seed dispersers could still be compromised, as in the case of Pacific flying foxes, which cease to provide seed dispersal services before they become rare (McConkey & Drake 2006).

It is becoming clear that dispersal services are a critical component of the recovery process of degraded landscapes and that seed availability may be a limiting factor to that recovery (Duncan & Chapman 1999; Neilan *et al.* 2006; Wijdeven & Kuzee 2000; Wunderle 1997; Zimmerman *et al.* 2000). Our research provides strong evidence that hornbills are providing effective seed dispersal services in both protected and disturbed Central African



forests and that those seed dispersal services impact resulting vegetation structure. The importance of this seed dispersal is elevated by the extirpation of large-bodied mammalian dispersers from the disturbed forests. Wild meat extraction from Congo Basin forests is reaching staggering proportions (Bennett et al. 2002; Fa et al. 2002; Milner-Gulland & Bennett 2003), and the large-bodied mammalian dispersers - including elephant, chimpanzee, monkeys, duiker, and red river hog - are all heavily hunted (Robinson et al. 1999; Wang et al. 2007; Wilkie et al. 2000). Compared to more preferred game animals, hornbills are relatively lightly hunted, and our results strongly suggest that they are one of the most important groups of seed dispersers that remain.

Our findings, however, should not be interpreted to imply that hornbills are a panacea for the recovery of human-degraded forests. Due to physiological constraints (such as gape width) and food preferences (birds tend to avoid fruits with sticky latex), hornbills do not consume many of the species that are normally dispersed by the extirpated animals. For example, Poulsen et al. (2002) found that although hornbill and primate species may have as many as 36 diet species in common, proportional dietary overlap is actually quite low, and these groups are not redundant as seed dispersers. While most of the species that are taken by hornbills are also taken by monkeys, the opposite is not true: monkeys feed on many species that are not dispersed by hornbills (Poulsen et al. 2002). For example, 74 of the 120 species of tree and liana consumed by monkeys (Poulsen et al. 2001) were not observed to be eaten by hornbills in either this study or a previous three-year study (Whitney et al. 1998). Nonetheless, our results support the idea that in both human-disturbed and protected forests, seed dispersal by hornbills is an important determinant of vegetation structure (Schupp & Fuentes 1995; Wang & Smith 2002), leading to two critically important

conservation questions for further research: (1) what is the fate of the tree species that have lost their dispersers? and (2) in the absence of other dispersers, will disturbed forests come to be dominated by hornbill-dispersed trees?

### *Conclusions*

Based on evidence from hornbill diversity, relative abundance, biomass and diet species richness, we find that human-disturbed forests in Central Africa are potentially suitable habitat for hornbills. Furthermore, feeding observations and collection of dispersed seeds at nest sites indicates that hornbills can provide essential seed dispersal services in disturbed forests, and seedling plots at hornbill nests demonstrate that hornbill activity can affect seedling composition in both protected and disturbed forests, suggesting that the birds play a crucial role in maintaining plant populations. Conservation managers should consider the home range and reproductive needs of hornbills when devising conservation and forest management plans in the region; they also should consider (but not overestimate) the potential of human-disturbed secondary forests to contribute to biodiversity conservation. Also, as other game becomes more scarce, hornbills are increasingly being shot for bushmeat (Fa et al. 2006) and/or exportation of their bodies and/or skulls as decorative trophies (Trail 2007) – these trends must be curbed. Further research should be directed towards determining the exact response of hornbills to varying levels of habitat disturbance and towards understanding how a seed disperser assemblage that is dominated by hornbills will affect the regeneration and recovery of human-impacted secondary forests.

**Table 1.1.** Density of hornbills in protected and disturbed forest, Dja Reserve 1999

Species	Mass (kg) <sup>d</sup>	Bouamit (protected forest)				Kompia (disturbed forest)			
		n	Density (ind/km <sup>2</sup> )	Density 95% CL (ind/km <sup>2</sup> )	Biomass (kg/km <sup>2</sup> )	n	Density (ind/km <sup>2</sup> )	Density 95% CL (ind/km <sup>2</sup> )	Biomass (kg/km <sup>2</sup> )
<i>Ceratogymna atrata</i>	1.20	3527	23.2 <sup>a</sup>	20.7 - 26.0	27.8	1187	28.5 <sup>e</sup>	24.0 - 33.8	34.2
<i>Bycanistes subcylindricus</i>	1.20	0	-	-	-	5	-	-	-
<i>Bycanistes cylindricus</i>	1.13	882	9.0 <sup>b</sup>	7.4 - 10.8	10.2	732	<b>15.3<sup>c</sup></b>	12.2 - 19.3	17.3
<i>Bycanistes fistulator</i>	0.55	101	6.5 <sup>a</sup>	3.4 - 10.3	3.6	311	<b>15.6<sup>c</sup></b>	10.5 - 23.3	8.6
<i>Tockus alboeristatus</i>	0.31	219	1.7 <sup>b</sup>	1.3 - 2.0	0.5	89	1.1 <sup>b</sup>	0.9 - 1.4	0.3
<i>Tockus fasciatus</i>	0.26	447	4.3 <sup>b</sup>	3.1 - 5.9	1.1	232	5.7 <sup>a</sup>	4.4 - 7.6	1.5
<i>Tockus camurus</i>	0.10	554	2.5 <sup>b</sup>	1.9 - 3.3	0.3	323	<b>4.7<sup>a</sup></b>	3.8 - 5.9	0.5
<i>Tockus hartlaubi</i>	0.10	3	-	-	-	1	-	-	-
<b>Total</b>			<b>47.2</b>		<b>43.5</b>		<b>70.9</b>		<b>62.4</b>

<sup>a</sup> hazard rate cosines model.

<sup>b</sup> uniform cosines model.

<sup>c</sup> half-normal cosines model.

<sup>d</sup> Mean body masses are the midpoint of the mean adult male mass and the mean adult female mass from Kemp (1995)

Densities in **boldface** are significantly higher at that site

Table 1.2. Fruit Diet Items of Hornbills at Protected Forest Site, Kompia - 1999

Fruit species	Plant type <sup>a</sup>	Observations <sup>b</sup>			
		<i>C. atrata</i>	<i>B. cylindrica</i>	<i>B. fistulata</i>	<i>T. fasciatus</i>
<b>ANACARDIACEAE</b>					
<i>Lanuca velutischi</i>	T	N	F, N	F	
<b>ANNONACEAE</b>					
<i>Clitopholis glauca</i>	T	F, N	F, N	F	F
<i>Clitopholis patens</i>	T	F, N	F, N	F	
<i>Enantia chlorantha</i>	T	F, N	F, N	F	
<i>Polyalthia suaveolens</i>	T	F, N	F, N	F	F
<i>Xylopia aethiopica</i>	T	F, N	N		F
<i>Xylopia hypoleuca</i>	T	F, N	F	F	F
<i>Xylopia rubescens</i>	T				
<b>APOCYNACEAE</b>					
<i>Rauvolfia macrophylla</i>	T		F		
<i>Tabernaemontana penduliflora</i>	T	F	F	F	F
<b>ARECACEAE</b>					
<i>Laccosperma secundiflorum</i>	L	N			
<i>Elais guineensis</i>	T	F, N			
<i>Raphia monbuttorum</i>	T	F, N			
<b>BURSERACEAE</b>					
<i>Canarium schweinfurthii</i>	T	F, N	F, N	F	F
<b>CAESALPINACEAE</b>					
<i>Dioscoreanthus benthianus</i>	T		F	F	F
<i>Erythrolobos suaveolens</i>	T	F			
<b>COMBRETACEAE</b>					
<i>Terminalia superba</i>	T	F	F, N		F
<b>CONNARACEAE</b>					
<i>Rouropis obliquifoliolata</i>	L	N	N		
<b>DRACAENACEAE</b>					
<i>Draecena arborea</i>	T	N	F, N		
<b>EUPHORBIACEAE</b>					
<i>Macaranga sp.</i>	T		F		
<i>Ricinus communis</i>	T	N			
<i>Uapaca sp.</i>	T	F		F	
<b>IRVINGIACEAE</b>					
<i>Desfontainia glaucescens</i>	T		F		
<i>Nuclea diderrichii</i>	T			F	
<b>LECYTHIDACEAE</b>					
<i>Pterocarpus macrocarpus</i>	T	F			
<b>MELIACEAE</b>					
<i>Guarea exaltata</i>	T	F, N	F, N	F	
<i>Guarea thompsonii</i>	T	F	F		
<i>Trichilia rubescens</i>	T		N		
<i>Trichilia velutischi</i>	T	F, N	F	F	F
<i>Pentaclethra macrophylla</i>	T		F		
<i>Piptadeniastrum africanum</i>	T	F	F	F	
<b>MORACEAE</b>					
<i>Ficus elastica</i>	L	F	F	F	F
<i>Ficus exasperata</i>	T	F	F	F	F
<i>Ficus sp.</i>	L	F	F	F	F
<i>Musa sapientum</i>	T	F, N	F, N	F	F
<i>Triplaris madagascariensis</i>	T		F	F	
<b>MYRISTICACEAE</b>					
<i>Coelocaryon prussii</i>	T	F, N	F, N		
<i>Pyrenanthe angolensis</i>	T	F, N	F, N	F	
<i>Standleya kamerunensis</i>	T	F, N	F	F	F
<b>OLACACEAE</b>					
<i>Hastiera gimmereri</i>	T	F, N	F, N	F	
<b>PAPILIONACEAE</b>					
<i>Baphia leptobotrys</i>	T		N		
<b>RHAMNACEAE</b>					
<i>Macropis emini</i>	T	F, N	F, N	F	F
<b>RUBIACEAE</b>					
<i>Morinda lucida</i>	T	F	F	F	
<i>Pausanystax brachyhyrsa</i>	T		F	F	F
<b>SAPINDACEAE</b>					
<i>Ericaceum macrocarpum</i>	T				F
<b>SIMARUBACEAE</b>					
<i>Odyndes gibbonensis</i>	T	N	N		
<b>STERCULIACEAE</b>					
<i>Eriobroma oblongum</i>	T	F	F		
<b>ULMACEAE</b>					
<i>Celtis adolfi-friederici</i>	T		F		
<i>Celtis mlabradii</i>	T	F	F	F	F
<b>VERBENACEAE</b>					
<i>Vitex sp.</i>	L	F			

