

is in. Adjusting effort levels in response to this information confers a selective advantage over a strategy that never updates its belief about the world (fig. S2). This evolutionary explanation complements an earlier suggestion that, in an uncertain environment, individuals should invest more in exploring alternative options when the current food source unexpectedly deteriorates, as compared to individuals used to experiencing poor foraging returns (10). Both of these explanations highlight the significance of uncertainty for successive contrast effects.

The magnitude of the contrast effects predicted by our model depends strongly on the pattern of temporal fluctuations to which the animal is adapted (Fig. 3 and fig. S1). The effects should be strongest in animals adapted to rapidly changing conditions (fig. S1), because this enhances the differential allocation of effort between favorable and unfavorable periods (26). Positive contrast effects should be strongest when bad habitats are likely (low ρ) and rich periods in such habitats are very brief (low t_{Br} ; Fig. 3, solid and dashed lines), because then it is particularly important to take advantage of a higher gain rate while it lasts. Negative contrast effects should be strongest when good habitats are likely (high ρ) and poor periods in such habitats are very brief (low t_{Gp} ; Fig. 3, solid and dotted lines), because the animal can easily afford to reduce its effort until rich conditions return. Consequently, positive contrast should dominate negative contrast when bad habitats have very brief rich periods and good habitats have long poor periods (low t_{Br} , high t_{Gp} ; Fig. 3, dashed lines), whereas negative contrast should dominate positive contrast when good habitats have very brief poor periods and bad habitats have long rich periods (low t_{Gp} , high t_{Br} ; Fig. 3, dotted lines).

Empirical evidence suggests that negative contrast effects are stronger or more prevalent than positive contrast effects (4). According to our model, this bias is expected in animals adapted to relatively benign environments that are favorable most of the time, with only brief exposures to unfavorable conditions (e.g., high t_{Br} combined with low t_{Gp} ; Fig. 3 and fig. S1). Arguably, such a pattern characterizes the typical laboratory conditions experienced by domesticated strains of rats and other animals commonly used in studies of instrumental learning.

Models of adaptive behavior have traditionally considered complex rules for responding in highly simplified, static environments, but it is becoming clear that to understand many features of behavior, we need to consider how phenotypes evolve in more complex, dynamic environments that better reflect the natural world (27). Stochastic fluctuations in conditions are a potentially important component of selection in real environments (24, 26). For fluctuations over a much longer time scale than the animal's lifetime, optimal behavior could be fully programmed (epi-)genetically. Here we have focused on more rapid changes, which select for individual plasticity. If it is un-

certain about the pattern of fluctuations, an animal's experience of past conditions may alter its future expectations and hence its optimal behavior.

Our evolutionary approach has potential applications to cognitive psychology, by offering a novel perspective on people's hedonic responses to a change in their circumstances (28). The model could be extended in several interesting directions. One would be to allow habitat type, which we assumed is stable over the animal's lifetime, to change with some small probability. Another would be to let decisions depend on energy reserves, which we ignored here to isolate the effect of past experiences on optimal behavior. Individuals with critically low reserves may not have the option to rest when conditions are poor (26).

References and Notes

1. J. Huber, J. W. Payne, C. Puto, *J. Consum. Res.* **9**, 90 (1982).
2. A. Tversky, I. Simonson, *Manage. Sci.* **39**, 1179 (1993).
3. K. V. Morgan, T. A. Hurlly, M. Bateson, L. Asher, S. D. Healy, *Behav. Processes* **89**, 115 (2012).
4. C. F. Flaherty, *Incentive Relativity* (Cambridge Univ. Press, Cambridge, 1996).
5. D. Kahneman, A. Tversky, *Econometrica* **47**, 263 (1979).
6. D. Kahneman, *Am. Psychol.* **58**, 697 (2003).
7. L. P. Crespi, *Am. J. Psychol.* **55**, 467 (1942).
8. D. Zeaman, *J. Exp. Psychol.* **39**, 466 (1949).
9. P. A. Couvillon, M. E. Bitterman, *J. Comp. Psychol.* **98**, 100 (1984).
10. E. Freidin, M. I. Cuello, A. Kacelnik, *Anim. Behav.* **77**, 857 (2009).
11. K. R. Kobre, L. P. Lipsitt, *J. Exp. Child Psychol.* **14**, 81 (1972).
12. M. R. Papini, A. E. Mustaca, M. E. Bitterman, *Anim. Learn. Behav.* **16**, 53 (1988).
13. A. E. Mustaca, M. Bentosela, M. R. Papini, *Learn. Motiv.* **31**, 272 (2000).
14. M. Bentosela, A. Jakovcovic, A. M. Elgier, A. E. Mustaca, M. R. Papini, *J. Comp. Psychol.* **123**, 125 (2009).
15. E. J. Capaldi, D. Lynch, *J. Exp. Psychol.* **75**, 226 (1967).
16. J. H. McHose, D. P. Peters, *Anim. Learn. Behav.* **3**, 239 (1975).
17. J. A. Gray, *The Psychology of Fear and Stress* (Cambridge Univ. Press, Cambridge, 1987).
18. A. Amsel, *Frustration Theory: An Analysis of Dispositional Learning and Memory* (Cambridge Univ. Press, Cambridge, 1992).
19. J.-Å. Nilsson, *Proc. R. Soc. London Ser. B* **269**, 1735 (2002).
20. A. I. Houston, J. M. McNamara, J. M. C. Hutchinson, *Philos. Trans. R. Soc. London Ser. B* **341**, 375 (1993).
21. A. I. Houston, J. M. McNamara, *Models of Adaptive Behaviour: An Approach Based on State* (Cambridge Univ. Press, Cambridge, 1999).
22. Materials and methods are available as supplementary materials on Science Online.
23. J. M. McNamara, A. I. Houston, *Am. Nat.* **127**, 358 (1986).
24. J. M. McNamara, P. C. Trimmer, A. Eriksson, J. A. R. Marshall, A. I. Houston, *Ecol. Lett.* **14**, 58 (2011).
25. J. M. McNamara, A. I. Houston, *J. Theor. Biol.* **85**, 673 (1980).
26. A. D. Higginson, T. W. Fawcett, P. C. Trimmer, J. M. McNamara, A. I. Houston, *Am. Nat.* **180**, 589 (2012).
27. J. M. McNamara, A. I. Houston, *Trends Ecol. Evol.* **24**, 670 (2009).
28. A. Tversky, D. Griffin, in *Subjective Well-being: An Interdisciplinary Perspective*, F. Strack, M. Argyle, N. Schwarz, Eds. (Pergamon Press, Oxford, 1991), pp. 101–118.

Acknowledgments: We thank A. Higginson, A. Radford, D. Mallpress, and P. Trimmer for discussion and the European Research Council for funding (Advanced Grant 250209 to A.I.H.). J.M.M. and A.I.H. conceived the project, J.M.M. built the model, and T.W.F. analyzed the model and wrote the paper with input from the other authors.

Supplementary Materials

www.sciencemag.org/cgi/content/full/340/6136/1084/DC1
Materials and Methods
Figs. S1 and S2
References (29–31)

24 September 2012; accepted 21 March 2013
10.1126/science.1230599

Functional Extinction of Birds Drives Rapid Evolutionary Changes in Seed Size

Mauro Galetti,^{1*} Roger Guevara,² Marina C. Côrtes,¹ Rodrigo Fadini,³ Sandro Von Matter,⁴ Abraão B. Leite,¹ Fábio Labecca,¹ Thiago Ribeiro,¹ Carolina S. Carvalho,⁵ Rosane G. Collevatti,⁵ Mathias M. Pires,⁶ Paulo R. Guimarães Jr.,⁶ Pedro H. Brancalion,⁷ Milton C. Ribeiro,¹ Pedro Jordano⁸

Local extinctions have cascading effects on ecosystem functions, yet little is known about the potential for the rapid evolutionary change of species in human-modified scenarios. We show that the functional extinction of large-gape seed dispersers in the Brazilian Atlantic forest is associated with the consistent reduction of the seed size of a keystone palm species. Among 22 palm populations, areas deprived of large avian frugivores for several decades present smaller seeds than nondefaunated forests, with negative consequences for palm regeneration. Coalescence and phenotypic selection models indicate that seed size reduction most likely occurred within the past 100 years, associated with human-driven fragmentation. The fast-paced defaunation of large vertebrates is most likely causing unprecedented changes in the evolutionary trajectories and community composition of tropical forests.

High rates of human-driven extinctions, estimated to be 100-fold greater than those of natural extinctions (1), have pervasive impacts on the functions and services of ecosys-

tems (2, 3). Despite efforts to understand the immediate and cascading effects of the loss of species on the persistence of other species and biotic interactions (4, 5), little is known about

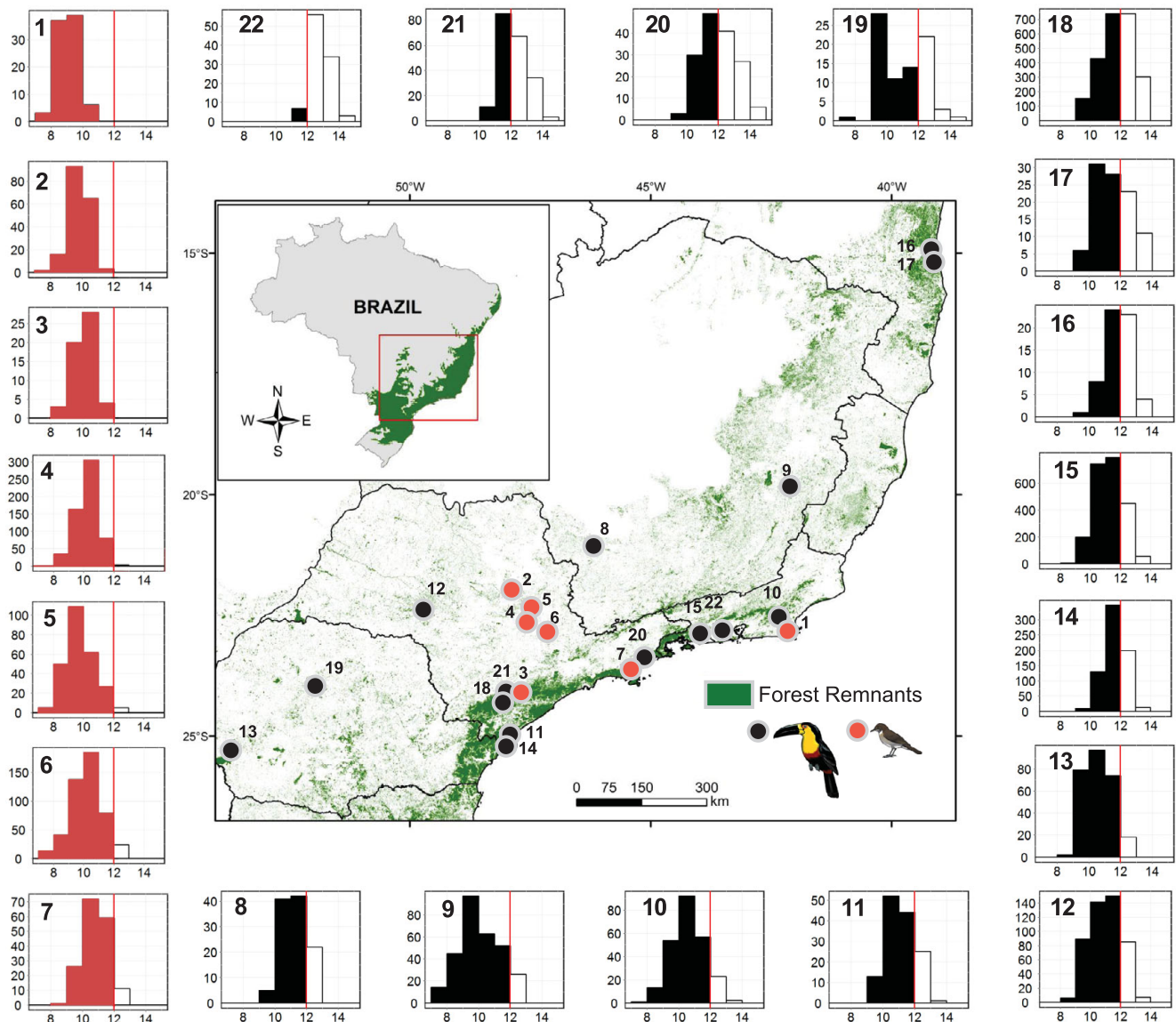


Fig. 1. Geographic variation in seed size in palm populations. Seed size (seed diameter in millimeters, x axis) frequency distributions (number of seeds, y axis) of 22 palm (*E. edulis*) populations in the remnants of the Brazilian Atlantic forest (green areas). The numbers refer to the population codes given in table S1. The red dots (codes 1 to 7) are defaunated sites,

where large-gape frugivores are locally extinct or rare; the black dots are nondefaunated sites (codes 8 to 22). The vertical red line marks the upper size limit for successful dispersal by small birds (gape size 12 mm) in the absence of large-gape frugivores. The solid bars in the histograms indicate seed sizes below this threshold.

¹Departamento de Ecologia, Universidade Estadual Paulista, Rio Claro, São Paulo, 13506-900, Brazil. ²Instituto de Ecologia, A. C. Red de Biología Evolutiva, Carretera Antigua a Coatepec 351, Xalapa, Veracruz, 91070, Mexico. ³Instituto de Biodiversidade e Florestas, Universidade Federal do Oeste do Pará, Santarém, Pará, 68035-110, Brazil. ⁴Departamento de Biologia Animal, Universidade Federal Rural do Estado do Rio de Janeiro, Seropédica, Rio de Janeiro, 23.897-000, Brazil. ⁵Laboratório de Genética & Biodiversidade, Universidade Federal de Goiás, Goiânia, Goiás, 74001-970, Brazil. ⁶Departamento de Ecologia, Universidade de São Paulo, São Paulo, São Paulo, 05508-90, Brazil. ⁷Departamento de Ciências Florestais, Escola Superior de Agricultura "Luiz de Queiroz," Universidade de São Paulo, Piracicaba, São Paulo, 13418-900, Brazil. ⁸Integrative Ecology Group, Estación Biológica de Doñana, EBD-CSIC, Sevilla, E-41092, Spain.

*Corresponding author. E-mail: mgaletti@rc.unesp.br

the potential for rapid evolutionary changes in human-modified ecosystems. Rapid evolutionary changes have been shown in short-lived organisms, such as commercially exploited species, microorganisms, and perennial plants (6–8).

Here we document the rapid evolutionary reduction of seed size in a keystone palm, *Euterpe edulis*, across the Atlantic rainforest, subsequent to human-driven extensive deforestation (9). Seed size is an important trait, positively correlated with seed reserve amount, germination success, seedling size, and reproductive output (10). At the same time, seed size constrains the range of effective seed dispersers, because only large-bodied frugivores have gapes wide enough to consume large seeds (11).

Populations of large-gape frugivorous birds are directly threatened by hunting. They require extensive tracts of forest and hence are prone to local extinction in smaller forest fragments (12). These frugivores disperse several plant species over distances of several kilometers and eat large-seeded species that cannot be swallowed and successfully dispersed by smaller birds, which often are the only species resilient to large-scale disturbances (13). The functional loss of large frugivores, either by local extinction or by the severe reduction of population abundance (functional extinction), can affect natural regeneration by impairing the main components of the dispersal process: escape, colonization, and recruitment (14). With the functional extinction of large-gape

birds, the fruit and seed traits of large-seeded plants might experience evolutionary changes within ecological time scales. We can expect shifts of the phenotypic selection regime and changes in the outcomes of selection after a substantial fraction of the selective agents (i.e., the large-gape frugivores) has been extirpated from their natural habitats.

We compared the seed size distributions of 22 palm populations in nondefaunated and defaunated areas of the two main physiognomic types (semideciduous and rainforest) in the Brazilian Atlantic forest (15) (Fig. 1). We found a consistent trend toward smaller seeds in defaunated forests (Fig. 1 and table S1). We classified an area as “defaunated” when large-gape frugivorous birds (those with a mean gape width >12 mm), such as toucans (*Ramphastos dicolorus* and *R. vitellinus*), toucanets (*Pteroglossus aracari*, *P. bailloni*, and *Selenidera maculirostris*), and large cotingas (*Procnias nudicollis*, *Carpornis* spp., and *Pyroderus scutatus*), are locally or functionally extinct (i.e., present with a very low abundance) (15) (fig. S1 and table S3).

Toucans and large cotingas are the major large seed dispersers in nondefaunated forests (the average local richness of large frugivorous birds that disperse palm fruits is 11.9 species; tables S2 and S3). Small-gape thrushes are the most common seed dispersers remaining in defaunated forests, and the species richness of large frugivorous birds is reduced to 5.1 species (tables S2 and S3). A few mammal species very infrequently act as legitimate seed dispersers (table S2). Small-gape frugivores (<12 mm) represent 38% of the species in nondefaunated areas but 49% in defaunated forests (table S2). This

distribution, in turn, results in 33% of the fruits being consumed by small-gape frugivores in nondefaunated areas and up to 98% of the fruits in the defaunated areas (table S4). Thus, there is ample potential for small-gape frugivores to have significant selective pressures on fruit traits in defaunated areas.

A nested analysis of variance revealed that variation in seed size is minimally accounted for by the forest physiognomic type (3.7%). In contrast, the defaunation status within each forest type accounted for more than 33.9% of the variance in seed size, with 0.1% accounted for by differences among sites. Most of the total variance in seed size (44.9%) was associated with individual palms within each site, with intra-individual variation (among-year variations and/or positional variation within the infructescence) representing 17.4% (overall nested analysis, $F_{[1, 9195]} = 909.8$, $P < 0.0001$). These results demonstrate the marked geographic patterns in seed size potentially related to the local selective regime (the fruit selection process) driven by frugivores according to the defaunation status, with ample among-individual trait variance for natural selection to operate. Many environmental factors can influence seed size. Thus, we modeled seed size as a function of defaunation status and 13 other environmental variables, including climate, soil fertility, relief complexity, and forest cover (table S5). Although biotic variables failed to explain the variation in seed size, the model including defaunation status nested within forest type yielded the best fit to the observed data (table S5) (15). These results show that local variation in seed size is unrelated to any of the abiotic predictors or landscape variables but

consistently relates to the defaunation status of each site.

The seeds of *E. edulis* are not successfully dispersed either when the fruits fall beneath the plant or when birds drop the fruits with the seeds still within the pulp. Seeds that remain with pulp are less likely to germinate (16), and fruits deposited beneath their parent palm usually experience high density-dependent mortality (17) (fig. S2E). The seeds dispersed by birds (defecated or regurgitated) collected in the field and from experiments with captive birds revealed that different bird species disperse seeds of different sizes [generalized linear model (GLM) $\chi^2_{[6]} = 94.1$, $P < 0.001$; Fig. 2A]. The seeds dispersed by thrushes were consistently ≤ 12 mm in diameter, whereas large-gape birds, mainly toucans, dispersed a broader range of seed sizes (Fig. 2A). To corroborate these findings of fruit size selection with bird fruit choice, we estimated the probability of seed dispersal by birds as a function of seed size by recording the diameter of successfully dispersed seeds (regurgitated) and nondispersed seeds (fruits with beak marks) at four pristine and three defaunated sites. The dispersal probability was near zero for seeds >12 mm at all defaunated sites, which is significantly lower than the estimated probability for nondefaunated sites [binomial generalized additive model (GAM) $\chi^2_{[1,20]} = 40.3$, $P < 0.001$; Fig. 2B]. Seeds wider than 12 mm represent approximately 32% of the overall seeds produced by *E. edulis* populations in nondefaunated forests. Our data show that defaunated areas have lost this large size range of the phenotypic seed size variation (Fig. 1), suggesting directional selection for reduced seed size of *E. edulis* at

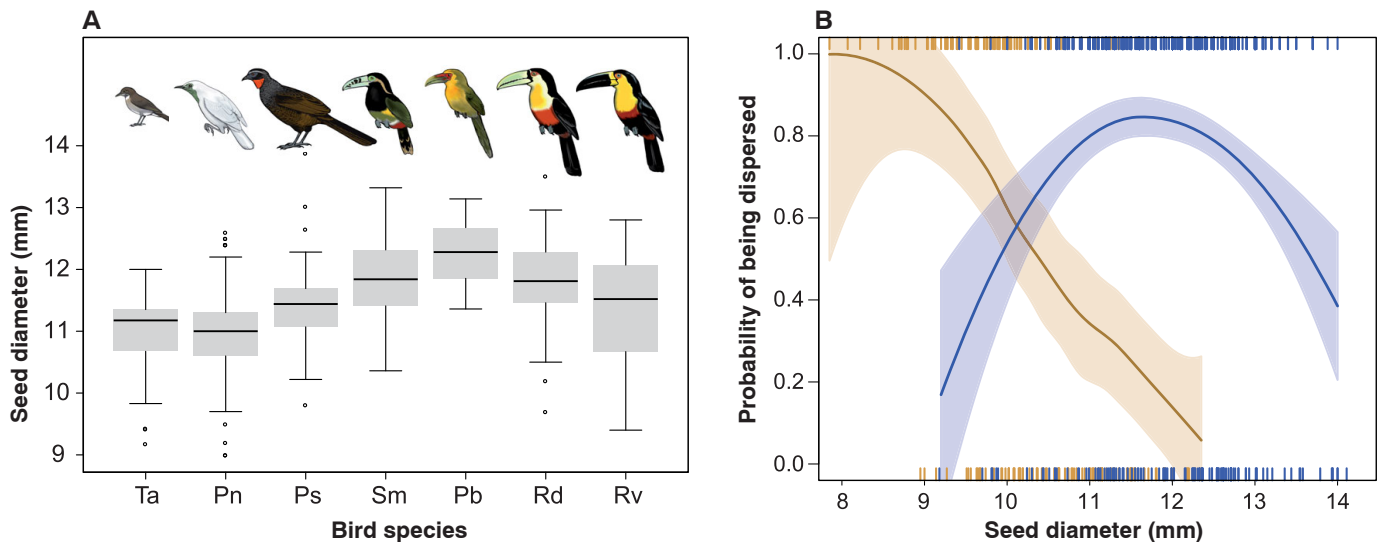


Fig. 2. Patterns of fruit preferences by frugivorous birds and consequences for phenotypic selection on seed size. (A) Seed size variation of the palm (*E. edulis*) fruits consumed by birds (from left to right): white-necked thrush (Ta, *Turdus albicollis*), bare-throated bellbird (Pn, *Procnias nudicollis*), rusty-margined guan (Ps, *Penelope superciliosus*), spot-billed toucanet and saffron toucanet (Sm, *Selenidera maculirostris*; Pb, *Pteroglossus bailloni*), and red-breasted and channel toucan (Rd, *Ramphastos dicolorus*; Rv, *R. vitellinus*). The

boxes include the mean (horizontal black line), ± 1 SE (gray box), the 95% confidence interval (vertical lines), and outlier values (circles). **(B)** The probability of the dispersal of palm seeds as a function of seed diameter in defaunated forest sites (orange), where large-gape frugivorous birds are functionally extinct, and in nondefaunated forests (blue) [see (15) for the trends in local areas; fig. S3]. The vertical lines in the rug plot indicate the individual seed sizes of undispersed and dispersed seeds.

defaunated sites that contrasts with the stabilizing selection observed in nondefaunated sites (Fig. 2B and fig. S3).

Given that resilient small-gape frugivores (thrushes) only successfully disperse small seeds (≤ 12 mm), we tested the potential of such differential selection to generate the observed striking reductions in the seed size of *E. edulis* over time in defaunated areas (Fig. 1, panels 1 to 7). We used a simple evolutionary model based on the breeder's equation (18) to estimate the number of generations of selection on seed size needed to result in such a size difference between nondefaunated and defaunated forests (19, 20). Our simulations indicate that such an evolutionary change in the seed size of *E. edulis* populations would be possible in less than 100 years after a disturbance event (such as defaunation due to hunting or fragmentation) causing the functional loss of large frugivores (Fig. 3). Our estimates highlight the fact that a period of <75 years after a severe defaunation would be sufficient to cause the observed seed size reduction in palm populations in defaunated areas (Fig. 1). The documented extensive forest conversion to agriculture (mainly coffee) in semideciduous defaunated forests dates back to the 1800s (21, 22), which agrees with the results of our phenotypic selection model

and indicates that the observed changes in seed size of *E. edulis* could have evolved very recently in relation to the remnant frugivore fauna (fig. S4). Thus, we argue that defaunation could have triggered the rapid evolutionary change of a phenotypic plant trait, resulting in a consistent size reduction of seeds in defaunated Atlantic forests.

From an ecological perspective, the reduction of seed size may have several negative consequences for plant recruitment and population dynamics (23). In *E. edulis*, it results in reductions in the total, shoot, and root biomasses of 1-year-old seedlings (24, 25). Our experiments indicate that the seed size reduction most likely resulted in the significantly increased vulnerability of *E. edulis* recalcitrant seeds to desiccation and decreased seedling size in both semideciduous forest and rainforest defaunated areas (15). Thus, seed size reduction may increase seed mortality in drier conditions and result in smaller seedlings, thereby tending to reduce the average fitness of the population. If regeneration becomes critically dependent on small seeds in defaunated areas, extended and intensified periods of drought induced by ongoing climate change, as predicted by climate models for South America (26), may be particularly harmful to the

seedling establishment of this threatened palm species.

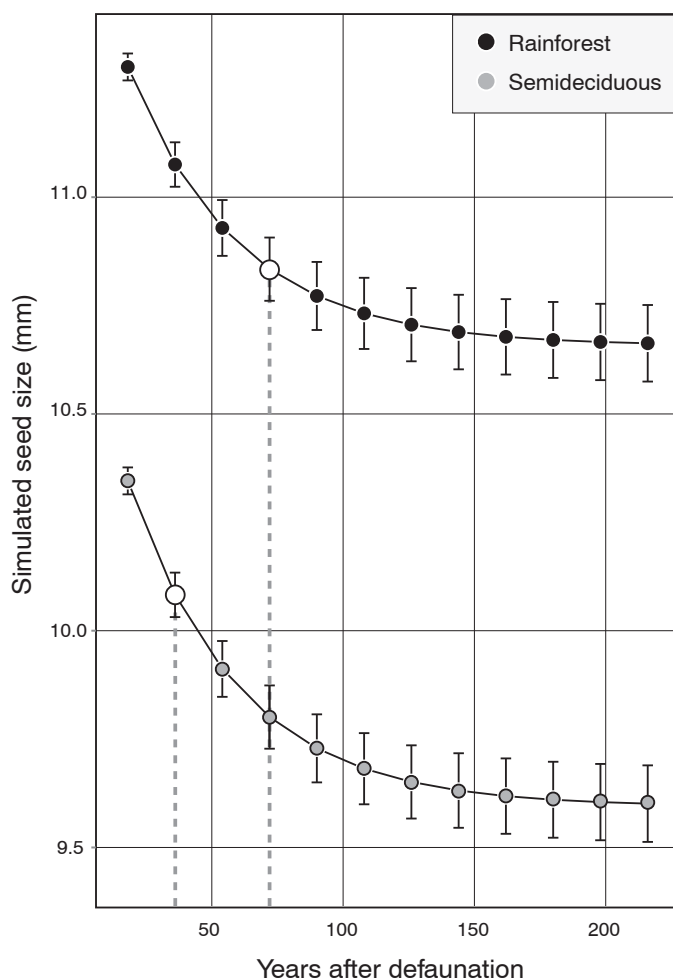
The seed size reduction documented here may be a generalized phenomenon in human-modified ecosystems where large frugivores that act as seed dispersers have been extinct for a long time. We thus foresee pervasive ecological and evolutionary effects of widespread vertebrate defaunation in tropical ecosystems. In particular, the rapid current defaunation in tropical forests will most likely result in unprecedented shifts of selection regimes on key life-history traits and in their evolutionary trajectories.