<sup>a</sup> Plant type: T = tree, L = liana

<sup>b</sup> Observations

F = direct observation of feeding on this species

N = seed of this species found in seed trap at hornbill nest

**Table 1.3.** Feeding Summaries of Hornbills in Protected and Disturbed Forest

	Hornbill species			
	<i>C. atrata</i>	<i>B. cylindricus</i>	<i>B. fistulator</i>	<i>T. fasciatus</i>
<b>Disturbed Forest (Kompia)</b>				
<i>Feeding obs species</i>	33	36	26	18
<i>total number of feeding obs.</i>	183	304	95	48
<i>observations of feeding on fruit</i>	177	276	80	33
<i>Fruit % of diet</i>	97%	91%	85%	69%
<i>Nest trap species</i>	23	19	N/A <sup>a</sup>	N/A <sup>a</sup>
<i>number of seeds counted<sup>b</sup></i>	1791	2137	N/A <sup>a</sup>	N/A <sup>a</sup>
<b>Total species (Feeding Obs &amp; Nests)</b>	<b>39</b>	<b>41</b>	<b>25</b>	<b>18</b>
<b>Number of Families</b>	<b>22</b>	<b>21</b>	<b>15</b>	<b>12</b>
<b>Protected Forest (Bouamir)</b>				
<i>Feeding obs species</i>	41	34	12	12
<i>total number of feeding obs.</i>	519	277	44	58
<i>observations of feeding on fruit</i>	504	261	42	34
<i>Fruit % of diet</i>	97%	94%	95%	59%
<i>Nest trap species</i>	29	28	N/A <sup>a</sup>	N/A <sup>a</sup>
<i>number of seeds counted<sup>b</sup></i>	3002	3143	N/A <sup>a</sup>	N/A <sup>a</sup>
<b>Total species (Feeding Obs &amp; Nests)</b>	<b>46</b>	<b>44</b>	<b>12</b>	<b>12</b>
<b>Number of Families</b>	<b>23</b>	<b>24</b>	<b>9</b>	<b>8</b>

<sup>a</sup>N/A - no nests found for these hornbill species

<sup>b</sup> 13 unidentified seeds were not included in this table

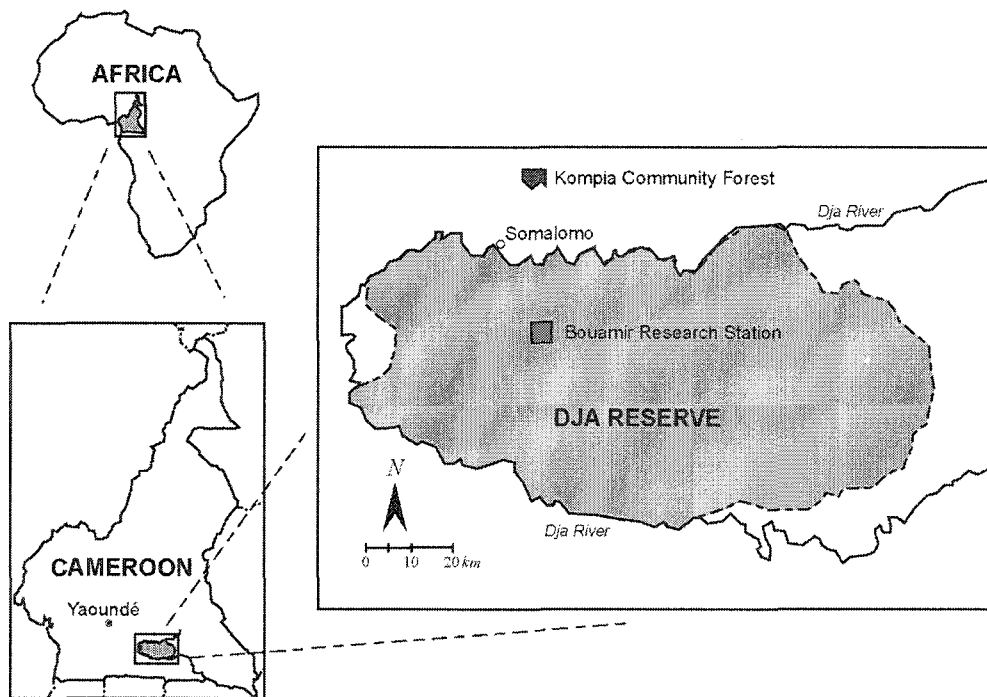
**Table 1.4.** Comparative Species Richness of Diets after Rarefaction

Hornbill species	Number of diet species		Rarefied "n"
	Disturbed forest	Protected forest	
<i>C. atrata</i>	33 *	26.3 (21-31)	177
<i>B. cylindricus</i>	35.4 (34-36)	34	261
<i>B. fistulator</i>	19.3 (16-22) *	12	42
<i>T. fasciatus</i>	18 *	12.8 (12-13)	33

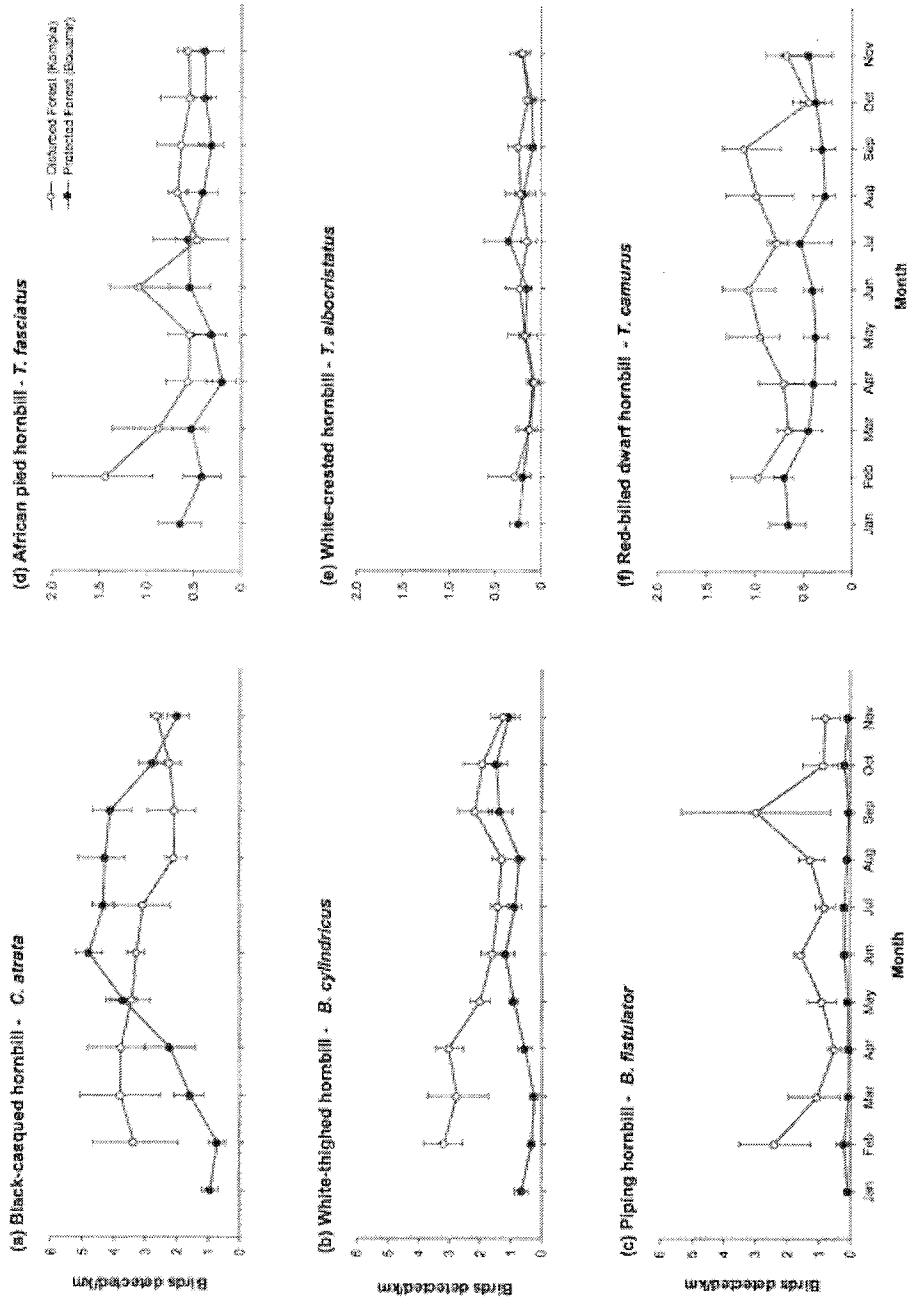
Numbers in italics are rarefied species richness - these are lower than observed species richness. Numbers in parentheses are 95% confidence intervals from the rarefaction analysis.