References and Notes

1. A. D. Barnosky *et al.*, *Nature* **471**, 51 (2011).
2. F. Isbell *et al.*, *Nature* **477**, 199 (2011).
3. A. D. Barnosky *et al.*, *Nature* **486**, 52 (2012).
4. S. H. Anderson, D. Kelly, J. J. Ladley, S. Molloy, J. Terry, *Science* **331**, 1068 (2011).
5. N. J. Cordeiro, H. F. Howe, *Proc. Natl. Acad. Sci. U.S.A.* **100**, 14052 (2003).
6. S. R. Palumbi, *Science* **293**, 1786 (2001).
7. C. T. Darimont *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **106**, 952 (2009).
8. S. A. B. Roels, J. K. Kelly, *Evolution* **65**, 2541 (2011).
9. The palmito or palm heart (*E. edulis*, Arecaceae) is a dominant palm species endemic to the Atlantic forest and dependent on birds for successful seed dispersal. It also occupies fragmented forest stands originated within the past 200 years since the establishment of extensive coffee plantations in São Paulo state (21, 22).
10. M. R. Leishman *et al.*, in *Seeds: The Ecology of Regeneration in Plant Communities*, M. Fenner, Ed. (CAB International, Wallingford, UK, 2000), pp. 31–57.
11. D. J. Levey, *Am. Nat.* **129**, 471 (1987).
12. N. S. Sodhi, L. H. Liow, F. A. Bazzaz, *Annu. Rev. Ecol. Evol. Syst.* **35**, 323 (2004).
13. K. M. Holbrook, B. A. Loiselle, *Ecology* **90**, 1449 (2009).
14. J. S. Markl *et al.*, *Conserv. Biol.* **26**, 1072 (2012).
15. Materials and methods are available as supplementary materials on Science Online.
16. A. de Barros Leite, P. H. S. Brancalion, R. Guevara, M. Galetti, *J. Trop. Ecol.* **28**, 615 (2012).
17. D. M. S. Matos, R. P. Freckleton, A. R. Watkinson, *Ecology* **80**, 2635 (1999).
18. D. S. Falconer, T. F. C. Mackay, *Introduction to Quantitative Genetics* (Addison Wesley Longman, Essex, UK, 1996).
19. We estimated the heritability (h^2) of seed size in *E. edulis* based on the variance of genetic relatedness between palms with available fruit phenotypic data (15). We used seed size data from genotyped individual palms in three populations, yielding $h^2 = 0.35$ (15), and the previously reported value of 18.7 years for the generation time of the palm (20).
20. M. Franco, J. Silvertown, *Ecology* **85**, 531 (2004).
21. W. Dean, *Rio Claro: A Brazilian Plantation System 1820–1920* (Stanford Univ. Press, Stanford, CA, 1976).
22. The defaunated semideciduous forests are located in the heart of traditional coffee plantations in Brazil. For example, Rio Claro, a typical city in our defaunated region, had 65 coffee farms by 1855.
23. A. T. Moles *et al.*, *Science* **307**, 576 (2005).
24. M. A. Pizo, C. von Allmen, L. P. C. Morellato, *Acta Oecol.* **29**, 311 (2006).
25. M. A. Pizo, I. Simão, *Acta Oecol.* **22**, 229 (2001).
26. J. A. Marengo, M. Rusticucci, O. Penalba, M. Renom, *Clim. Change* **98**, 509 (2010).

Acknowledgments: We thank the Fundação de Amparo do Estado de São Paulo (BIOTA - FAPESP); Conselho Nacional de Desenvolvimento Científico (CNPq), (Excellence Grant-Junta Andalucía (to P.J.); and Programa Iberoamericano de Ciencia y Tecnología para el Desarrollo (CYTED) for funding support. We thank J. Bascompte, R. Dirzo, D. Hansen, D. Levey, E. Bruna, D. Lapola, D. McCauley, M. A. Pizo, and three reviewers for useful comments and suggestions; T. A. Ferreira, E. Cazetta,

Fig. 3. Simulated phenotypic trends in seed size after loss of major frugivores. Expected trajectories over time, estimated from the phenotypic selection model, of the seed size reduction after defaunation in two Atlantic forest types (rainforest and semideciduous forest) (15). The vertical dashed lines with larger open dots denote the position along the mean trajectory when the predicted seed diameter is the closest to the present observed mean seed diameter in defaunated forests. The position where the dashed lines intercept the x axis represents the minimum time for the seeds to reach the present-day seed diameter mean value according to the model. Solid dots show the mean values (± 1 SD) of the simulated seed size for consecutive years after defaunation.



M. J. Campos, D. Rother, G. Ambar, C. Draxler, E. R. Castro, R. Laps, P. Develey, M. R. Francisco, and staff from PN Iguaçu for sending us palm seeds and data on frugivores; Fundação Florestal for allowing our study in the Protected Areas; and S. Nazareth and R. Brandolim for field and laboratory assistance. M.G. and P.R.G. receive a research fellowship from Conselho Nacional de Desenvolvimento Científico e Tecnológico.

Data supporting this study are available in the DRYAD repository (<http://dx.doi.org/10.5061/dryad.2pm42>). Bird plates were done by Carl Buell.

Supplementary Materials

www.sciencemag.org/cgi/content/full/340/6136/1086/DC1
Materials and Methods

Figs. S1 to S4
Tables S1 to S6
References (27–61)

7 December 2012; accepted 5 April 2013
10.1126/science.1233774

Tracking Individuals Shows Spatial Fidelity Is a Key Regulator of Ant Social Organization

Danielle P. Mersch,^{1*} Alessandro Crespi,² Laurent Keller^{1*}

Ants live in organized societies with a marked division of labor among workers, but little is known about how this division of labor is generated. We used a tracking system to continuously monitor individually tagged workers in six colonies of the ant *Camponotus fellah* over 41 days. Network analyses of more than 9 million interactions revealed three distinct groups that differ in behavioral repertoires. Each group represents a functional behavioral unit with workers moving from one group to the next as they age. The rate of interactions was much higher within groups than between groups. The precise information on spatial and temporal distribution of all individuals allowed us to calculate the expected rates of within- and between-group interactions. These values suggest that the network of interaction within colonies is primarily mediated by age-induced changes in the spatial location of workers.

Ant colonies, with their complex and efficient social organization, have long fascinated humans (1). Essential to their ecological success are high levels of cooperation and sophisticated division of labor. Although workers must perform a multitude of tasks such as foraging, nest construction, and brood rearing, it has become clear that there is no central control of how work is allocated among individuals. Therefore, workers must allocate themselves to tasks in a self-organized manner following simple behavioral rules that incorporate local stimuli received directly from the environment and from interactions with other workers (2–4). Despite extensive work on division of labor in social insects (1, 3, 5–7), the connection between individual task specialization and the social network remains unknown. Another important, yet little studied aspect of social organization is spatial organization. In honeybees, workers change tasks over the course of their lifetime, starting as nurses in the nest and generally ending as foragers outside (2, 8, 9). This suggests that the rate of interactions between group members may be affected by the task performed and its associated localization in the colony. In our experiment, we used an automated video tracking system based on fiducial identification labels to track all individuals in six colonies of the ant *Camponotus fellah* and to identify individual interactions and patterns of social organization (movie S1).

All colonies were established from a single queen collected after a mating flight. The experiment started when queens were 4 years old, out of a maximum life span of 26 years (10). We determined the age of all workers (122 to 192 per colony) by weekly color-coding all newly eclosed workers more than 60 weeks before the experiment began. A month before the start of the experiment, we individually marked all ants with a distinct barcode-like matrix (11), enabling individual identification (12). Colonies were kept in a constantly dark nest chamber that was connected by a tunnel to a foraging chamber exposed to daily light-dark cycles (fig. S1). The temperature, humidity, light, and food supply were computer-controlled, and both chambers were filmed from above with high-resolution monochrome cameras operating under infrared light (fig. S1) (12). We recorded the position and orientation of all individuals twice per second to reconstruct spatial movement and infer all social interactions occurring over the 41 days of the experiment. A pair of ants was considered to interact when the front end of one ant was located within the trapezoidal shape representing the other ant (fig. S4) (12). The data set we obtained consisted of a total of 2,433,250,580 ant positions and 9,363,100 social interactions (movies S2 and S3).

We used this data set to first investigate whether workers organize themselves into cohesive social groups by using the Infomap community detection algorithm (13). To facilitate data analysis, we split the 41 experimental days into four periods of 11, 10, 10, and 10 days. In each of these periods, we identified pairs of interacting ants. Analyses on the daily interaction networks of the first 11 days (see supplementary text) revealed two robust groups to which the same set

of workers was affiliated on almost all days. The first group always comprised the queen and $41 \pm 12\%$ of the workers (percentage \pm SD across the six colonies) (Fig. 1A), whereas the second group represented $31 \pm 11\%$ of the colony's workforce. Depending on days and colonies, we also identified zero to five other groups of workers. A visual analysis of the daily networks of interactions suggested that workers affiliated with the two robust groups on only a few days may form a third group with less marked within-group preferential interactions. These workers represented $28 \pm 4\%$ of the colony's workforce and were consistently located between the two other groups in the network (Fig. 1A and figs. S6 to S10). An additional analysis of the interaction frequencies (supplementary text) confirmed that workers of the third group interacted significantly more with members of their group than with workers of the other two groups. Together, these results indicate that colonies of *C. fellah* are structured in three interconnected social groups and that these groups differ in their interaction patterns.

Workers from the three social groups exhibited distinct behavioral signatures (Fig. 2). Workers of the first group performed most of the interactions with the queen [Kruskal-Wallis (KW): $\chi^2 = 514.05$, $P < 10^{-101}$] and visits to the brood. By contrast, workers from the second group performed most ($87.3 \pm 18.6\%$) of the foraging trips, whereas workers from the third group exhibited a significantly higher propensity to visit the rubbish pile. For simplicity, we hereafter refer to these three groups as nurses, foragers, and cleaners. Comparison of the normalized age of workers revealed an age-based division of labor. Nurses were younger than cleaners who, in turn, were younger than foragers (KW: $\chi^2 = 108.7$, $P < 10^{-23}$) (fig. S11). However, in all colonies, there was great overlap among the three groups, with some nurses being older and some foragers being younger than the workers' average age. Despite a wide distribution in worker body size (6 to 16 mm), no consistent size difference exists between workers of the three groups (fig. S12).

Our data also allowed us to track temporal changes among the three behavioral groups by performing community detection analyses on the three subsequent 10-day periods of the experimental data (Fig. 3). Workers exhibited a preferred behavioral trajectory, moving from nursing to cleaning to foraging as they age. The most common transition was from cleaner to forager (supplementary text). Such age-related behavioral transitions have been documented in honeybees, in which young bees nurse the brood, then move on to perform various other in-hive tasks and

¹Department of Ecology and Evolution, University of Lausanne, Switzerland. ²Biorobotics Laboratory, Ecole Polytechnique Fédérale de Lausanne, Switzerland.

*Corresponding author. E-mail: danielle.mersch@unil.ch (D.P.M.); laurent.keller@unil.ch (L.K.)



Supplementary Materials for

Functional Extinction of Birds Drives Rapid Evolutionary Changes in Seed Size

Mauro Galetti,* Roger Guevara, Marina C. Côrtes, Rodrigo Fadini, Sandro Von Matter, Abraão B. Leite, Fábio Labecca, Thiago Ribeiro, Carolina S. Carvalho, Rosane G. Collevatti, Mathias M. Pires, Paulo R. Guimarães Jr., Pedro H. Brancalion, Milton C. Ribeiro, Pedro Jordano

*Corresponding author. E-mail: mgaletti@rc.unesp.br

Published 31 May 2013, *Science* **340**, 1086 (2013)
DOI: 10.1126/science.1233774

This PDF file includes:

Materials and Methods
Figs. S1 to S4
Tables S1 and S3 to S6
Caption for Table S2
References (27–61)

Other Supplementary Material for this manuscript includes the following:
(available at www.sciencemag.org/cgi/content/full/340/6136/1086/DC1)

Table S2

Materials and Methods

Studied system and sampling sites:

The seed size of *Euterpe edulis* (Arecaceae) was studied in 22 populations: seven defaunated and 15 non-defaunated forests from two forest types, semideciduous and rainforest (table S1). See below for a definition of defaunation status for each area. We have chosen these areas (i) to cover large rainfall and elevation variation, (ii) to sample different forest types (semideciduous and ombrophilous, here rainforest), (iii) to sample areas that the bird community is well known and (iv) to ensure that the sites would have enough seeds to measure. Semideciduous forests have marked rainfall seasonality and are located in the upland Atlantic forest, whereas the rainforest sites have no rainfall seasonality and are located along the coastal areas (27). Historically, all sites shared a similar assemblage of seed dispersers (28), but forest fragmentation (particularly in the semideciduous forests) and hunting (in both semideciduous forests and rainforests) have impoverished the assemblage of large frugivores in many sites (29, 30).