Rarefied "n" = number of feeding observations at the site with fewer observations. The feeding observations at the site with more observations were rarefied to this number of observations to compare species richness. *See text for more details.*

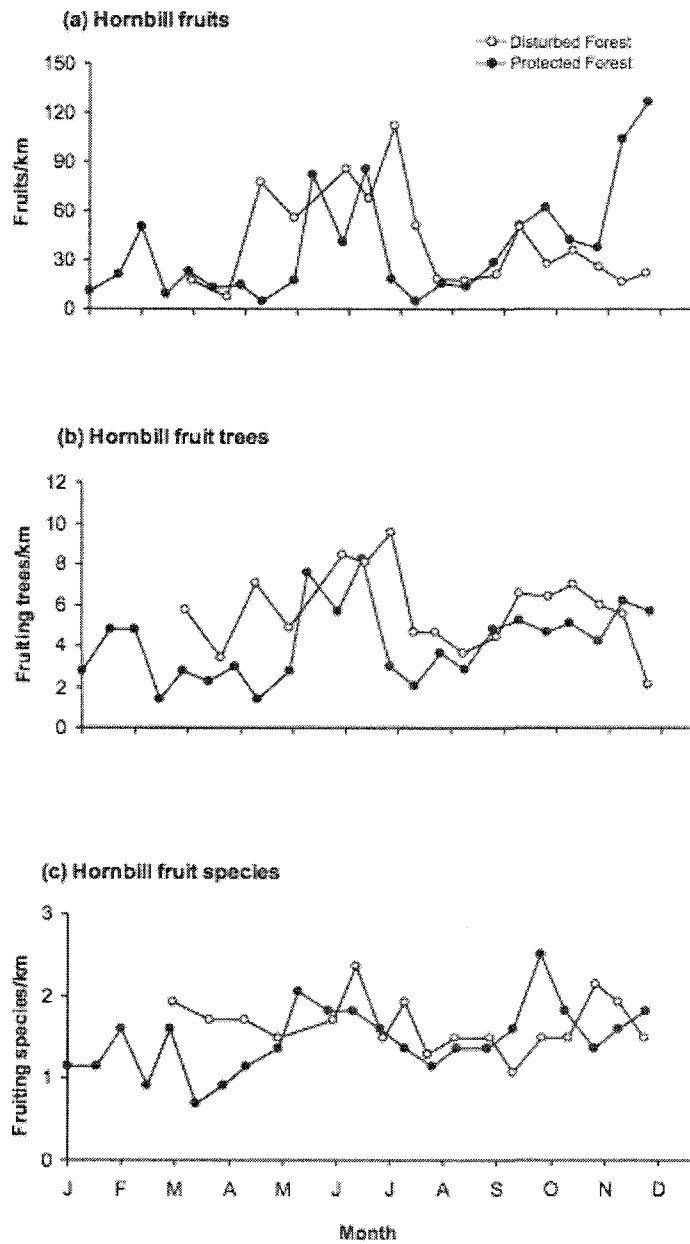
\* Indicates significantly higher hornbill diet species richness at this site



**Figure 1.1** Study site locations - Kompia (human-disturbed forest site) is 22 km north of Bouamir (protected forest site) and shares the same rainfall and climate patterns.

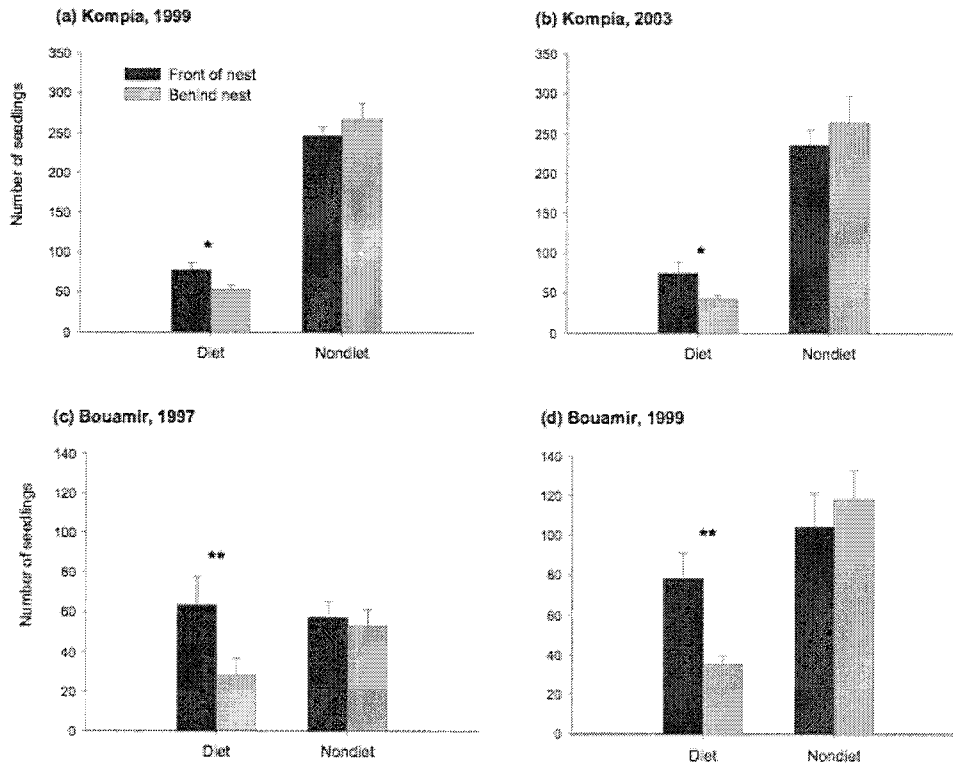


**Figure 1.2.** Hornbill abundances at Bouamir (protected forest) and Kompia (disturbed forest), 1999. Error bars represent 95% confidence intervals. Note scale differences between (a), (b), (c) and (d), (e), (f). Bootstrap probability of no significant difference between-sites summed across the year: *C. atrata* -  $p=0.67$ ; *B. cylindricus* -  $p<0.0002^{**}$ ; *B. fistulator* -  $p<0.0002^{**}$ ; *T. albocristatus* -  $p=0.62$ ; *T. camurus* -  $p<0.0002^{**}$ .



**Figure 1.3.** Hornbill diet fruit availability from raked fruit trail, 1999. (a) Hornbill fruits –  $t$ -test:  $t=0.56$ ;  $df=28$ ;  $p=0.58$ . (b) Hornbill fruit trees – repeated measures ANOVA using co-variance matrix (see text):  $t=-1.36$ ;  $df=5.09$ ;  $p=0.23$ . (c) Hornbill fruit species – repeated measures ANOVA using co-variance matrix:  $t=0.70$ ;  $df=6.81$ ;  $p=0.51$ .





**Figure 1.4.** Number of seedlings in plots in front of and behind hornbill nests, in disturbed forest (Kopia) (a) 1999, (b) 2003, and in protected forest (Bouamir) (c) 1997, (d) 1999. Significantly more seedlings of diet species in front of than behind hornbill nests for all years at all sites – one-tailed paired samples *t*-tests (a)  $t=2.42$ ;  $df=6$ ;  $p=0.026$  (b)  $t=2.44$ ;  $df=9$ ;  $p=0.019$  (c)  $t=2.66$ ;  $df=21$ ;  $p=0.008$  (d)  $t=3.81$ ;  $df=9$ ;  $p=0.002$ . No difference between plots in front of and behind nests for nondiet species. Error bars represent standard errors, note scale differences between (a), (b) and (c), (d).

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## CHAPTER 2

### **Hunting of mammals reduces seed removal and dispersal of an Afrotropical tree**

#### **Introduction**

For plant populations, seed dispersal links the end of the reproductive cycle with the establishment of offspring (Wang & Smith 2002) and serves as the main conduit for both gene flow (Ouborg *et al.* 1999) and colonization of new habitats (Cain *et al.* 2000). Regrettably, throughout most of the tropics, anthropogenic hunting of seed-dispersing vertebrates may be disrupting these critical seed dispersal processes (Chapman & Onderdonk 1998; Wright *et al.* 2000; Roldan & Simonetti 2001; Wright *et al.* 2007). This phenomenon is particularly problematic for tropical forest restoration and regeneration, as degraded forests often rely on the input of dispersed seeds to begin or accelerate their recovery (Martinez-Ramos & Soto-Castro 1993; Wunderle 1997; Duncan & Chapman 1999).

Hunting pressure in tropical forests is rapidly increasing due to burgeoning human populations, increasingly sedentary lifestyles, more technologically advanced weapons, increased accessibility of forests, and rising commercial demand for wild meat in urban centers (Peres & Lake 2003; Robinson & Bennett 2004). In the Congo Basin, over 4.9 million tons of wild meat are harvested annually (Fa *et al.* 2002) and in many Afrotropical forests, hunting pressure is driving populations of large mammals to local extinction, resulting in “empty forests” with relatively intact vegetation, but extremely reduced wildlife

(Redford 1992; Muchaal & Ngandjui 1999; Fa *et al.* 2005). The ecological ramifications go even further because the animals sought by hunters are often the most important mammalian seed dispersers in the forest (Gautier-Hion 1990; Poulsen *et al.* 2002). A recent study compiling hunting data from 36 sites in West and Central Africa found that of the harvested species, 82.0% of the carcasses and 80.4% of the biomass were frugivores (Fa *et al.* 2005). Given that approximately 80% of woody plants in African rainforests have fruits and seeds that are adapted for dispersal by vertebrates (Jordano 1992), it is of critical conservation importance to assess the consequences that this disperser loss will have for the seed dispersal of plants.

The seed dispersal process is notoriously complex, involving wide arrays of plants and animals and comprising many discrete stages and processes, each of which is contingent upon the previous one (Howe & Smallwood 1982; Schupp & Fuentes 1995; Wang & Smith 2002). Following fruit production, the first stage of dispersal is fruit and/or seed removal by dispersers. Fruits and seeds can be removed from the plant itself, or from the ground below the plant, but without this removal, fruits (with their seeds) remain directly below the plants, and seeds and seedlings may be unable to “escape” the high density-dependent mortality due to pathogens, seed predators, herbivores and eventually seedling competition under the parent plant (Janzen 1970; Connell 1971). In tropical systems, both arboreal and terrestrial seed removal are now relatively well-documented (e.g. Sork 1987; Forget 1996; Feer & Forget 2002) and it has been shown that loss of dispersers can lead to significantly reduced seed removal, resulting in greater numbers of seeds remaining directly underneath the mother tree (Asquith *et al.* 1999; Guariguata *et al.* 2000).

After seed removal, the next stage of the dispersal cycle is the movement of seeds away from the parent plant. Since the study of seed dispersal bloomed in the 1980's (Levey & Benkman 1999), tracking seeds to calculate dispersal distances and study their ultimate fate has remained one of its most difficult challenges. Many researchers have attempted to track seeds by following their movement from parent plant to dispersal destination (Howe & Smallwood 1982; Forget & Millerton 1991; Levey & Sargent 2000; Gomez 2003), but this is extremely difficult for dispersal via animals due to the difficulty of physically following dispersers through natural habitats (Dalling *et al.* 2002; Wang & Smith 2002). More recently, advances in molecular genetics have allowed researchers to retrace the path of dispersed seeds and/or seedlings back to their maternal source(s) (Ouborg *et al.* 1999). In a direct genetic maternity analysis, maternally-inherited endocarp tissue of dispersed seeds is matched with genotypes of maternal source plants to calculate exact dispersal distances (e.g. Godoy & Jordano 2001; Grivet *et al.* 2005; Jones *et al.* 2005; Paireon *et al.* 2006). Successful application of this technique requires high resolution genetic markers, large sample sizes of progeny, genotypes of all or most potential seed source trees, and spatial locations of those potential sources (Smouse & Sork 2004). The latter two conditions are especially difficult to meet, and Grivet *et al.* (2005) provide an alternative methodology that only requires clusters (or pools) of dispersed seeds scattered across the landscape. This approach, called Probability of Maternal Identity (PMI), ignores the location and identity of the source trees and instead considers the probability that two seeds drawn from the same cluster have the same maternal source, subsequently employing genetic structure statistics to estimate genetic neighborhood size and seed dispersal distances.

Each of these approaches have advantages and disadvantages (reviewed in Smouse & Sork 2006), but they both require large numbers of dispersed seeds, which may be difficult or impossible to find at sites where dispersal agents, and by extension seed dispersal, have been reduced. An alternative approach is to determine the origin of seeds where they *can* be found, underneath the canopies of fruiting trees. Fruiting trees are attractive to dispersal agents as a food source, and can serve as dispersal foci (or seed sinks) when animals bring seeds from elsewhere and drop or eliminate them at that tree (Hamann & Curio 1999; Clark *et al.* 2004). Under this scenario, some proportion of the conspecific seeds underneath a fruiting tree will be from sources other than the tree above, and the maternally-inherited seed endocarp DNA of those dispersed seeds will not match that of the putative mother above. One can predict that in habitats where dispersal agents have been reduced, the proportion of these dispersed seeds will be lower and a higher percentage of seeds will have endocarp DNA that matches the mother tree above.

In this paper, we compare two rainforest sites in southern Cameroon -- one site with heavy hunting pressure and one site protected from hunting -- to appraise the loss of mammalian seed dispersal agents and to assess the impact of this loss on seed removal and dispersed seed arrival beneath the crowns of *Antrocaryon klaineinum* (Anacardiaceae), a mammal-dispersed tree. Specifically, we attempt to establish the link between loss of dispersal agents and loss of seed dispersal by addressing three questions: (1) Has hunting reduced populations of arboreal mammalian seed dispersal agents? (2) At the heavily-hunted site, are there more diaspores (the dispersal unit of the plant) underneath the *A. klaineinum* canopies, indicating less seed removal? And (3) Of the *A. klaineinum* diaspores underneath

those canopies, is there a reduced proportion of dispersed diaspores in the heavily-hunted site, indicating less seed dispersal?

## Methods

### *Study sites*

The protected forest site is a 25 km<sup>2</sup> square centered at the Bouamir Research Station (3°11'N, 12°48'E) in the west-central region of the 526,000 ha Dja Biosphere Reserve in Southern Cameroon. Although hunting has been documented inside the boundaries of the Dja Reserve (Muchaal & Ngandjui 1999), Bouamir has never been logged, has experienced no agriculture for at least 100 years, and has traditionally been relatively well protected from poaching, due to continuous presence of researchers, and its location 23 km from the nearest road or village. The habitat consists of semi-deciduous lowland forest interrupted by *Raphia* and *Uapaca* swamps and punctuated by rock inselberg outcroppings, which rise up to 400m above the forest floor (Whitney *et al.* 1998).

The hunted forest site is the Kompia Community Forest, centered around the village of Kompia, pop. 317, located at (3°32'N, 12°52'E). Situated about 22 km north of the Dja Reserve, and 37 km from the Bouamir study site, the Kompia Community Forest encompasses an area of 16.3 km<sup>2</sup>. Commercial selective logging operations were active there until 1995, small-scale slash-and-burn agriculture continues to be practiced, and hunting pressure is very intense: hunters bring >18 tons of bushmeat per year into the village – but the biomass of animals actually killed is probably much greater, as this figure does not

include animals that decompose in snares or animals that are sold and/or eaten before arriving in the village (Dethier 1998). The habitat at Kompia is a mosaic of relatively mature forests that have never been under cultivation (44%), abandoned fields/secondary forests (20%), swamps (26%) and active plots - mostly manioc, peanuts, coffee, and cocoa (10%) (Tchatchou 1997). More details about the sites are given in Whitney *et al.* (1998).

#### *Study species*

*Antrocaryon klaineianum* (Anacardiaceae) is an upper canopy rainforest tree produces commercially important softwood timber (it is listed as a Level 3 timber species by the Cameroonian government). From about September to December it also produces edible, fleshy, sugar-rich 2 - 3 cm green-yellow fruits each containing a single 1.5 - 2.5 cm diaspore – a stone containing 4 – 5 seeds encased in woody endocarp tissue. *A. klaineianum* diaspores (and the seeds contained within) are dispersed by gorilla (*Gorilla gorilla*) and chimpanzee (*Pan troglodytes*) (Poulsen *et al.* 2001) as well as monkeys (*Cercopithecus spp.*, *Lophocephus albigina*), duiker (*Cephalophus spp.*), and elephant (*Loxodonta cyclotis*) (Gautier-Hion *et al.* 1985; Yumoto & Maruhashi 1995). Large and small rodents are the primary seed predators (Gautier-Hion *et al.* 1985). With the exception of small rodents (<500kg), all of these animals are or have been heavily hunted in the Kompia Community Forest (Dethier 1998, B. Wang, *pers. obs.*)

#### *Surveys of arboreal frugivores*

Arboreal frugivores (large-bodied birds and primates) were censused using modified line-transects in 1999 and 2004, following methods described by Whitney & Smith (1998), Poulsen *et al.* (2001) and Wang & Smith (*in rev.*). At the hunted forest site, frugivores were

surveyed on four routes, ranging in distance from 4.4 to 5.8 km, created from a combination of village and hunting trails and transects from a prior logging survey. At the protected forest site, surveys were conducted on seven routes, ranging in distance from 6.4 to 7.9 km, created from a network of pre-existing trails. In 1999, we surveyed the protected forest from January to November, and the hunted forest from February to November. All routes in both forest sites were surveyed 3 times per month, resulting in a total of 640 km and 1,727 km surveyed in the hunted and protected forests, respectively. In 2004, all routes in both forest sites were surveyed 2 times during a period from mid-October to mid-November, resulting in a total of 43 km and 105 km surveyed in the hunted and protected forests, respectively.

All surveys were conducted between 06:00 and 12:00 by one local guide and one researcher working together. To avoid the bias of sampling the same part of the route at the same time of day, the direction of each route was alternated so that it was never walked in the same direction in two consecutive surveys. Trails were walked at a pace of between 1.5 - 2.5 km/hr, and censuses were suspended or aborted during rain. Observers occasionally left the trail to confirm frugivore species, group size, or diet item, but all groups were initially detected from the transect.