Seed dispersers of *Euterpe edulis*:

Fifty-eight birds and 21 mammal species have been recorded feeding on the fruits of *E. edulis* in the entire geographic distribution of the palm, but only 32 bird and six mammal species can act as legitimate seed dispersers (animals that regurgitate or defecate the seeds) (table S2). The main seed dispersers are birds ranging from small thrushes (*Turdus* spp.), with gape size up to 12 mm, to large toucans (*Ramphastos* spp.) with a mean gape size of 30 mm (table S2). Small-gape birds (e.g., *Euphonia* spp., *Tangara* spp., and *Saltator similis*) try to feed on fruits but always drop them beneath the parent palm. Several parrots and parakeets (e.g., *Pionus maximiliani*, *Triclaria malachitacea* and *Pyrrhura frontalis*), mid-size rodents (e.g., *Cuniculus paca* and *Dasyprocta* spp.), several species of small rodents, deer and peccaries are seed predators, and six mammals (*Didelphis aurita* and *D. albiventris*, *Tapirus terrestris*, *Nasua nasua*, *Eira barbara* and *Cerdocyon thous*) are infrequent consumers.

For each species that feeds on *E. edulis* fruits, we defined a “frugivory score”, aiming to characterize their reliance on fruits. The values were 1 (sporadic), 2 (moderate) and 3 (extensive frugivory), based on published accounts and our own data on the frequency of the appearance of fruits in the diet and the relative proportional contribution of the fruits to the diet (table S2).

Definition of defaunation status:

We assigned areas to the defaunated and non-defaunated categories based on a combination of criteria relying on occurrence data (presence/absence information; table S2), quantitative abundance (only available for nine areas; table S3, also see Fig. S1) and traits of the frugivore species that eat the fruits of *E. edulis* (table S4; see Fig. S1). Classification of areas as defaunated and non-defaunated was first based on a cluster analysis done on the total sums of four metrics describing each bird species weighted by their presence/absence in each area. The four metrics used were dispersal effectiveness (i.e., the product of visitation rate, number of fruits handled per visit, and probability of dispersing a handled seed), body mass (g), gape size (mm) and frugivory score (table S4). Dissimilarity between areas was calculated following a modification of the Bray-Curtis distance (31) and we used Ward's agglomerative procedure (32). Table S3 summarizes the available data on species richness (all 22 areas) and abundance (nine areas) of legitimate seed dispersers. Non-defaunated areas, established according to the above criteria, have almost twice

the abundance of large frugivorous birds than defaunated areas, and the abundance of toucans is almost five times higher (table S3).

By our definition, an area is “defaunated” when large-gape frugivorous birds (i.e., with a mean gape size >12 mm), such as toucans (*Ramphastos dicolorus* and *R. vitelinus*), toucanets (*Pteroglossus aracari*, *P. bailloni*, and *Selenidera maculirostris*) and large cotingas (*Procnias nudicollis*, *Carpornis* spp., and *Pyroderus scutatus*), are locally extinct or present with very low abundances (functionally extinct). Evidence for local extinction comes from the failure to detect large-gape seed dispersers during extensive focal observations on palm fruits, when the area is too small to support large-bodied frugivores (29), or the failure to detect these birds in bird surveys performed by us or in information provided by field ornithologists who have extensively worked on these sites (tables S1, S2).

Thrushes (*Turdus* spp.) and guans (*Penelope* spp.) are the main seed dispersers that may thrive in some defaunated forests. Thrushes can disperse *E. edulis* seeds through regurgitation, but only seeds < 12 mm. Guans defecate seeds significantly smaller (average width of 11.43 mm) than the seeds regurgitated by the toucans and cotingas (11.94 mm) ($F_{1,240} = 23.3$, $P < 0.0001$) although they are able to disperse seeds up to 13.88 mm. In addition, guans deliver clumps of seeds together with decomposing organic matter (mostly fruit pulp) (Fig. S2D) and this deposition pattern has two detrimental effects on seedling establishment. First, the presence of pulp inhibits *E. edulis* germination (16). Second, the clumping of seeds can increase density-dependent mortality due to a further increase in the incidence of pathogens and competition between establishing seedlings, an effect evidenced experimentally in previous studies (25). Moreover, frugivory by guans is very low in defaunated sites, and actually no visits were recorded during focal observations in defaunated areas (table S4). This indicates that their contribution to removal of seeds in defaunated sites is very limited. The toco toucan (*Ramphastos toco*) may occasionally visit some defaunated areas, but they are recent colonizers from open areas (33) and do not forage in the closed canopy forests for *E. edulis* fruits.

The classification analysis of the avifauna based on the dissimilarity in composition among the 22 studied areas (Fig. S1) showed a consistent assignment of area status with two exceptions: area #3 (Jurupará, SP), and area #7 (Caraguatatuba, SP) (table S1). These areas were grouped with the non-defaunated areas when in fact they were assigned as defaunated. The reason we kept these two areas as defaunated is that their avifauna is severely impoverished relative to other rainforest sites (34), have a low abundance of toucans (point count abundance index, IPA= 0.01 for Caraguatatuba; 0.26 for Jurupará) and very low species richness of large frugivores with frugivory score= 3. In comparison with the other defaunated areas, which are all semideciduous, the avifauna of Caraguatatuba and Jurupará appear better preserved, however these two areas show clear signs of defaunation compared to other rainforest sites (tables S2, S3, Fig. S1), suggesting a situation of functional extinction of the large frugivores. These two areas are known to have high impact of illegal hunting (35). Therefore, palms in defaunated areas have impaired dispersal, with seeds > 12 mm diameter failing to disperse successfully due to a combination of absence of large legitimate dispersers or their functional extinction.

Fruit measurements:

We recorded the diameter of the smallest axis of the roundish *E. edulis* seeds ($N= 9359$ seeds; table S1). This measure defines whether a seed can be swallowed by a bird depending on its gape size (36) because most avian frugivores are gape-limited. The mean fruit diameter (± 1 SD) of *E. edulis* fruits is 12.72 ± 1.40 mm (ranging from 6.70 to 16.62 mm) and mean seed diameter (± 1

SD) is 11.17 ± 1.15 mm (ranging from 6.25 to 14.61 mm). These two fruit traits are significantly correlated ($r = 0.8884$, $t = 139.6$, $df = 5201$, $P < 0.0001$). We used seed diameter as a proxy for seed size.

Fruit removal and seed dispersal:

We compiled information of 2326 h of monitoring fruiting *E. edulis* palms, from which 936 h were obtained from literature (37, 38, 39) and 1363 h were from our own observations. Data were from ten of the 22 sites sampled for seed size (table S4). Each palm population was sampled for at least 60 h, with different observers focusing on distinct palms to enable several palms to be monitored. In each area, we estimated the frugivore assemblage composition and the quantitative component of seed dispersal effectiveness of the different frugivore species (40). We assessed four different components: visitation frequency, mean number of fruits handled/visit (fruits that were touched by the frugivores), mean number of seeds dispersed/visit (only seeds that were swallowed) and the observed dispersal probability (the probability that a handled fruit results in a dispersed seed, i.e., regurgitated or defecated, away from the parent palm by a given frugivore species). These different components allowed an estimate of the quantitative component of seed dispersal, which measures the quantitative contribution of each bird to the seed dispersal of *E. edulis* (table S4).

Probability of seed dispersal as a function of fruit size:

To estimate the probability of dispersal for seeds of different sizes, we collected dispersed (regurgitated) and non-dispersed seeds (dropped fruits with beak marks in the pulp) (Fig. S2C) on the forest floor along 1-5 km trails in three defaunated sites (sites 2, 5, and 7, table S1) and four non-defaunated sites (sites 12, 14, 20, and 22, table S1) spanning the variety of forest physiognomic types. Regurgitated seeds were easily separated from non-dispersed, pecked fruits (Fig. S2C).

We then tested the hypothesis that defaunation jeopardizes the successful dispersal of large seeds by fitting a generalized additive model with a binary response variable (dispersed/non-dispersed seeds) and seed diameter (covariate) and site status (defaunated/non-defaunated) as main factors. We used GLM with a gamma error distribution and log link function to address whether different species of birds dispersed seeds of different sizes. All statistical analyses were performed in R (41).

Germination experiments:

The seeds of *E. edulis* are highly recalcitrant and start losing viability when their seed moisture content is less than 39%, with the complete loss of viability whenever the seed water content is less than 21% (42). This recalcitrant behavior results in rapid desiccation whenever the seeds are exposed to dry conditions. Humid environments can also reduce viability; accelerated respiration rates cause physiological deterioration, and fungal attacks are common. We have additional data on *E. edulis* germination trials that confirm this assumption.

We compared the germination viability between non-defleshed (whole fruits) and clean seeds (performed under chamber conditions on sand substrate; 90% air humidity and 25°C) to evaluate the effect of removing pulp from seeds, which could for example release seeds from germination inhibitors (43). All of the defleshed seeds germinated within eight weeks after sowing, whereas the seeds left within the fruits did not germinate. In some species seeds may remain dormant for years even when still surrounded by the pulp (44). To assure that seeds were not viable at the end of the

experiment, we analyzed all non-germinated seeds present in the substrate for embryo integrity with a tetrazolium chloride test. This test indicated that the embryos were dead.

Environmental models:

To test whether environmental factors and defaunation status were associated with seed size, we conducted linear mixed effect model analyses using the maximum likelihood estimator with the *bbmle* package within the R language (41, 45). The set of explanatory covariates included the following: climate (precipitation seasonality, temperature seasonality, annual mean precipitation, annual mean temperature, and temperature of the wettest quarter), landscape (percentage of forest cover and forest type), elevation, longitude and latitude, soil texture, soil fertility, relief complexity, and defaunation status. The climatic data were extracted using Worldclim 1.4, with 900 m of spatial resolution (46). The landscape model included the forest amount estimated at the sub-watershed scale, which used a forest cover available with a 50 m spatial resolution (47). The soil texture, soil fertility and relief complexity were obtained from the IBGE official database (<http://mapas.ibge.gov.br/>), and the data were resampled at a 900 m spatial resolution to match the scale of the climatic data. The elevation data were obtained from the shuttle radar topographic mission database (srtm, <http://www-radar.jpl.nasa.gov/srtm/>).

To select the best competing model, we used the Akaike Information Criterion (AICc)-based approach for the comparison of multiple hypotheses (48). The model with the lowest AICc is considered the best among all defined models, and models with $\Delta AICc < 2$ were considered as equally plausible to explain the observed patterns. To guarantee that the models were not good by chance, a neutral model that considers the absence of effects was included in the model competing list (49). Weights for AICs ($wAIC$) were estimated for each model to assess its support relative to all models. The results (table S5) suggest that the three models that included defaunation and forest type were the best to explain seed size variation. The cumulative $wAIC$ was then computed as the sum of $wAIC$ s of these best, equally plausible models (table S5). Altogether, they had an 85% of probability of best explaining the seed size relative to the set of competing models, given the data. Bioclimatic variables, forest amount, soil texture and relief did not improve the fit of these models (table S5).

Interannual variation in seed size and fecundity:

One possible alternative reason why seed size varied in the populations is inter-year variation or fecundity trade-offs, i.e., palms that produce more fruits have smaller fruits than palms with fewer fruits (table S6). We harvested seeds from 20 *E. edulis* individuals in two large, non-defaunated regions – one Atlantic rainforest (site 21, table S1) and one semideciduous forest (site 12, table S1) in May 2008 and May 2009. We placed a plastic tarpaulin in the projected area for infructescence fall from each mother tree to obtain all the seeds present in the ripe infructescence. Unripe infructescences present on the plant were not harvested. The stem diameter at breast height (dbh), a proxy of palm age, of all mother palms was measured in 2009. The pulp was removed from the *E. edulis* fruits by placing them on a wire-mesh screen, followed by rinsing abundantly in running water. The fruits were then processed separately for each infructescence, avoiding seed mixtures among different mother palms and provenances. The seed dry mass (oven method - 105 \pm 3°C for 24 h; two replicates of 20 seeds) and number of seeds per infructescence were evaluated for each palm. There was no significant correlation, in either year, among the number of infructescences, number of seeds, and seed dry mass within populations, except for the positive association between seed dry mass and number of seeds per infructescence in the Atlantic

Rainforest population in 2009 (table S6). Hence, no seed size-number trade-off was evident at the population level. These variables were also not correlated with the estimated palm age within a population (table S6).

Seed size heritability and coalescence estimation:

The heritability of seed size (seed diameter) was estimated using the software Mark – Genetic Marker Analysis version 3.1 (50-52) with 10,000 bootstrap resamplings. The estimation is based on the actual variance of the genetic relatedness coefficient between individual palms with seeds that have been measured and directly incorporates the environmental patchiness under isolation-by-distance assumptions. We used a linear model for phenotypic similarity, $Z_i = 2 r_{ij} h^2 + a_e - b_e d_{ij} + e_{ij}$ (50), where r_{ij} is the pairwise genetic relatedness between individuals sharing the same environment; h^2 is the heritability; a_e is the average environmental correlation between individuals; b_e is the linear decrease of environmental correlation, which is a function of the physical distance between individuals (d_{ij}); and e_{ij} is the error term, assumed to be normally distributed. The analyses were performed using data from two defaunated populations (sites 5 and 6, table S1) and one non-defaunated population (site 14, table S1). In each population, all adult individuals in a 25 x 50 m plot were mapped to determine the spatial distances among them. Pairwise relatedness was estimated based on the multilocus genotype at eight microsatellite loci (53). The estimated heritability was $h^2 = 0.35$ (95% CI 0.289-0.499).