#### *Relative abundance estimates*

To calculate monthly relative abundance estimates, we first divided the number of primates of each species encountered during each survey by the length of that survey to generate a per-km estimate for each species. Following Whitney & Smith (1998), transect width was set at 200m: primates estimated to be more than 100m from the trail were not included in our



estimates. Estimates for replicate surveys of the same route in a given month were averaged to obtain the best estimate for that survey route for that month. Since each survey route was assumed to be a representative sample of that site, for each primate species, monthly survey route estimates for each site ( $n = 7$  routes at the protected forest site;  $n = 4$  routes at the hunted forest site) were also averaged, yielding a monthly relative abundance estimate for that primate species at that site.

We used the STATA software package (StataCorp 2003) to perform a Monte Carlo bootstrap analysis to generate confidence intervals for these estimates. For each month and each species, we performed 10,000 bootstrap simulations using the per-km estimates for each route ( $n = 7$  for protected forest,  $n = 4$  for hunted forest), and generated 95% confidence intervals from the distribution of the 10,000 values.

#### *Quantifying seeds under *A. klaineianum* canopies*

In November, 2004, near the end of the *Antrocaryon klaineianum* fruiting season, we assessed seed removal by quantifying seeds under the crowns of six fruiting *A. klaineianum* at each site. Focal fruiting trees were opportunistically chosen as they were discovered, but they were spatially distributed across the sites, with inter-tree distances ranging from 24 m to 4.6 km. At each tree, we randomly placed ten 1 m<sup>2</sup> quadrats and counted the number of “fresh” and “old” seeds in each quadrat. “Fresh” seeds from the 2004 season were distinguished from “old” seeds of previous seasons by the change in color of endocarps over time. These counts were averaged to obtain an estimate of the density of fresh and old seeds for each tree. Some (~50) of the fresh seeds as well as a leaf sample from each of the six “mother”

trees (dried and preserved with silica gel desiccant) were collected for genetic analysis (see below).

#### *DNA extraction and amplification*

We assayed 133 seeds; ~11 (range 10-12) seeds from underneath each of the six trees at each site (66 seeds from Kompia; 67 seeds from Bouamir). First, we used a high-speed rotary Dremel tool to cut ~20 mg of dry, woody endocarp tissue from each seed, changing the disposable blade after each seed. Each sample was then frozen with liquid nitrogen and ground into a fine powder in a clean mortar and pestle, and the DNEasy Plant Mini Kit (Qiagen) was used to extract DNA from the powdered samples. The same procedure was used to extract DNA from the leaf samples of each of the 12 (6 from each site) fruiting “mother” trees, except the Dremel tool was not needed to cut the leaf tissue.

Following DNA extraction, we used dye-labeled primers in conjunction with the Qiagen Multiplex Kit for Polymerase Chain Reaction (PCR) to amplify seven microsatellite markers that were developed specifically for *A. klaineanaum* (See Appendix I). From preliminary optimization work, we had already determined that the markers are variable and that they amplify well with unambiguous reads for allele sizes. Furthermore, preliminary testing of DNA extracted from endocarps of individuals with known mothers confirmed that the seed coat genotypes accurately reflect maternal genotypes. However, DNA yields from woody tissue (e.g. *A. klaineanaum* endocarp) are often lower than from leaf tissue, and the quality of this DNA is often less than optimal. Therefore, to generate more confident allele reads, 2 – 4 amplifications were performed for each marker for each sample. PCR product was analyzed on an ABI 3700 automatic sequencer at the UCLA Genotyping CORE

Facility. Results were imported into Genemapper v.3.7 (Applied Biosystems, Inc.) and electropherogram peaks for all samples were read and scored by BCW.

#### *Maternity exclusion analysis*

In our maternity exclusion analysis, we included all seed endocarp samples for which we could confidently genotype at least 3 of the 7 assayed markers. Our criteria for a confident genotype was duplicate identical genotype results from multiple PCRs and/or a single genotype result without conflicting genotype results from other PCRs. Because DNA from woody endocarp tissue can have null and/or weakly amplifying alleles due to its poor quality (Dakin & Avise 2004), we occasionally detected a non-amplifying allele of a heterozygote pair from replicate PCRs of the same sample. In these few cases, we did not consider a homozygous result to conflict with a heterozygous result, provided the homozygous allele was one of the alleles contained in the heterozygous genotype.

Because seed endocarp tissue is maternally-derived, its DNA should perfectly match that of the mother that produced it. However, random mutation and/or DNA degradation, as well as null and/or weakly amplifying alleles can cause mismatched genotype results from mother-offspring pairs. Therefore, to avoid over-estimation of seed dispersal, a seed was considered to have a source other than the tree above only when at least 2 of the 3-7 successfully genotyped markers did not match the putative mother tree above. Furthermore, as with the analysis of multiple PCRs of the same sample, we did not consider non-amplifying alleles of a heterozygote pair to be mismatches; in order to be assigned mismatch status, a genotype had to include at least one “novel” allele. While these measures bias us towards Type II errors (assignment of seed to mother above when source is actually another

tree), they limit Type I errors (assignment of seed to another source when its maternal origin is actually tree above), allowing for a more conservative analysis of seed dispersal (Dakin & Avise 2004).

## Results

### *Relative abundance of primates*

During 1999, the relative abundances of six primate species were significantly greater at Bouamir, the site that was protected from hunting, than at Kompia, the site with hunting (see Fig. 2.1). Three species of monkeys (*Cercopithecus nictitans*, *Colobus guereza*, *Lophocephus albigena*) as well as the chimpanzee (*Pan troglodytes*) have been effectively extirpated from Kompia (Fig. 2.1a - d), and an additional monkey species *Cercopithecus pogonias* (Fig. 2.1e) has been significantly reduced. Gorillas (*Gorilla gorilla*) have also been extirpated from the hunted forest site: they were never observed at that site during the study period, whereas they were observed 10 times during surveys at the protected forest site. Of the locally occurring diurnal primates, only *Cercopithecus cephus*, the moustached monkey, seems to maintaining a viable population in the hunted forest (Fig 1f) – there is even a trend towards higher abundances of moustached monkeys in the hunted forest, although for the majority of months, this difference is not statistically significant.

Surveys from October-November, 2004 were compared with averaged values of October and November of 1999. Relative abundances of all species presented in Fig. 2.1 in

2004 were within the confidence intervals calculated for 1999, indicating that relative abundances of primates had not changed significantly in the intervening years.

#### *Seed Removal of *Antrocaryon klaineinum**

We found dramatically different numbers of diaspores underneath the crowns of *A. klaineinum* at the two sites (Fig. 2.2). The ground underneath canopies in Kompia, the site where mammalian dispersers are heavily hunted, had significantly more diaspores from both the current fruiting season ( $t=-2.40$ ;  $df=5$ ; 1-tailed  $p=0.03$ ) and from previous seasons ( $t=-5.46$ ;  $df=5$ ; 1-tailed  $p=0.001$ ). This analysis does not distinguish between arboreal and terrestrial seed removal, nor does it distinguish seed dispersal from seed predation; however it is a clear indication of cumulative disruption of some or all of these seed removal processes.

#### *Dispersal of *A. klaineinum* diaspores to conspecific fruiting trees*

Of the diaspores assayed, 53 from the hunted forest and 48 from the protected forest yielded positive genotypes for at least 3 loci. For both sites, the eligible diaspores were distributed fairly evenly amongst the fruiting trees (average per tree: hunted site  $8.83 \pm 1.17$ , protected site  $8.00 \pm 1.26$ ). Our maternity exclusion analysis revealed that in the hunted forest with reduced mammal dispersal agents, only 1 of the 53 diaspores (2%) had a source that was other than the “mother” tree above. By contrast, 20 of the 48 diaspores (42%) from the protected forest had endocarps that did not match the fruiting tree above and were determined to be from dispersed diaspores (Fig. 2.3). This finding indicates that significantly

more seeds are dispersed to *A. klaineianum* trees in the protected forest than in the heavily hunted forest ( $\chi^2=24.20$ ,  $df=1$ ,  $p<0.001$ ).

## Discussion

This informative case study shows that the loss of mammalian seed dispersal agents can disrupt the seed dispersal process. Our results indicate that Kompia, the site experiencing heavy hunting pressure, is rapidly becoming an “empty forest”. Four of the five monkey species, as well as chimpanzee and gorilla, have been either extirpated or reduced in that forest. Tropical forests have a limited capacity to produce wild meat (Barnes 2002), and the large mammals targeted by hunters are particularly vulnerable to local extinction due to relatively low annual production and prolonged developmental periods (Robinson & Bodmer 1999; Brashares *et al.* 2001; Jerozolimski & Peres 2003). The only diurnal primate species that seems to be doing well at the hunted site is *Cercopithecus cephus*, the moustached monkey, and its persistence might be attributed its relatively high fecundity, and relatively short gestation and developmental period (Kingdon 1997). This species may be experiencing some ecological release due to the loss of other primates with which it competes for food and space. We have observed the same phenomenon for hornbills (see Chapter 1), and it is likely with the loss of mammalian seed dispersal agents, these birds, along with large turacos, will be the most important group of seed dispersers that remain.

Many tree species are not dispersed by those birds, and the loss of mammals is clearly affecting seed removal of some of those species. The dramatic differences in

diaspore abundance under the crowns of *A. klaineianum* adults between the two sites suggests the loss of seed predators as well as the loss of arboreal and terrestrial dispersal agents. We did not survey terrestrial dispersers or seed predator species in this study, but many of these species (notably elephant, duiker, cane rat, and brush-tailed porcupine) are also heavily hunted (Dethier 1998, B. Wang, *pers. obs.*), and their populations are likely to be reduced at the hunted forest site. Thus, the difference in seed removal could be due to an overall defaunation. Beckman and Muller-Landau (2007) found a similar difference in seed removal of *Oenocarpus mapora* (Arecaceae) seeds in hunted and unhunted sites in central Panama, and they used an experimental approach to demonstrate that this difference was due to both predation and dispersal. In general, reduced seed removal is good evidence that vertebrate populations are reduced, but not sufficient evidence that seed dispersal is diminished just as dramatically.

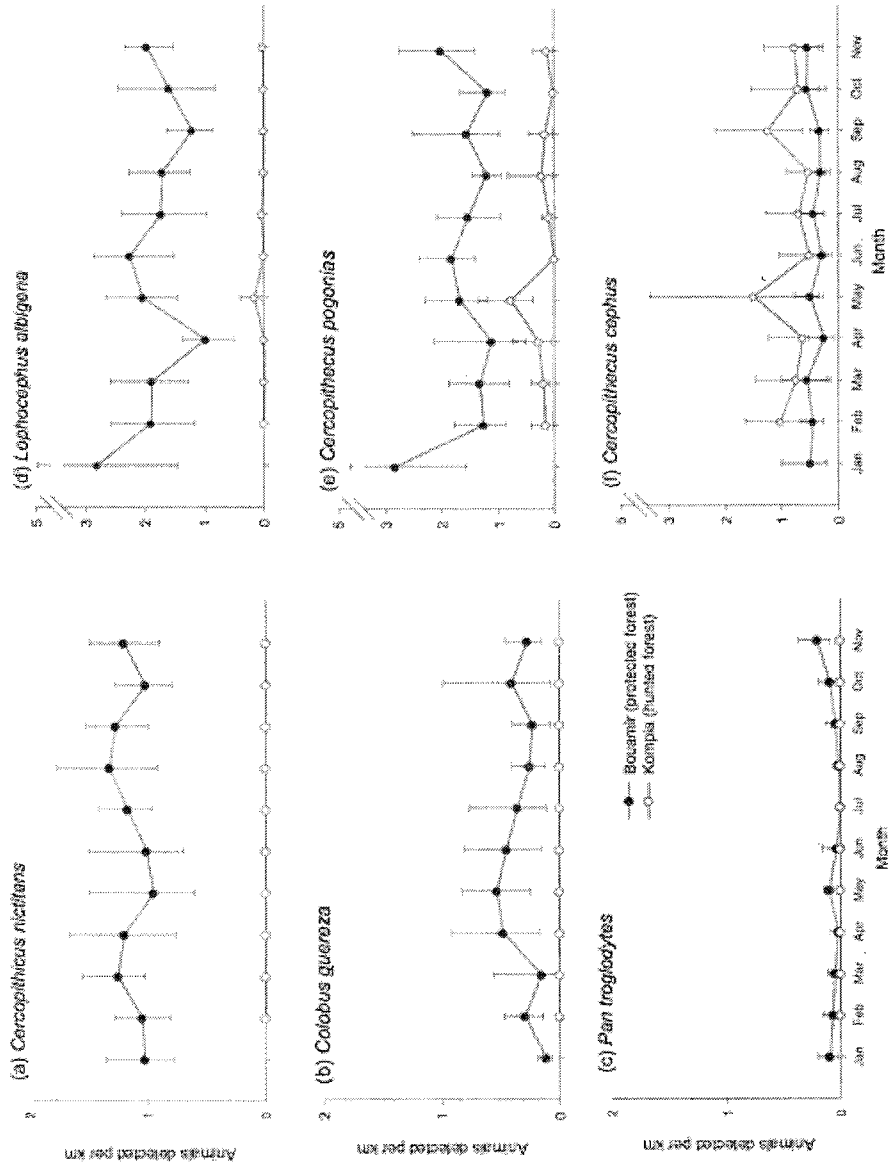
Our genetic analyses corroborate our interpretation that lack of seed removal signifies lack of seed dispersal, providing strong evidence of disruption of the seed dispersal loop in the hunted forest. These results show that seed dispersal is a prevalent and extensive process at Bouamir, the protected forest site. Even when using conservative criteria for maternity exclusion, a full 42% of the eligible *A. klaineianum* diaspores from that site were determined to come from sources other than the tree above, indicating that the fruiting trees are serving as dispersal foci for an active community of seed dispersers. By contrast, at Kompia, the hunted forest site with reduced dispersers, only one (2%) of the eligible diaspores was determined to have a source other than the “mother” tree above, indicating that alteration of the seed disperser community has disrupted the seed dispersal process.

This disruption will ultimately have consequences for vegetation composition, although the consequences appear to differ from site to site. Chapman and Onderdonk (1998) found that loss of mammalian dispersers in the Ugandan forest favors smaller-seeded and wind-dispersed species, whereas Wright *et al.* (2007) demonstrate that hunting in central Panama favors large seeded species, lianas, and seeds dispersed by bats, birds, and wind. Despite differing results about which species are favored, both of these empirical studies, as well as modeling studies (Muller-Landau 2007), suggest that plant species diversity decreases when mammalian seed dispersers are lost.

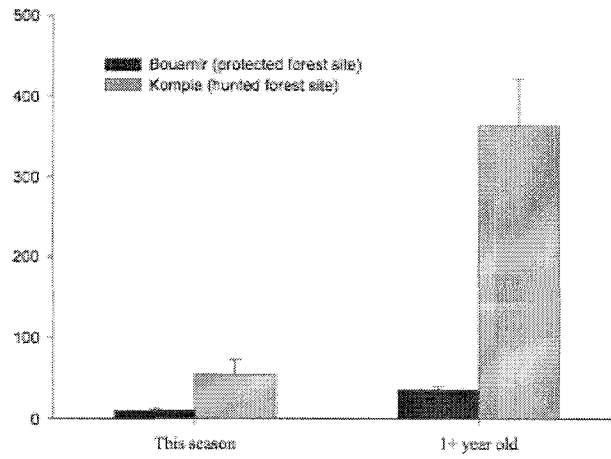
Ecologists who study seed dispersal have often commented on the difficulty of linking the activity of seed dispersing animals with the composition and structure of the resulting vegetation (Herrera *et al.* 1994; Schupp & Fuentes 1995; Wang & Smith 2002). Ironically, just as empirical studies that make that connection are emerging (e.g. Julliot 1997; Kinnaird 1998; Fragoso *et al.* 2003; Russo & Augspurger 2004), we are simultaneously finding that the loss of seed dispersal may reduce the viability of plant populations (Nunez-Iturri & Howe 2007; Wright *et al.* 2007). Our study indicates that hunting is disrupting the seed dispersal loop for vertebrate-dispersed seedlings. The genetic analyses not only provide concrete evidence that less dispersers equals less dispersal (leaving high densities of seeds undispersed and subject to density-dependent mortality due to factors such as competition, seed predation, and or pathogens), but they also indicate that hunting may change the spatial genetic structure of populations. The lack of dispersal can increase the genetic structure of plant populations and lower the size of the local genetic neighborhood, thus making these populations more at risk to genetic drift through smaller population size (Smouse & Sork 2004). A lack of dispersal could also reduce the overall demographic and genetic



connectivity of plant populations and may threaten long-term survival, especially in times of increased fragmentation (Sork & Smouse 2006). The long-term demographic and genetic impact of the loss of vertebrate dispersal agents will require future work across many species and locations. Meanwhile, the case study that we present here demonstrates that hunting interrupts the early phases of the seed dispersal loop of a mammal-dispersed African tropical forest tree species..

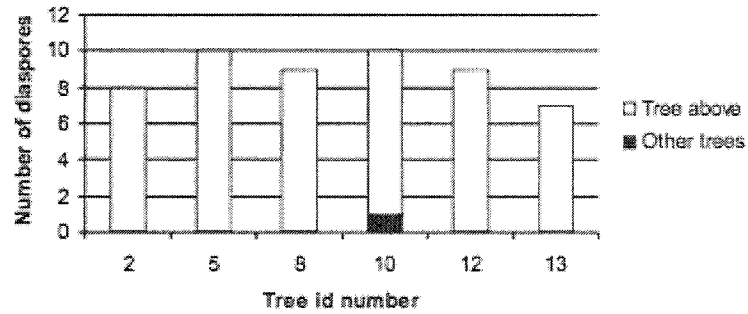


**Figure 2.1.** Relative abundances of primates at Bouamir (protected forest) and Kompia (hunted forest), 1999. (a) White-nosed guenon - *Cercopithecus nictitans*, (b) Black-and-white colobus - *Colobus guereza*, (c) Chimpanzee - *Pan troglodytes*, (d) Grey-checked mangabey - *Lophoceros albigena*, (e) Crowned guenon - *Cercopithecus pogonias*, (f) Moustached monkey - *Cercopithecus cephus*. Error bars represent 95% confidence intervals. Note scale differences between graphs (a), (b), (c) and graphs (d), (e), (f).