To decouple the effects of migration and time since isolation, we used a coalescence model (54). The demographic parameters $\theta = 4\mu Ne$ (coalescent or mutation parameter for diploid genome), $M = 4N_{em}/\theta$ (migration parameter) and g (exponential growth rate, $\theta_t = \theta_{now} \exp[-gt]$, where t is the time to coalescence in the mutational unit (55)) were estimated using the Markov Chain Monte Carlo (MCMC) approach (56) implemented in LAMARC 2.0.2 software (57). The analyses were run with 10 initial chains of 10,000 and two final chains of 100,000 steps, and the chains were sampled every 100 steps following a 10,000 step burn-in. We used the default settings for the initial estimate of θ . Additionally, to improve the run performance, we added heating (metropolis-coupled MCMC), with one heated chain and a temperature of 1.2. The program was run four times to assess convergence and validate the analyses. Then, we generated combined results using Tracer v1.4.1 (58) and considered the results only when $ESS \geq 200$ (effective sample size). The most probable estimates (MPE) were obtained, i.e., the highest point on the posterior probability curve for a given parameter, which is the best solution found by a Bayesian run, and the credibility interval around the estimate of each parameter was also obtained (55). The time to the most recent common ancestor (TMRCA) was estimated from the overall θ using the lowest mutation rate reported for a microsatellite marker in plants, 2.4×10^{-4} mutations per allele per generation (95% CI = $[1.4 \times 10^{-4}; 4.2 \times 10^{-4}]$) (59), which is often quoted in the range of 10^{-3} to 10^{-4} per locus per generation and a generation time of 18.7 years (20).

The coalescence analysis performed with LAMARC 2.0.2 software showed constant population growth $g = 0.280$ (95% CI -1.019-2.183) and a high mutation parameter ($\theta = 7.905$ [95% CI 1.714-10.000]), indicating a historically large effective population size. The TMRCA estimated from the coalescence parameter for the overall population dated from 32,937 generations or 615,921 yrs BP (95% CI = 76,313.80 – 1,335,714 yrs BP).

The number of migrants per generation among population pairs ranged from 0.43 to 7.93 migrant events. Although the coalescent time dated from the Pleistocene, the most recent common ancestor (MRCA) of the haplotypes (their coalescence) does not necessarily correspond to the actual temporal split of the populations but may precede the actual divergence of the populations

(60). In fact, the high migration among populations showed that populations of *E. edulis* shared a common evolutionary history until very recently, and thus the difference in the seed size characteristics is most likely due to recent changes caused by increasing isolation in a progressively fragmented landscape. These changes may be driving population divergence due to the combination of drift and strong divergent selection.

Phenotypic models of seed size trends over time after defaunation:

We modeled evolutionary changes in seed size as $z_{t+1} = z_t + h^2 (P - z_t)$, in which z_t is the seed size at generation t , h^2 is the heritability of seed size, P is the mean seed size successfully dispersed by the frugivore species remaining at the defaunated sites, and $h^2 (P - z_t)$ is the response to selection at generation t . We estimated the heritability (h^2) of seed size in *E. edulis* based on the variance of genetic relatedness between palms with available fruit phenotypic data (see Seed size heritability and coalescence estimation). We used previously-reported value of 18.7 yr for the generation time of the palm (20). The simulations examined the expected trajectories for seed size, z_t , given the palm generation time, the empirically estimated h^2 value and differential selection by small-gape frugivores in the absence of large-gape species from disturbed areas. We ran two models, one for the initial conditions of seed size similar to non-defaunated semideciduous forests and a second with the initial condition similar to non-defaunated rainforest. Initial seed sizes were bootstrapped from our data on seed size for each type of forest. We estimated P using information on the size of regurgitated seeds only, because defecators (guans) are less effective as seed dispersers, as discussed above (Definition of defaunation status). Therefore, the results from our simulations hold in two situations: (i) if defecators are not relevant as dispersers relative to species that regurgitate seeds; (ii) if defecators select for the same seed size as species that regurgitate the seeds. In fact, defecators moved a relatively small amount of seeds in this system, as revealed by Table S4 data on visitation and quantitative component of seed dispersal effectiveness, and their visitation frequency to palms in defaunated areas is indeed very low (point abundance index, IPA, of *Penelope superciliosus* in defaunated areas is 27 times lower than in non-defaunated areas; visitation rate by cracids is 0.29 visits/10 h, compared to toucans and cotingas with 3.2 visits/10h). In addition, guans defecate seeds significantly smaller than the seeds regurgitated by the toucans and cotingas (see Definition of defaunation status).

Consequences of seed size reduction for seedling establishment:

Seed size reduction and desiccation vulnerability: Because *E. edulis* seeds are recalcitrant (i.e., seeds that do not survive drying or freezing) (42), their desiccation is one of the main limitations for seedling survival during extended and intensified periods of drought, as predicted by future scenarios of climate change (61). Based on this assumption, we studied how seed size reduction would affect the vulnerability of *E. edulis* seeds to desiccation. We measured the individual seed diameter and seed mass of 200 seeds randomly picked from 50 palms in 2012. Considering that *E. edulis* seeds are spherical, we used the mean seed diameter to calculate the seed surface using the formula $Surface = 4\pi r^2$, where r is the mean seed radius. By dividing the surface of the seed by its mass, we obtained its specific surface (mm^2/mg). The specific surface of live organisms has been widely reported in the literature as a determinant of their propensity for water and temperature exchange with the environment, such that the higher the specific surface, the higher the exchange. We then evaluated the association between the *E. edulis* seed diameter (ranging from 8.1 to 12.9 mm) and its specific surface, a predictor of seed desiccation vulnerability, and found a strong negative association (linear regression: $R^2 = 0.39$; $P < 0.0001$).

Using the mean seed diameter observed in non-defaunated (11.9 mm) and defaunated forest remnants (10.0 mm) (table S1) in the regression model, we observed an increase of 16.3% in the specific surface of seeds in defaunated sites, thereby indicating a higher vulnerability to desiccation. This observation is an added factor to the possible lower fitness of small seeds under natural conditions (25). The combination of reduced seed dispersal success for larger seeds and reduced seedling size due to smaller seeds (which could increase mortality) in defaunated areas is consistent with the pattern of a close association between mean seed size and frugivore species richness across areas (Fig. S4).

Seed size effect on seedling growth: To assess the effects of seed size on seedling size, direct seeding was performed in May 2008, seven days after seed harvesting, to avoid viability loss of *E. edulis* seeds. Eight plots were established along two different trails for each forest type (four plots per trail) (sites 12 and 21, table S1). Ninety seeds were sown in each plot (three seeds from each of thirty mother palms), and all seeds were individually measured and weighed. We used seeds from the same mother palms in all plots. The exact location of the seeds from each mother palm was marked with a plastic colored tag. The plots were protected by exclusion cages to avoid seed removal by mammals and from seed deposition by dispersal agents. The cages were made with iron rods that were 110 cm long x 70 cm wide x 15 cm high and covered by a wire mesh with round 1 cm diameter openings. The seeds were half buried in the soil, and the other half of each seed was left exposed. The passage from the seedling to sapling stage, which is indicated by complete endosperm exhaustion, was evaluated to determine the experiment duration. We observed that endosperm exhaustion occurred ca. 270 days after sowing. Because our goal was to evaluate the effect of seed size on early seedling establishment and growth, we removed the shoots of the seedlings from the plots 270 days after sowing. These shoots were dried in an oven at 72°C for 48 h for the evaluation of shoot dry mass. The results indicated that larger seed size was correlated with heavier seedling shoots in both the Atlantic rainforest (Pearson's correlation: $r = 0.22$; $P = 0.0006$) and semideciduous forest ($r = 0.22$; $P = 0.0012$). Seedlings from seeds >12 mm in diameter were 1.5 times larger (dry mass) than those emerging from <12 mm diameter seeds ($t = -8.2$, $gl = 67$, $P < 0.0001$). At 270 days after sowing, seedling emergence was more than twice as high in the Atlantic rainforest (75.8%) compared with the semideciduous forest (35.1%), where a stronger drier season is observed. Therefore, seed size variation is a critical trait determining early seedling size and increasing the establishment probabilities under drier conditions.

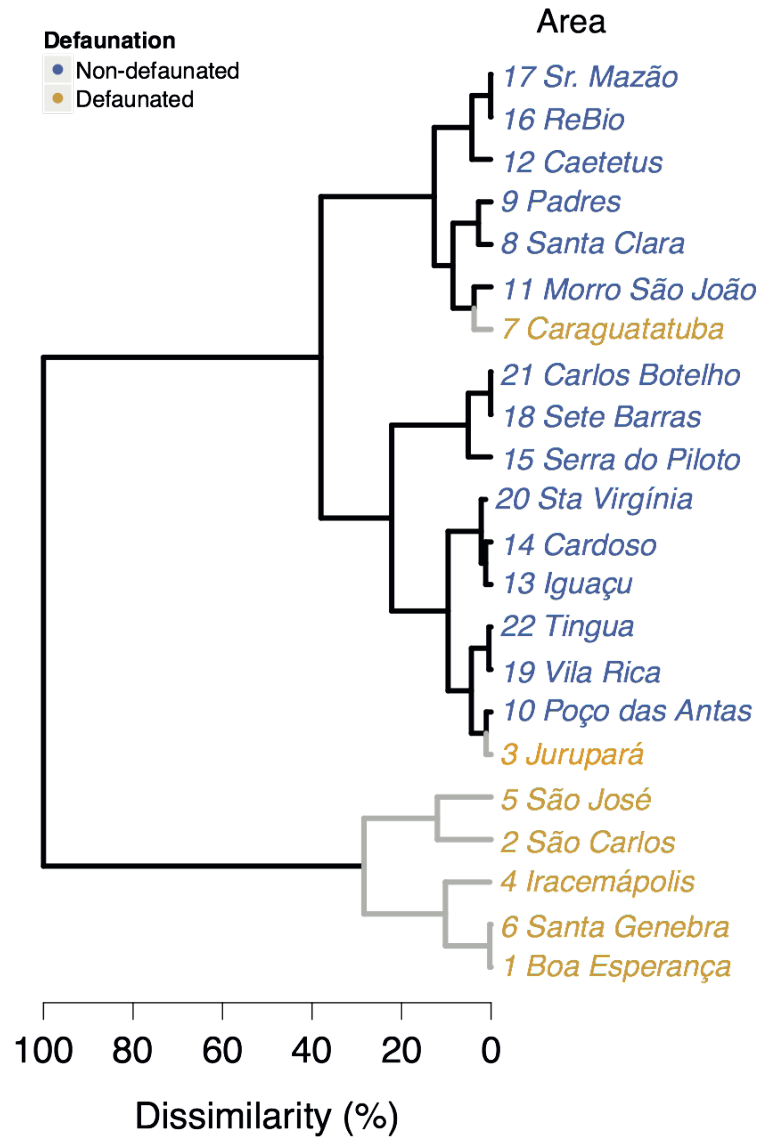


Fig. S1.

Classification of areas based on a cluster analysis of four avian frugivore traits describing each bird species weighted by their presence/absence in each area. Bird traits included the quantity component of dispersal effectiveness (i.e., the product of visitation rate, number of fruits handled per visit, and probability of dispersing a handled seed), body mass (g), gape size (mm) and frugivory score (table S4). The numbers are area codes (see table S1, Fig. 1).

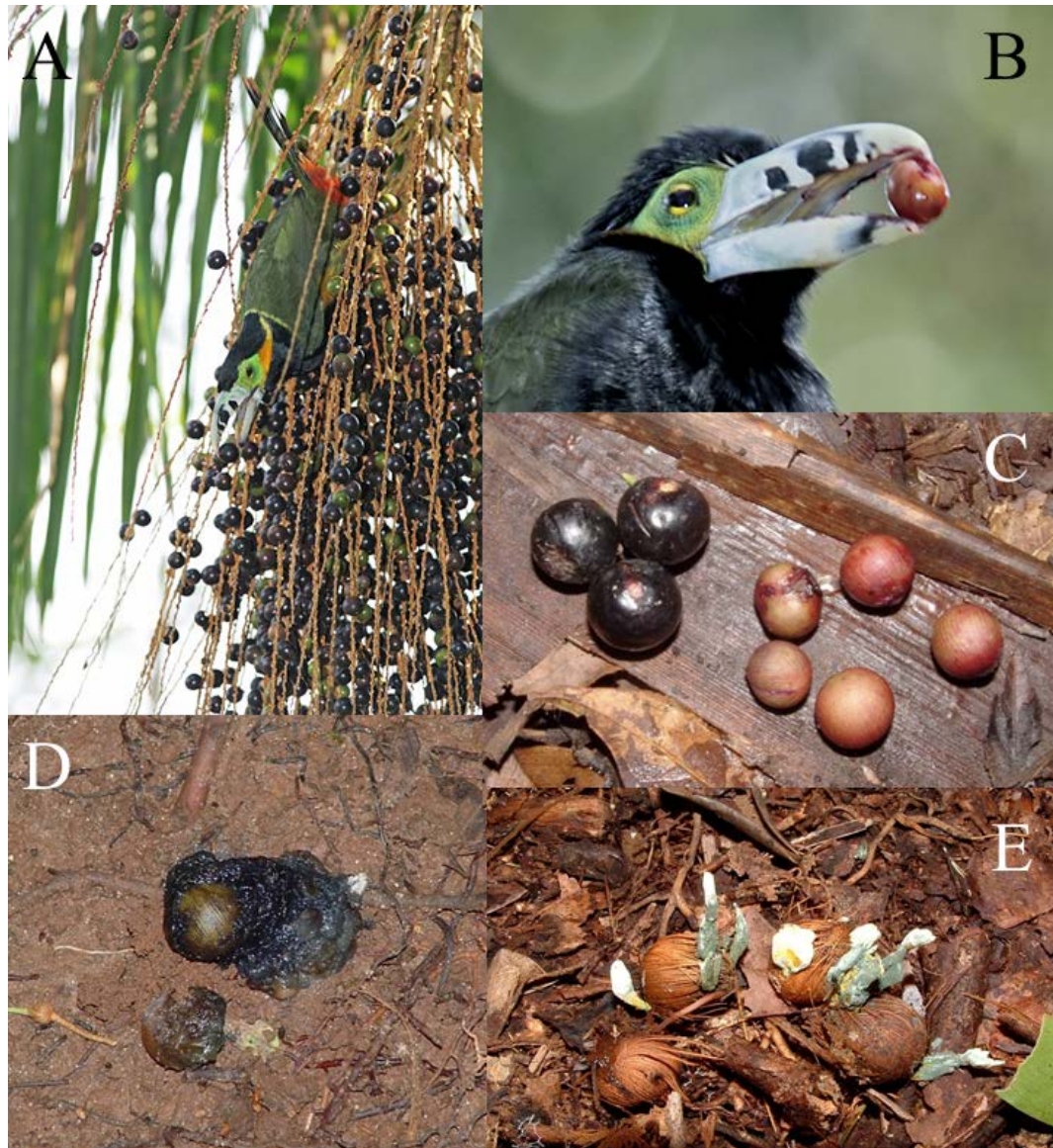


Fig. S2.