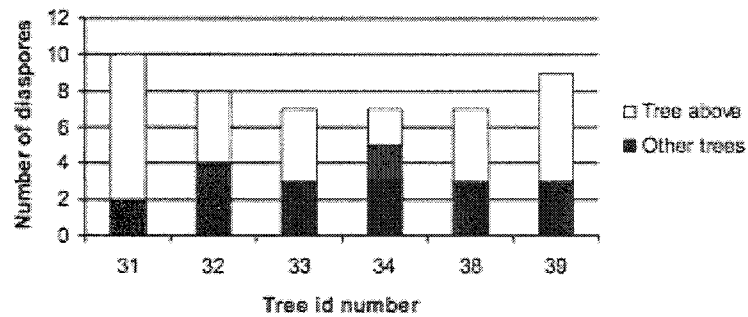


**Figure 2.2.** Density of *A. klaineianum* diaspores under crowns of fruiting trees. Significantly more diaspores were found under canopies at hunted forest site for both this season ( $t=-2.40$ ;  $df=5$ ; 1-tailed  $p=0.03$ ) and previous seasons ( $t=-5.46$ ;  $df=5$ ; 1-tailed  $p=0.001$ ). Error bars = Mean + 1 SEM.

**a. Hunted Forest**



**b. Protected Forest**



**Figure 2.3.** Origin of *A. klaineunum* diaspores found under fruiting “mother” trees in (a) Kompia (hunted forest) and (b) Bouamir (protected forest). Between 10 to 12 diaspores found under each tree were assayed for use in maternity exclusion analysis. Diaspores whose origin could not be determined (did not yield confident results at 3 or more loci) are not depicted on the graph.

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## CHAPTER 3

**There goes the neighborhood:**

**hunting reduces the genetic neighborhood of an Afrotropical tree**

### **Introduction**

Recent research has sounded the alarm that hunting of seed-dispersing animals may have consequences for recruitment and species composition of tropical forest trees (1); however, the genetic effects of this dispersal loss remain poorly understood. Tropical forests are dominated by angiosperms, which have only two opportunities for gene movement: movement of haploid pollen from paternal plant to maternal plant, and movement of diploid seeds from maternal plant to the site of germination. Because seeds carry twice the amount of genetic material, we hypothesize that loss of seed dispersal will result in a substantial reduction in genetic neighborhood size, even while pollen dispersal remains intact.

Although studies of gene flow through pollen and seed dispersal are emerging from temperate zone sites (2, 3), direct measures in the tropics have been hindered by the difficulty of sampling all putative pollen and seed donors. Two recently developed techniques, TwoGener and Probability of Maternal Identity (PMI) circumvent the need to identify and locate all sources of pollen or seed, and instead capitalize on the clustered nature of pollen and seed distribution, computing the probability that two propagules drawn from the same cluster have the same source tree, and subsequently employing genetic structure statistics to calculate neighborhood sizes and dispersal areas (3, 4). Here we use TwoGener

and PMI with microsatellite markers to measure the effective neighborhood sizes created through dispersal of pollen ( $N_{ep}$ ) and seeds ( $N_{em}$ ) of *Antrocaryon klaineianum*, an insect-pollinated, mammal-dispersed canopy tree, in hunted and protected tropical forest in Cameroon, Africa. We then apply the two-sex, plant variant of Wright's neighborhood model (whereby genetic neighborhood  $N_e = 4\pi\sigma^2d_e$  (5), and total variance in gene dispersal  $\sigma^2 = (\sigma_{\text{pollen}}^2/2) + \sigma_{\text{seed}}^2$ ) to combine  $N_{ep}$  and  $N_{em}$  in each forest (6), and we assess the effects of hunting by comparing resulting  $N_e$  values for the two forest types.

## Methods

### *Study sites and species*

We conducted fieldwork and sampling of *Antrocaryon klaineianum* (ANACARDIACEAE) trees and seeds between October and December, 2004 at Bouamir Research Station (protected forest site) and Kompia Community Forest (hunted forest site). Details on study sites and characteristics of *A. klaineianum* are given in ref. 7.

### *Fates of seeds*

We calculated total fruit production of 6 focal trees at each site using methods described in ref. 8. We then calculated the number of seeds that remain underneath the canopy (category (A) in Fig. 3.1) as the difference between the number of "current-season" seeds found under the canopy (data from ref. 7) and the number of those seeds that did not originate from the mother above (42% and 2% in protected and disturbed forest, respectively, from genetic

maternity analysis in ref. 7). By assuming that focal trees contribute seeds to conspecific canopies in the same percentage that those conspecific trees contribute seeds to theirs (idealized trees), we could calculate the number of seeds dispersed to conspecific canopies – category (B). Subtracting seeds in (A) and (B) from total production yields category (C) - seeds dispersed away from conspecific canopies.

#### *Pollen neighborhood*

Following DNA extraction and PCR protocols described in ref. 7, we used 7 microsatellite markers to genotype 16 fruit-bearing “mother” *A. klaineianum* trees from Kompia (from silica-dried leaf tissue) and 252 of their offspring (fresh leaf tissue from greenhouse-grown seedlings of known maternity). We then used GenAlEx (9) to conduct a TwoGener analysis (4) which subtracts the maternal genotypic contribution from the seedling genotypes to obtain the paternal genotypic contributions and subsequently runs an Analysis of Molecular Variance (AMOVA) on a genetic distance matrix of those paternal genotypes and to estimate pollen pool structure -  $\Phi_{it}$  and number of effective pollen donors  $N_{ep} = 1/2\Phi_{it}$ .

#### *Seed neighborhood*

We estimated  $N_{em}$  using PMI analysis (3). For “away-canopy” (see Fig. 3.1)  $N_{em}$  calculation, we used genotypes from endocarps (maternal tissue) of 176 seeds in 20 dispersed clusters collected at Bouamir. We followed DNA extraction and PCR protocols in ref. 7 with two modifications: (1) 4 microsatellite markers [A2, C2, C108, C121] were added and one marker [C103] was dropped (see Appendix I), and (2) double PCR technique (10) was employed to

increase genotype readability. All included samples had >5 loci typed and all included clusters had >6 usable samples.

For our “under-canopy”  $N_{em}$  calculations, we applied PMI calculations to endocarp genotypes from ref. 7 ( $n = 101$  seeds from under 6 canopies at each site). We scaled the “away-canopy” and “under-canopy”  $N_{em}$  estimates by multiplying by the percentage of seeds of that category (see Table 3.1) and summed the scaled estimates to obtain combined  $N_{em}$  values.

### *Genetic neighborhood sizes*

According to Wright’s neighborhood model, effective genetic neighborhood  $N_e = 4\pi\sigma^2 d_e$ , where  $d_e$  is the density of reproducing adults and variance in gene dispersal  $\sigma^2 = (\sigma_p^2/2) + \sigma_s^2$ , whereby  $\sigma_p^2$  and  $\sigma_s^2$  are variances in pollen and seed movement, respectively (6). We calculated  $\sigma_p^2$  and  $\sigma_s^2$  from our estimates of  $N_{ep}$  and combined  $N_{em}$ , then combined them with Wright’s formulas to calculate  $N_e$ . We then obtained estimates of  $d_e$  by multiplying stem density estimates from (Fogiel, *unpublished data*) and ref. 11 by the percentage of trees bearing fruit (8 of 41 and 23 of 52 in Bouamir and Kompia, respectively), and used them to calculate Wright’s effective neighborhood area  $A_e = N_e/d_e$ .

## **Results**

Seed dispersal was dramatically reduced at Kompia Community Forest, the hunted site where large mammalian dispersers have been virtually extirpated (Fig. 1). Far fewer seeds were dispersed away from conspecific canopies when compared with Bouamir Research Station, the protected forest site located in the Dja Biosphere Reserve (4% vs. 88%).

Furthermore, among the seeds found underneath *A. klaineianum* canopies at Kompia, a lower percentage had a source other than the putative mother above, resulting in a lower under-canopy  $N_{em}$  value from PMI analysis (1.04 vs. 2.23,  $n = 101$  seeds from under 6 canopies in each forest – Table 3.1). PMI analysis of  $n = 176$  seeds dispersed in 20 clusters away from conspecific canopies at Bouamir revealed an away-canopy  $N_{em}$  value of 6.04. When scaled by the percentage of seeds in each category, these under-canopy and away-canopy  $N_{em}$  estimates yielded combined  $N_{em}$  values of 5.58 and 1.24, for the protected and hunted forests, respectively. These are the effective maternal neighborhoods, which can be visualized as the number of females, each contributing equally to a given seed cluster, that would yield the inter-cluster genetic variation among seed pools that we observed.

TwoGener analysis from the hunted forest yielded the analogous effective paternal neighborhood ( $N_{ep}=4.50$ ). Combining  $N_{ep}$  with  $N_{em}$  revealed that the total effective neighborhood ( $N_e$ ) is 55% smaller in the hunted forest with reduced seed dispersal (3.49 vs. 7.83). Moreover, because the hunted forest also had a higher density of reproducing trees,  $d_e$ , (8.40 vs. 2.54 stems/km<sup>2</sup>), the resulting effective neighborhood area ( $A_e = N_e/d_e$ ) is less than one-sixth that in the protected forest (0.42 vs 3.09 km<sup>2</sup>) (Table 3.1).