(A) Toucans and toucanets (such as *Selenidera maculirostris*) are the major large-gape, legitimate seed dispersers of *Euterpe edulis* in the Atlantic forest. (B) By defleshing the fruits, seeds regurgitated by toucans (and thrushes) germinated better than non-regurgitated seeds that are dropped beneath the palm (note the beak marks), (C) On the forest floor, regurgitated seeds and non-dispersed fruits (i.e., with the beak marks of small-gape birds) can be readily sampled and measured. (D) Seeds defecated by cracids (e.g., *Penelope superciliaris*) are attached to a mass of pulp and disseminated in a highly clumped pattern. (E) Together with non-dispersed fruits falling beneath the parent palm, their seeds are heavily attacked by pathogens or post-dispersal seed predators (Photos A and B by Edson Endrigo, and C, D and E by Marina C. Côrtes, with permission).

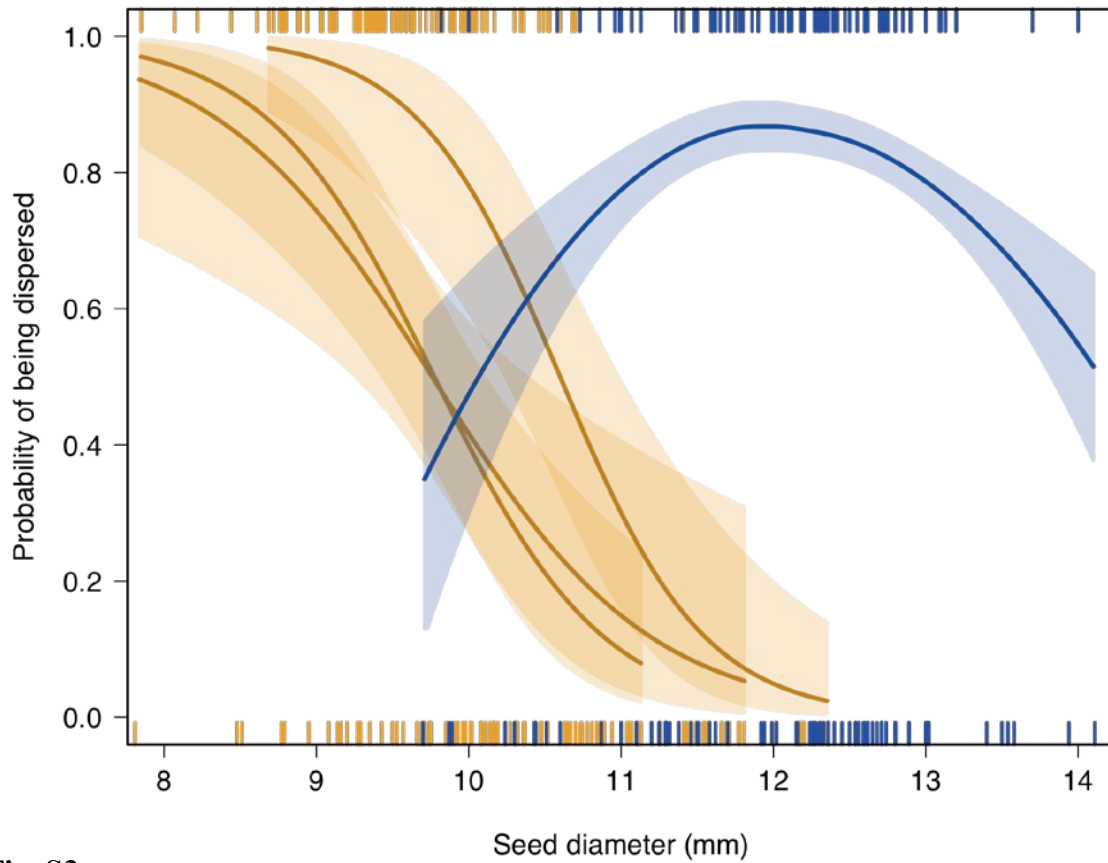


Fig. S3.

Phenotypic selection functions for palm seeds with different seed diameters in three defaunated forest fragments (orange; sites 2, 5, and 7, table S1) where large frugivorous birds are functionally extinct and in four pristine forests (blue; sites 12, 14, 20, and 22, table S1; to facilitate visualization, a pooled spline is shown). The vertical lines in the rugged plot indicate the individual seed size of undispersed (beak-marked fruits) and dispersed seeds (regurgitated).

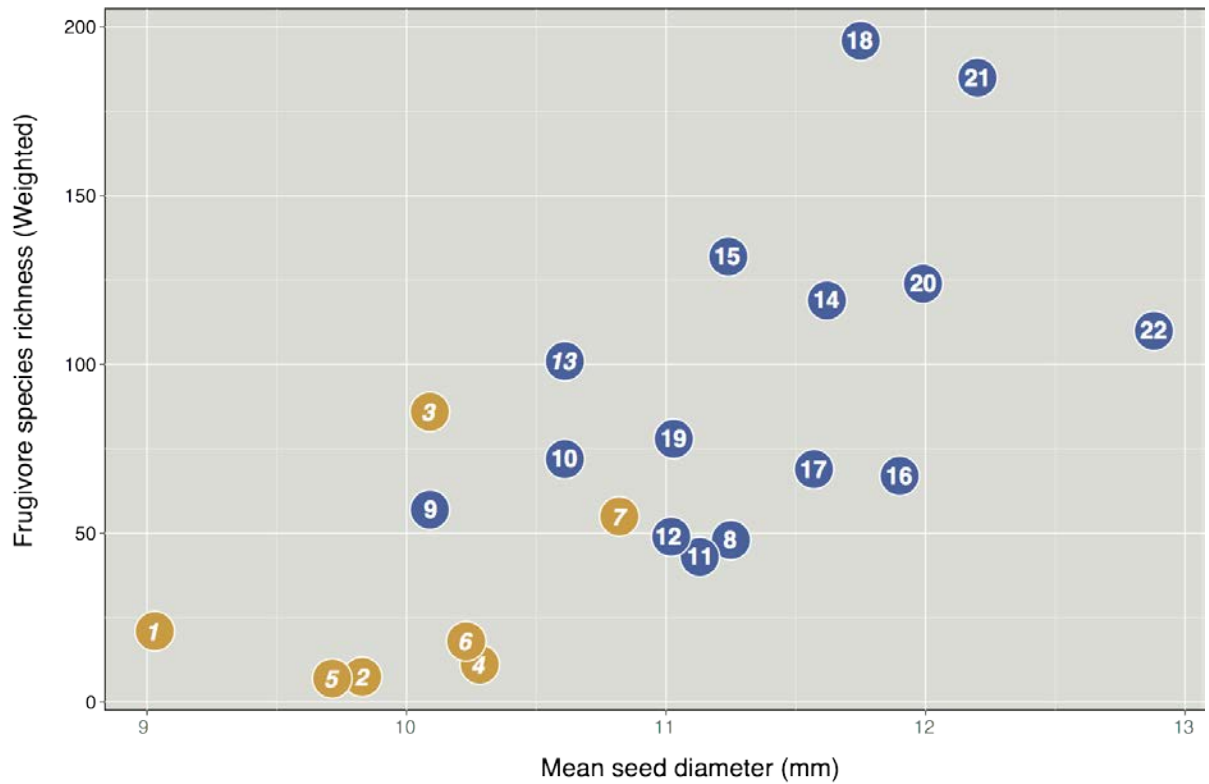


Fig. S4.

Relationship between frugivore species richness (all species of legitimate seed dispersers pooled, table S2) and mean seed size (maximum diameter, mm) of *Euterpe edulis* across the studied areas ($r= 0.6971$; $P= 0.00031$). Numbers correspond to the area codes listed in table S1 and Fig. 1.

Colors of symbols indicate the defaunation status: orange is defaunated and blue is non-defaunated. Italicized numbers represent semideciduous forest areas and normal type numbers are rainforest areas.

Table S1.

Location, defaunation status and mean (\pm SD) diameter (mm) of *Euterpe edulis* seeds in the Atlantic forest in Brazil. *N*, sample size. (*) sites where focal observations were conducted.

Site Code	Site name and location (Brazilian State)	<i>N</i>	Seed diameter	Status	Forest type
			(mm) Mean \pm SD		
1	Faz. Boa Esperança, Silva Jardim (RJ)	85	9.03 \pm 0.60	Defaunated	Rainforest
2	Mata UFSCar-São Carlos (SP)*	179	9.78 \pm 0.63	Defaunated	Semideciduous
3	PE Jurupará (SP)	55	10.09 \pm 0.68	Defaunated	Rainforest
4	Iracemápolis (SP)	588	10.27 \pm 0.74	Defaunated	Semideciduous
5	Faz. São José, Rio Claro (SP)*	258	9.75 \pm 1.02	Defaunated	Semideciduous
6	Mata Santa Genebra, Campinas (SP)*	480	10.23 \pm 1.03	Defaunated	Semideciduous
7	PESM, Caraguatatuba (SP)*	169	10.82 \pm 0.79	Defaunated	Rainforest
8	Sítio Santa Clara, Conceição Aparecida (MG)	110	11.25 \pm 0.79	Non-defaunated	Semideciduous
9	Mata dos Padres, Piedade de Caratinga (MG)	297	10.09 \pm 1.26	Non-defaunated	Semideciduous
10	EE Poço das Antas, Silva Jardim (RJ)	242	10.61 \pm 1.00	Non-defaunated	Rainforest
11	Morro São João, Cananéia (SP)*	135	11.13 \pm 0.87	Non-defaunated	Rainforest
12	EE Caetetus, Gália (SP)*	479	11.02 \pm 1.03	Non-defaunated	Semideciduous
13	PARNA Iguaçu (PR)	270	10.61 \pm 0.83	Non-defaunated	Semideciduous
14	Ilha do Cardoso, Cananéia (SP)*	701	11.62 \pm 0.70	Non-defaunated	Rainforest
15	Serra do Piloto, Rio Claro (RJ)	2250	11.24 \pm 0.92	Non-defaunated	Rainforest
16	ReBio, Una (BA)	60	11.90 \pm 0.82	Non-defaunated	Rainforest
17	Fragmento Sr. Mazão, Una (BA)	99	11.57 \pm 1.07	Non-defaunated	Rainforest
18	PE Intervalles, Sete Barras (SP)*	2366	11.75 \pm 1.07	Non-defaunated	Rainforest

Site Code	Site name and location (Brazilian State)	<i>N</i>	Seed diameter	Status	Forest type
			(mm) Mean \pm SD		
19	PE Vila Rica, Fênix (PR)	80	11.03 \pm 1.40	Non-defaunated	Semideciduous
20	PESM, Sta Virgínia (SP)	156	11.99 \pm 1.14	Non-defaunated	Rainforest
21	PE Carlos Botelho (SP)*	200	12.20 \pm 0.84	Non-defaunated	Rainforest
22	ReBio Tingua (RJ)*	100	12.88 \pm 0.60	Non-defaunated	Rainforest

PE = Parque Estadual (State Park), ReBio = Reserva Biológica (Biological Reserve), EE=Estação Ecológica (Ecological Station), PESM=Parque Estadual da Serra do Mar (Serra do Mar State Park), Faz. = Fazenda (farm).

Additional data table S2 (separate file)

Occurrence of all known frugivore species that feed on *Euterpe edulis* fruits in the 22 palm populations sampled. [Spreadsheet document]

Table S3.

Summary table for avian dispersers occurrence data in the 22 study areas (species richness; seven defaunated, 15 non-defaunated; table S2) and abundance data (four defaunated, and six non-defaunated). Abundance is given as the total, summed point abundance index (IPA) value for the large-gape dispersers and the toucans.

	Species richness	Species richness (weighted)*	Total abundance (IPA, mean)	IPA range
Large dispersers				
Defaunated	5.1±3.9	6.7±9.3	3.69	[0.004-0.94]
Non-defaunated	11.9±3.4	32.5±17.8	7.18	[0.01-1.00]
Toucans				
Defaunated	0.0	0.0 [†]	0.33	[0.01-0.11]
Non-defaunated	2.47±0.99	4.81±2.05 [†]	1.48	[0.01-0.28]

* mean species richness, with occurrence of each species weighted by its frugivory score. For toucans, occurrence was weighted by the quantitative component of seed dispersal effectiveness (table S4).

[†] only *Rhamphastos toco* has been recorded in defaunated areas, as the species infrequently enters small fragments.

Table S4. Summary of visitation data and fruit feeding behavior for frugivorous birds that feed on *Euterpe edulis* fruits in defaunated and non-defaunated Atlantic forests in Brazil. The data include information for each species from focal observations in different areas of the defaunated and non-defaunated sites, totaling 2326 h of observation. Small, small-gape (≤ 12 mm) frugivores; Large, large-gape (> 12 mm) frugivores.

Defaunation status/bird size	Family	Species	Common name	Behavior	Mean gape size (mm)	No visits/10h	No fruits handled/visit ¹	No fruits dispersed/visit ²	Dispersal probability ³	Quantitative component ⁴
Defaunated										
Large	Momotidae	<i>Baryphthengus ruficapillus</i>	Rufous-capped Motmot	regurgitate	17.2	0.60	0.83	0.83	1.00	0.50
Large	Trogonidae	<i>Trogon viridis</i>	White-tailed Trogon	regurgitate	19.6	0.10	1.00	1.00	1.00	0.10
Large	Tyrannidae	<i>Myiodynastes maculatus</i>	Streaked Flycatcher	regurgitate	17.0	0.24	1.00	1.00	1.00	0.24
Small	Turdidae	<i>Turdus albicollis</i>	White-necked Thrush	regurgitate	11.2	0.51	1.27	1.18	0.93	0.60
Small	Turdidae	<i>Turdus amaurochalinus</i>	Creamy-bellied Thrush	regurgitate	11.4	1.37	0.94	0.94	1.00	1.28
Small	Turdidae	<i>Turdus flavipes</i>	Yellow-legged Thrush	regurgitate	12.1	19.00	1.67	1.63	0.97	31.00
Small	Turdidae	<i>Turdus leucomelas</i>	Pale-breasted Thrush	regurgitate	11.9	1.28	1.21	1.21	1.00	1.55
Small	Turdidae	<i>Turdus rufiventris</i>	Rufous-bellied Thrush	regurgitate	12.0	1.20	1.29	1.21	0.94	1.45
Large	Psittacidae	<i>Pionus maximiliani</i>	Scaly-headed Parrot	pulp consumer	16.2	0.40	19.25	0.00	0.00	0.00

Large	Psittacidae	<i>Pyrrhura frontalis</i>	Maroon-bellied Parakeet	seed predator	11.2	0.10	70.00	0.00	0.00	0.00
Large	Psittacidae	<i>Triclaria malachitacea</i>	Blue-bellied Parrot	seed predator	13.6	0.20	86.00	0.00	0.00	0.00

Non-defaunated

Large	Cotingidae	<i>Carpornis cucullata</i>	Hooded Berryeater	regurgitate	14.4	0.30	2.97	2.12	0.71	0.64
Large	Cotingidae	<i>Lipaugus lanioides</i>	Cinnamon-vented Piha	regurgitate	18.7	0.83	2.10	2.10	1.00	1.74
Large	Cotingidae	<i>Procnias nudicollis</i>	Bare-throated Bellbird	regurgitate	24.5	0.42	5.54	5.41	0.98	2.29
Large	Cotingidae	<i>Pyroderus scutatus</i>	Red-ruffed Fruitcrow	regurgitate	26.7	0.12	2.20	2.20	1.00	0.25
Large	Momotidae	<i>Baryphthengus ruficapillus</i>	Rufous-capped Motmot	regurgitate	17.2	0.30	1.67	1.57	0.94	0.47
Large	Picidae	<i>Celeus flavescens</i>	Blond-crested Woodpecker	regurgitate	12.9	0.03	2.00	2.00	1.00	0.06
Large	Ramphastidae	<i>Pteroglossus bailloni</i>	Saffron Toucanet	regurgitate	23.6	0.45	3.60	3.60	1.00	1.64
Large	Ramphastidae	<i>Ramphastos dicolorus</i>	Red-breasted Toucan	regurgitate	30.7	0.14	4.44	4.37	0.98	0.63
Large	Ramphastidae	<i>Ramphastos vitellinus</i>	Channel-billed Toucan	regurgitate	31.0	0.34	6.95	6.44	0.93	2.20
Large	Ramphastidae	<i>Selenidera maculirostris</i>	Spot-billed Toucanet	regurgitate	25.2	0.51	6.28	6.02	0.96	3.05
Large	Tityridae	<i>Tityra cayana</i>	Black-tailed Tityra	regurgitate	18.7	0.04	2.25	2.00	0.89	0.08
Large	Trogonidae	<i>Trogon viridis</i>	White-tailed Trogon	regurgitate	19.6	1.22	1.09	0.55	0.50	0.66

Small	Turdidae	<i>Turdus albicollis</i>	White-necked Thrush	regurgitate	11.2	1.57	1.99	1.42	0.71	2.22
Small	Turdidae	<i>Turdus amaurochalinus</i>	Creamy-bellied Thrush	regurgitate	11.4	0.42	1.16	0.95	0.82	0.40
Small	Turdidae	<i>Turdus flavipes</i>	Yellow-legged Thrush	regurgitate	12.0	2.69	1.41	1.07	0.76	2.87
Small	Turdidae	<i>Turdus leucomelas</i>	Pale-breasted Thrush	regurgitate	11.9	1.52	0.97	0.71	0.73	1.08
Small	Turdidae	<i>Turdus rufiventris</i>	Rufous-bellied Thrush	regurgitate	12.0	0.20	1.75	1.50	0.86	0.30
Small	Turdidae	<i>Turdus subalaris</i>	Eastern Slaty Thrush	regurgitate	12.0	0.11	3.00	3.00	1.00	0.33
Large	Tyrannidae	<i>Myiodynastes maculatus</i>	Streaked Flycatcher	regurgitate	17.0	0.06	2.00	2.00	1.00	0.12
Large	Tyrannidae	<i>Pitangus sulphuratus</i>	Great Kiskadee	regurgitate	15.5	0.05	1.50	1.50	1.00	0.07
Large	Cracidae	<i>Aburria jacutinga</i>	Black-fronted Piping-Guan	defecate	24.0	0.18	30.09	30.09	1.00	5.46
Large	Cracidae	<i>Penelope obscura</i>	Dusky-legged Guan	defecate	23.4	0.12	8.91	8.27	0.93	0.97
Large	Corvidae	<i>Cyanocorax caeruleus</i>	Azure Jay	pulp consumer	17.2	0.12	2.38	1.13	0.47	0.14
Small	Fringillidae	<i>Euphonia pectoralis</i>	Chestnut-bellied Euphonia	pulp consumer	6.5	0.17	3.60	0.00	0.00	0.00
Small	Fringillidae	<i>Euphonia violacea</i>	Violaceous Euphonia	pulp consumer	6.5	0.10	4.00	0.00	0.00	0.00
Large	Psittacidae	<i>Pionus maximiliani</i>	Scaly-headed Parrot	pulp consumer	16.2	0.24	11.80	0.00	0.00	0.00
Small	Thraupidae	<i>Dacnis cayana</i>	Blue Dacnis	pulp consumer	7.0	0.11	3.00	0.00	0.00	0.00
Small	Thraupidae	<i>Orthogonys chloricterus</i>	Olive-green Tanager	pulp consumer	9.8	0.05	2.67	0.00	0.00	0.00

Small	Thraupidae	<i>Tachyphonus coronatus</i>	Ruby-crowned Tanager	pulp consumer	9.1	0.05	1.00	0.40	0.40	0.02
Small	Thraupidae	<i>Tangara cayana</i>	Burnished-buff Tanager	pulp consumer	8.8	0.07	6.50	0.00	0.00	0.00
Small	Thraupidae	<i>Tangara cyanocephala</i>	Red-necked Tanager	pulp consumer	6.6	0.06	1.00	0.00	0.00	0.00
Small	Thraupidae	<i>Tangara cyanopectera</i>	Azure-shouldered Tanager	pulp consumer	10.9	0.21	7.17	0.00	0.00	0.00
Small	Thraupidae	<i>Tangara ornata</i>	Golden-chevroned Tanager	pulp consumer	NA	0.43	1.00	0.00	0.00	0.00
Small	Thraupidae	<i>Tangara seledon</i>	Green-headed Tanager	pulp consumer	7.0	0.16	1.08	0.08	0.08	0.01
Small	Vireonidae	<i>Cyclarhis gujanensis</i>	Rufous-browed Peppershrike	pulp consumer	NA	0.07	1.50	0.00	0.00	0.00
Small	Psittacidae	<i>Brotogeris tirica</i>	Plain Parakeet	seed predator	9.9	0.07	3.50	0.00	0.00	0.00
Large	Psittacidae	<i>Pyrhura frontalis</i>	Maroon-bellied Parakeet	seed predator	11.2	0.30	18.88	0.00	0.00	0.00
Large	Psittacidae	<i>Triclaria malachitacea</i>	Blue-bellied Parrot	seed predator	13.6	0.07	32.00	0.00	0.00	0.00

1. Handled fruits are all fruits touched by the birds, and include fruits that were swallowed, dropped, carried in the beak, predated and that had the pulp consumed.

2. Dispersed fruits include only the fruits that were swallowed by the birds.

3. Dispersal probability = Total dispersed fruits / Total handled fruits

4. Quantitative component = (Number visits/10 hours) x (Number of fruits handled/visit) x (Dispersal probability)

Table S5.

AICc-based model selection with a set of candidate models for predicting the *Euterpe edulis* seed size considering defaunation, forest type, soil texture, relief, landscape structure and climatic data. The models are ranked from best to worst fit based on the AICc. For each model, the number of parameters (K), the AIC score corrected for small sample size (AICc), AICc difference from the best model (Δ AICc), and Akaike weight (w AIC) are shown. Models with Δ AICc<2 are equally plausible to explain the observed patterns. The most plausible models, which together have an 85% probability (cumulative w AIC) of best explaining the seed size given the data and relative to all competing models, are shown in bold.

Model rank	Model	K	Δ AICc	w AIC	Cumulative w AIC
1	Defaunation within Forest Type	5	0	0.4180	0.4180
2	Defaunation Status	3	1.2	0.2273	0.6453
3	Forest Type (rainforest or semideciduous)	3	1.5	0.2014	0.8467
4	Percent of Forest within sub-watershed	3	5.1	0.0323	-
5	Soil Texture	3	5.7	0.0243	-
6	Null Model	2	5.9	0.0216	-
7	Elevation	3	7.1	0.0120	-
8	Precipitation Seasonality	3	7.8	0.0084	-
9	Latitude	3	7.9	0.0080	-
10	Longitude	3	8	0.0075	-
11	Temperature Seasonality	3	8.1	0.0073	-
12	Annual Mean Precipitation	3	8.1	0.0073	-
13	Temperature of Wettest Quarter	3	8.1	0.0073	-
14	Soil Fertility	3	8.5	0.0060	-
15	Relief Complexity	3	8.5	0.0059	-
16	Annual Mean Temperature	3	8.6	0.0056	-

Table S6.

Pearson's correlations among *Euterpe edulis* palm seed dry mass, number of seeds per infructescence, number of infructescences per palm, and perimeter at breast height (pbh) (a proxy of palm age) in each year and forest types.

Variables	Year	Rainforest		Semideciduous Forest	
		<i>r</i>	<i>p</i> - value	<i>r</i>	<i>p</i> - value
Seed dry mass x seeds/infruct.	2008	-0.26	0.27	-0.20	0.40
Seed dry mass x infruct./palm		-0.12	0.10	0.35	0.13
Seeds/infruct. x infruct./palm		-0.12	0.61	-0.09	0.71
Seed dry mass x seeds/infruct.	2009	0.51	0.02	0.17	0.48
Seed dry mass x infruct./palm		0.02	0.99	0.22	0.33
Seeds/infruct. x infruct./palm		0.67	0.78	0.23	0.33
Seed dry mass x pbh	2009	0.28	0.91	0.14	0.56
Seeds/infruct. x pbh		0.23	0.32	0.08	0.74
infruct./palm x pbh		-0.43	0.06	0.23	0.33