## Discussion

Our findings demonstrate that hunting of seed-dispersing animals can dramatically reduce genetic neighborhood sizes of trees that rely on them for dispersal. Wright surmised that populations with  $N_e < 20$  will experience random local genetic differentiation (5); *A.*

*klaineianum* populations are below that threshold and removal of their seed dispersers creates an acute risk of loss of genetic variability and fitness due to inbreeding and/or genetic drift

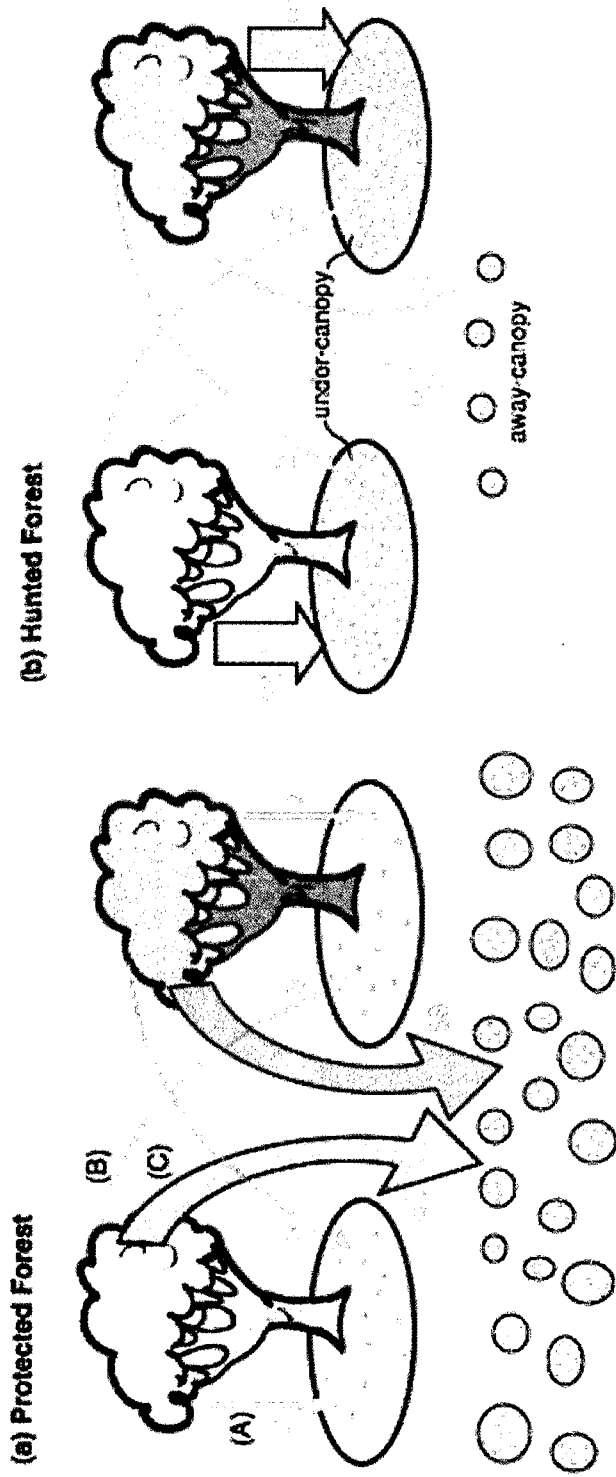


(4). This pattern is consistent with the one created by forest fragmentation (12), suggesting that the two forces are working in concert to reduce genetic diversity in human-disturbed tropical forests. The implications stretch beyond ecology – *A. klaineianum* is listed by the Cameroon government as a level three timber species, and its edible fruits are a potential non-timber forest product (NTFP). Stochastic environmental conditions created by global climate change and other factors can create challenges for which tree populations need genetic diversity to survive. By compromising genetic neighborhoods of tropical trees, hunting of seed-dispersing animals may be reducing their ability to respond to those challenges, with potentially serious economic ramifications as those populations decline or even disappear.

**Table 3.1.** Genetic neighborhood size in protected and hunted forest

Forest type	$N_{ep}$	$N_{em}$			$N_e$ total	$d_e$ (stems/km <sup>2</sup> )	$A_e$ (km <sup>2</sup> )
		under-canopy	away-canopy	combined			
Protected	4.50 <sup>a</sup>	2.23 (12%)	6.04 (88%)	5.58	<b>7.83</b>	2.54	<b>3.09</b>
Hunted	4.50	1.04 (96%)	6.04 <sup>b</sup> (4%)	1.24	<b>3.49</b>	8.40	<b>0.42</b>

<sup>a</sup>estimate from hunted forest; <sup>b</sup>estimate from protected forest



**Figure 3.1.** Fate of 100 seeds of two idealized *A. kalmianum* trees in (a) protected and (b) hunted forest. All seeds either (A) remain underneath canopy of mother trees, (B) are dispersed and deposited under the canopies of conspecific trees or (C) are dispersed and deposited away from conspecific canopies. Percentages of seeds with the three fates were derived from tree production estimates, numbers of seeds on the ground under the canopies, and genetic analyses to determine if seeds came from the mother above. In these diagrams, dispersal from ground is combined with dispersal from canopy. Two types of seed pools form for PMI analysis: (1) under-canopy – combination of seeds in category (A) and (B), with canopy as sampling unit and (2) away-canopy – seeds in category (C), with seed cluster as sampling unit.

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## Appendix I. Microsatellite primers for *Antrocaryon klaineanum*

### Appendix I.a. Microsatellite primers used on samples collected at Bouamir, protected forest site

Locus	$k$	Primer sequences (5' - 3')	Repeat	$H_O$	$H_E$	PIC	$F_{(null)}$	HW
B3	8	F: TAC TGG TGG TGG TTT AGG TAT G R: AAT GCT TAG AGC CAA GTG AAC T	GA	0.252	0.762	0.722	0.511	ND
B107	16	F: CAG TCC ACT ATC AAC CAC AAA R: TGG GAT TGC TGT ATT TAT GC	GA	0.476	0.808	0.785	0.253	ND
D104	6	F: AGG TCA AGT CGT CCC TGA CTA R: ATC CAT GAT TTG ATC CTG ACT G	TAGA	0.272	0.606	0.540	0.367	***
A101	5	F: GCC AAA ACC AAC TCA TGT GA R: GCC TAT TGT TTG ATG GTG GAC	CA	0.211	0.569	0.522	0.447	ND
B4	12	F: AGA GGA GCG TCA CTA CTT CAG R: AAA CCA GAC GTT TAT TTC AGT G	GA	0.337	0.757	0.727	0.387	ND
D109	4	F: CCT TCA AGT TTT GGG CTA AA R: GGA ATG TCC ATT TGA CTT CTG	TAGA	0.268	0.668	0.599	0.418	ND
C2	11	F: TTC CGC AGG TTC ATT CTT TAC R: GCA AAC TTG ACT TTT CCG TCT A	AAT	0.415	0.780	0.752	0.290	ND
C108	6	F: TGG GAG GGA AGA TTA GAT T R: TGG AGT AGA AGA TTC ATC ATT C	AAT	0.183	0.646	0.607	0.556	ND
A2	9	F: TTG CTG TGT CAT TTA CGA AAT C R: TGC AGA TCC TTT GTG TGA CTA A	CA	0.400	0.749	0.713	0.322	ND
C121	11	F: TCA TCC ACT TCA TTC TGA TTA C R: ACT TTT GTT CTG TGC TGT CTT	AAT	0.122	0.729	0.699	0.723	ND

Data from  $n=246$  samples (endocarp tissue from 238 dispersed seeds, leaf tissue from 8 adult trees)

### Appendix I.b. Microsatellite primers used on samples collected at Kompia, hunted forest site

Locus	$k$	Primer sequences (5' - 3')	Repeat	$H_O$	$H_E$	PIC	$F_{(null)}$	HW
B3	7	F: TAC TGG TGG TGG TTT AGG TAT G R: AAT GCT TAG AGC CAA GTG AAC T	GA	0.636	0.717	0.663	0.053	ND
B107	14	F: CAG TCC ACT ATC AAC CAC AAA R: TGG GAT TGC TGT ATT TAT GC	GA	0.818	0.838	0.818	0.009	ND
D104	4	F: AGG TCA AGT CGT CCC TGA CTA R: ATC CAT GAT TTG ATC CTG ACT G	TAGA	0.502	0.572	0.483	0.063	ND
A101	3	F: GCC AAA ACC AAC TCA TGT GA R: GCC TAT TGT TTG ATG GTG GAC	CA	0.230	0.271	0.244	0.071	ND
B4	13	F: AGA GGA GCG TCA CTA CTT CAG R: AAA CCA GAC GTT TAT TTC AGT G	GA	0.804	0.776	0.745	-0.024	ND
D109	4	F: CCT TCA AGT TTT GGG CTA AA R: GGA ATG TCC ATT TGA CTT CTG	TAGA	0.491	0.572	0.480	0.064	ND
C103	8	F: AAG GGT ACA AAG AAG ATT GTC C R: CCC AAA TCC TAT ACT CCA GAT T	AAT	0.419	0.559	0.481	0.120	*

Data from  $n=291$  samples (leaf tissue from 273 offspring from 18 known mothers)

$k$ =number of alleles;  $H_O$ =observed heterozygosity,  $H_E$ =expected heterozygosity;  
PIC=polymorphic information content;  $F_{(null)}$ =estimated null allele frequency  
HW=Hardy-Weinberg equilibrium (ND=no difference; \*sig different  $p<0.01$ )