References and Notes

1. A. D. Barnosky *et al.*, Has the Earth's sixth mass extinction already arrived? *Nature* **471**, 51 (2011). [doi:10.1038/nature09678](https://doi.org/10.1038/nature09678) [Medline](#)
2. F. Isbell *et al.*, High plant diversity is needed to maintain ecosystem services. *Nature* **477**, 199 (2011). [doi:10.1038/nature10282](https://doi.org/10.1038/nature10282) [Medline](#)
3. A. D. Barnosky *et al.*, Approaching a state shift in Earth's biosphere. *Nature* **486**, 52 (2012). [doi:10.1038/nature11018](https://doi.org/10.1038/nature11018) [Medline](#)
4. S. H. Anderson, D. Kelly, J. J. Ladley, S. Molloy, J. Terry, Cascading effects of bird functional extinction reduce pollination and plant density. *Science* **331**, 1068 (2011). [doi:10.1126/science.1199092](https://doi.org/10.1126/science.1199092) [Medline](#)
5. N. J. Cordeiro, H. F. Howe, Forest fragmentation severs mutualism between seed dispersers and an endemic African tree. *Proc. Natl. Acad. Sci. U.S.A.* **100**, 14052 (2003). [doi:10.1073/pnas.2331023100](https://doi.org/10.1073/pnas.2331023100) [Medline](#)
6. S. R. Palumbi, Humans as the world's greatest evolutionary force. *Science* **293**, 1786 (2001). [doi:10.1126/science.293.5536.1786](https://doi.org/10.1126/science.293.5536.1786) [Medline](#)
7. C. T. Darimont *et al.*, Human predators outpace other agents of trait change in the wild. *Proc. Natl. Acad. Sci. U.S.A.* **106**, 952 (2009). [doi:10.1073/pnas.0809235106](https://doi.org/10.1073/pnas.0809235106) [Medline](#)
8. S. A. B. Roels, J. K. Kelly, Rapid evolution caused by pollinator loss in *Mimulus guttatus*. *Evolution* **65**, 2541 (2011). [doi:10.1111/j.1558-5646.2011.01326.x](https://doi.org/10.1111/j.1558-5646.2011.01326.x) [Medline](#)
9. The palmito or palm heart (*E. edulis*, Arecaceae) is a dominant palm species endemic to the Atlantic forest and dependent on birds for successful seed dispersal. It also occupies fragmented forest stands originated within the past 200 years since the establishment of extensive coffee plantations in São Paulo state (21, 22).
10. M. R. Leishman *et al.*, in *Seeds: The Ecology of Regeneration in Plant Communities*, M. Fenner, Ed. (CAB International, Wallingford, UK, 2000), pp. 31– 57.
11. D. J. Levey, Seed size and fruit-handling techniques of avian frugivores. *Am. Nat.* **129**, 471 (1987). [doi:10.1086/284652](https://doi.org/10.1086/284652)
12. N. S. Sodhi, L. H. Liow, F. A. Bazzaz, Avian extinctions from tropical and subtropical forests. *Annu. Rev. Ecol. Evol. Syst.* **35**, 323 (2004). [doi:10.1146/annurev.ecolsys.35.112202.130209](https://doi.org/10.1146/annurev.ecolsys.35.112202.130209)
13. K. M. Holbrook, B. A. Loiselle, Dispersal in a Neotropical tree, *Virola flexuosa* (Myristicaceae): Does hunting of large vertebrates limit seed removal? *Ecology* **90**, 1449 (2009). [doi:10.1890/08-1332.1](https://doi.org/10.1890/08-1332.1) [Medline](#)
14. J. S. Markl *et al.*, Meta-analysis of the effects of human disturbance on seed dispersal by animals. *Conserv. Biol.* **26**, 1072 (2012). [doi:10.1111/j.1523-1739.2012.01927.x](https://doi.org/10.1111/j.1523-1739.2012.01927.x) [Medline](#)
15. Materials and methods are available as supplementary materials on *Science Online*.

16. A. de Barros Leite, P. H. S. Brancalion, R. Guevara, M. Galetti, Differential seed germination of a keystone palm (*Euterpe edulis*) dispersed by avian frugivores. *J. Trop. Ecol.* **28**, 615 (2012). [doi:10.1017/S0266467412000594](https://doi.org/10.1017/S0266467412000594)
17. D. M. S. Matos, R. P. Freckleton, A. R. Watkinson, The role of density dependence in the population dynamics of a tropical palm. *Ecology* **80**, 2635 (1999). [doi:10.2307/177246](https://doi.org/10.2307/177246)
18. D. S. Falconer, T. F. C. Mackay, *Introduction to Quantitative Genetics* (Addison Wesley Longman, Essex, UK, 1996).
19. We estimated the heritability (h^2) of seed size in *E. edulis* based on the variance of genetic relatedness between palms with available fruit phenotypic data (15). We used seed size data from genotyped individual palms in three populations, yielding $h^2 = 0.35$ (15), and the previously reported value of 18.7 years for the generation time of the palm (20).
20. M. Franco, J. Silvertown, A comparative demography of plants based upon elasticities of vital rates. *Ecology* **85**, 531 (2004). [doi:10.1890/02-0651](https://doi.org/10.1890/02-0651)
21. W. Dean, *Rio Claro: A Brazilian Plantation System 1820-1920* (Stanford Univ. Press, Stanford, CA, 1976).
22. The defaunated semideciduous forests are located in the heart of traditional coffee plantations in Brazil. For example, Rio Claro, a typical city in our defaunated region, had 65 coffee farms by 1855.
23. A. T. Moles *et al.*, A brief history of seed size. *Science* **307**, 576 (2005). [doi:10.1126/science.1104863](https://doi.org/10.1126/science.1104863) [Medline](#)
24. M. A. Pizo, C. von Allmen, L. P. C. Morellato, Seed size variation in the palm *Euterpe edulis* and the effects of seed predators on germination and seedling survival. *Acta Oecol.* **29**, 311 (2006). [doi:10.1016/j.actao.2005.11.011](https://doi.org/10.1016/j.actao.2005.11.011)
25. M. A. Pizo, I. Simão, Seed deposition patterns and the survival of seeds and seedlings of the palm *Euterpe edulis*. *Acta Oecol.* **22**, 229 (2001). [doi:10.1016/S1146-609X\(01\)01108-0](https://doi.org/10.1016/S1146-609X(01)01108-0)
26. J. A. Marengo, M. Rusticucci, O. Penalba, M. Renom, An intercomparison of observed and simulated extreme rainfall and temperature events during the last half of the twentieth century: Part 2: Historical trends. *Clim. Change* **98**, 509 (2010). [doi:10.1007/s10584-009-9743-7](https://doi.org/10.1007/s10584-009-9743-7)
27. A. T. Oliveira-Filho, M. A. L. Fontes, Patterns of floristic differentiation among Atlantic forests in southeastern Brazil and the influence of climate. *Biotropica* **32**, 793 (2000).
28. D. F. Stotz, J. W. Fitzpatrick, T. A. Parker III, D. K. Moskovits, *Neotropical Birds: Ecology and Conservation* (Univ. of Chicago Press, London, 1996).
29. E. O. Willis, The composition of avian communities in remanescent woodlots in southern Brazil. *Papéis Avulsos Zool.* **33**, 1 (1979).

30. M. B. Christiansen, E. Pitter, Species loss in a forest bird community near Lagoa Santa in southeastern Brazil. *Biol. Conserv.* **80**, 23 (1997). [doi:10.1016/S0006-3207\(96\)00073-0](https://doi.org/10.1016/S0006-3207(96)00073-0)
31. A. Aguirre, R. Guevara, M. García, J. C. López, Fate of epiphytes on phorophytes with different architectural characteristics along the perturbation gradient of *Sabal mexicana* forests in Veracruz, Mexico. *J. Veg. Sci.* **21**, 6 (2010). [doi:10.1111/j.1654-1103.2009.01131.x](https://doi.org/10.1111/j.1654-1103.2009.01131.x)
32. J. H. Ward Jr., Hierarchical grouping to optimize an objective function. *J. Am. Stat. Assoc.* **58**, 236 (1963). [doi:10.1080/01621459.1963.10500845](https://doi.org/10.1080/01621459.1963.10500845)
33. L. Short, J. Horne, *Toucans, Barbets and Honeyguides* (Oxford Univ. Press, New York, 2001).
34. R. F. Fadini, M. Fleury, C. I. Donatti, M. Galetti, Effects of frugivore impoverishment and seed predators on the recruitment of a keystone palm. *Acta Oecol.* **35**, 188 (2009). [doi:10.1016/j.actao.2008.10.001](https://doi.org/10.1016/j.actao.2008.10.001)
35. M. Galetti *et al.*, Priority areas for the conservation of Atlantic forest large mammals. *Biol. Conserv.* **142**, 1229 (2009). [doi:10.1016/j.biocon.2009.01.023](https://doi.org/10.1016/j.biocon.2009.01.023)
36. N. T. Wheelwright, Fruit size, gape width, and the diets of fruit-eating birds. *Ecology* **66**, 808 (1985). [doi:10.2307/1940542](https://doi.org/10.2307/1940542)
37. E. R. de Castro, thesis, Universidade Estadual Paulista, Rio Claro, Brazil (2003).
38. R. R. Laps, thesis, Universidade Estadual de Campinas, Campinas, Brazil (1996).
39. D. C. Rother, thesis, Universidade Estadual Paulista, Rio Claro, Brazil (2010).
40. E. W. Schupp, P. Jordano, J. M. Gómez, Seed dispersal effectiveness revisited: A conceptual review. *New Phytol.* **188**, 333 (2010). [doi:10.1111/j.1469-8137.2010.03402.x](https://doi.org/10.1111/j.1469-8137.2010.03402.x) [Medline](#)
41. R Core Team, *R: A Language and Environment for Statistical Computing* (R Foundation for Statistical Computing, Vienna, Austria, 2012); www.R-project.org/.
42. C. C. Martins, J. Nakagawa, M. L. A. Bovi, Desiccation tolerance of four seedlots from *Euterpe edulis* Mart. *Seed Sci. Technol.* **28**, 101 (2000).
43. I. A. Samuels, D. J. Levey, Effects of gut passage on seed germination: Do experiments answer the questions they ask? *Funct. Ecol.* **19**, 365 (2005). [doi:10.1111/j.1365-2435.2005.00973.x](https://doi.org/10.1111/j.1365-2435.2005.00973.x)
44. A. W. Robertson, A. Trass, J. J. Ladley, D. Kelly, Assessing the benefits of frugivory for seed germination: The importance of the deinhibition effect. *Funct. Ecol.* **20**, 58 (2006). [doi:10.1111/j.1365-2435.2005.01057.x](https://doi.org/10.1111/j.1365-2435.2005.01057.x)
45. B. Bolker and R Development Core Team, *bbmle: Tools for general maximum likelihood estimation*. R package version 1.0.5.2 (2012); <http://CRAN.R-project.org/package=bbmle>.

46. R. J. Hijmans, S. E. Cameron, J. L. Parra, P. G. Jones, J. A. Jarvis, Very high resolution interpolated climate surfaces for global land areas. *Int. J. Climatol.* **25**, 1965 (2005). [doi:10.1002/joc.1276](https://doi.org/10.1002/joc.1276)
47. M. C. Ribeiro *et al.*, in *Biodiversity Hotspots. Distribution and Protection of Conservation Priority Areas*, F. E. Zachos, J. C. Habel, Eds. (Springer, New York, 2011), pp. 405–434.
48. K. P. Burnham, D. R. Anderson, *Model Selection and Multimodel Inference. A Practical Information-Theoretical Approach* (Springer, New York, 2002).
49. A. G. Zuur *et al.*, *Mixed Effects Models and Extensions in Ecology With R* (Springer, New York, 2009).
50. K. Ritland, A marker-based method for inferences about quantitative inheritance in natural populations. *Evolution* **50**, 1062 (1996). [doi:10.2307/2410647](https://doi.org/10.2307/2410647)
51. K. Ritland, Marker-inferred relatedness as a tool for detecting heritability in nature. *Mol. Ecol.* **9**, 1195 (2000). [doi:10.1046/j.1365-294x.2000.00971.x](https://doi.org/10.1046/j.1365-294x.2000.00971.x) [Medline](#)
52. K. Ritland, S. Travis, Inferences involving individual coefficients of relatedness and inbreeding in natural populations of *Abies*. *For. Ecol. Manage.* **197**, 171 (2004). [doi:10.1016/j.foreco.2004.05.012](https://doi.org/10.1016/j.foreco.2004.05.012)
53. F. A. Gaiotto, R. P. V. Brondani, D. Grattapaglia, Microsatellite markers for heart of palm - *Euterpe edulis* and *E. oleracea* Mart. (Arecaceae). *Mol. Ecol. Notes* **1**, 86 (2001). [doi:10.1046/j.1471-8278.2001.00036.x](https://doi.org/10.1046/j.1471-8278.2001.00036.x)
54. J. F. C. Kingman, The coalescent. *Stoch. Proc. Appl.* **13**, 235 (1982).
55. M. K. Kuhner, L. P. Smith, Comparing likelihood and Bayesian coalescent estimation of population parameters. *Genetics* **175**, 155 (2007). [doi:10.1534/genetics.106.056457](https://doi.org/10.1534/genetics.106.056457) [Medline](#)
56. P. Beerli, J. Felsenstein, Maximum likelihood estimation of a migration matrix and effective population sizes in *n* subpopulations by using a coalescent approach. *Proc. Natl. Acad. Sci. U.S.A.* **98**, 4563 (2001). [doi:10.1073/pnas.081068098](https://doi.org/10.1073/pnas.081068098) [Medline](#)
57. M. K. Kuhner, LAMARC 2.0: Maximum likelihood and Bayesian estimation of population parameters. *Bioinformatics* **22**, 768 (2006). [doi:10.1093/bioinformatics/btk051](https://doi.org/10.1093/bioinformatics/btk051) [Medline](#)
58. A. Rambaut, A. J. Drummond, <http://beast.bio.ed.ac.uk/Tracer> (2007).
59. A. C. Thuillet *et al.*, Direct estimation of mutation rate for 10 microsatellite loci in durum wheat, *Triticum turgidum* (L.) Thell. ssp *durum* desf. *Mol. Biol. Evol.* **19**, 122 (2002). [doi:10.1093/oxfordjournals.molbev.a003977](https://doi.org/10.1093/oxfordjournals.molbev.a003977) [Medline](#)
60. B. S. Arbogast, S. V. Edwards, J. Wakeley, P. Beerli, J. B. Slowinski, Estimating divergence times from molecular data on phylogenetic and population genetic timescales. *Annu. Rev. Ecol. Syst.* **33**, 707 (2002). [doi:10.1146/annurev.ecolsys.33.010802.150500](https://doi.org/10.1146/annurev.ecolsys.33.010802.150500)

61. J. C. Tweddle, J. B. Dickie, C. C. Baskin, J. M. Baskin, Ecological aspects of seed desiccation sensitivity. *J. Ecol.* **91**, 294 (2003). [doi:10.1046/j.1365-2745.2003.00760.x](https://doi.org/10.1046/j.1365-2745.2003.00760.x)