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 uncertain 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. Hurly, 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. Jakovcevic, 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.

Functional Extinction of Birds Drives Rapid Evolutionary Changes in Seed Size

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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 ecosystems (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
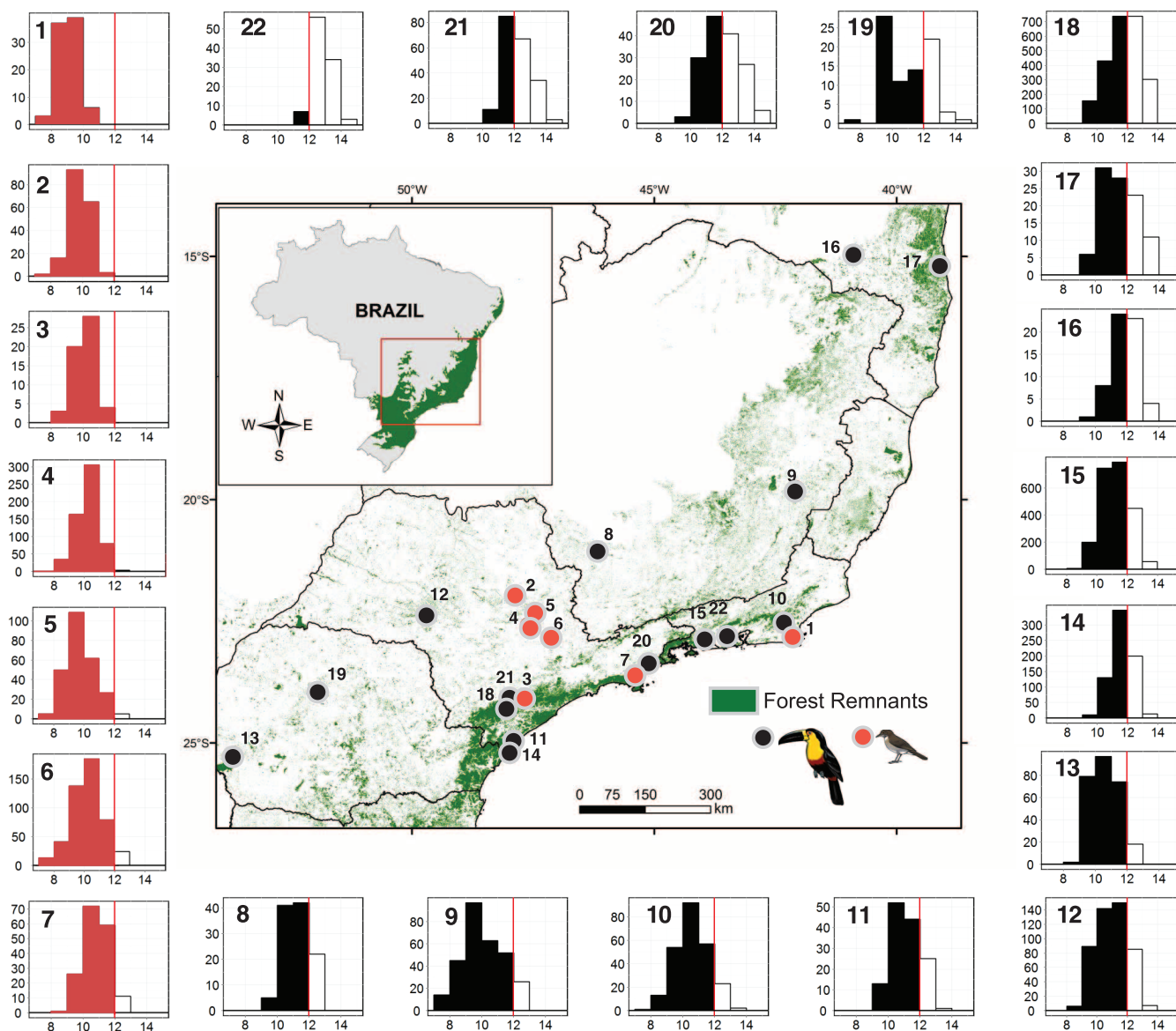
Fig. 1. Geographic variation in seed size in palm populations. Seed size (seed diameter in millimeters, *x* axis) frequency distributions (number of seeds, *y* axis) of 22 palm (*E. edulis*) populations in the remnants of the Brazilian Atlantic forest (green areas). The numbers refer to the population 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.

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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. vitelinus), 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
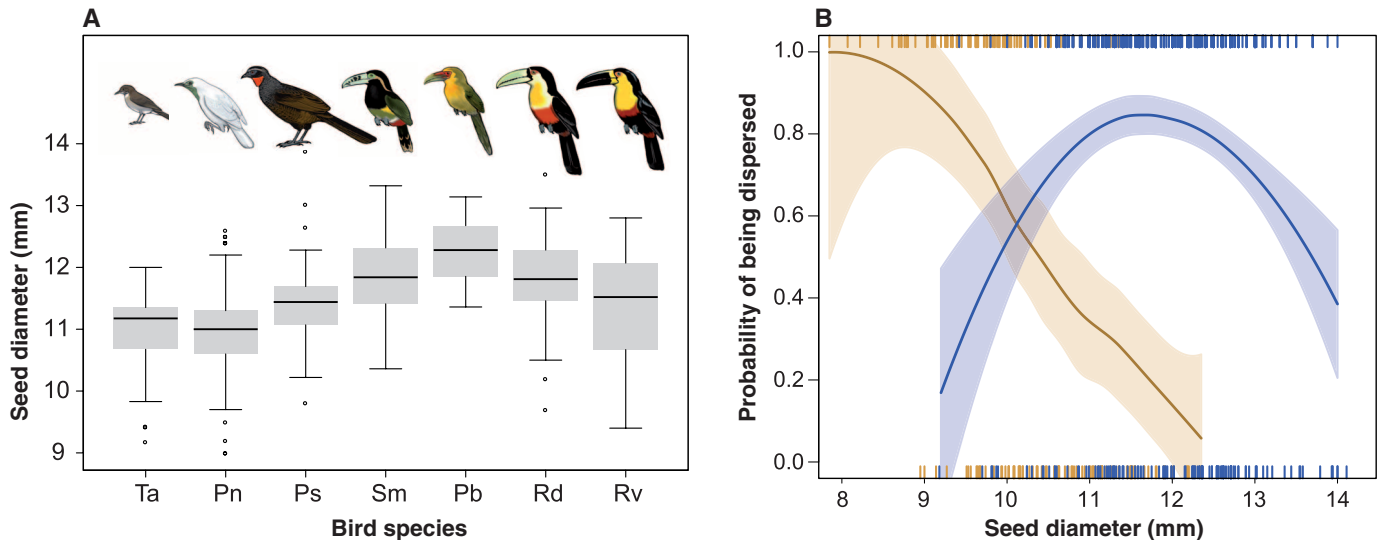
Fig. 2. Patterns of fruit preferences by frugivorous birds and consequences for phenotypic selection on seed size. (**A**) Seed size variation of the palm (E. edulis) fruits consumed by birds (from left to right): white-necked thrush (Ta, Turdus albicollis), bare-throated bellbird (Pn, Procnias nudicollis), rusty-margined guan (Ps, Penelope superciliaris), 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 rugged 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 non-defaunated 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

el 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

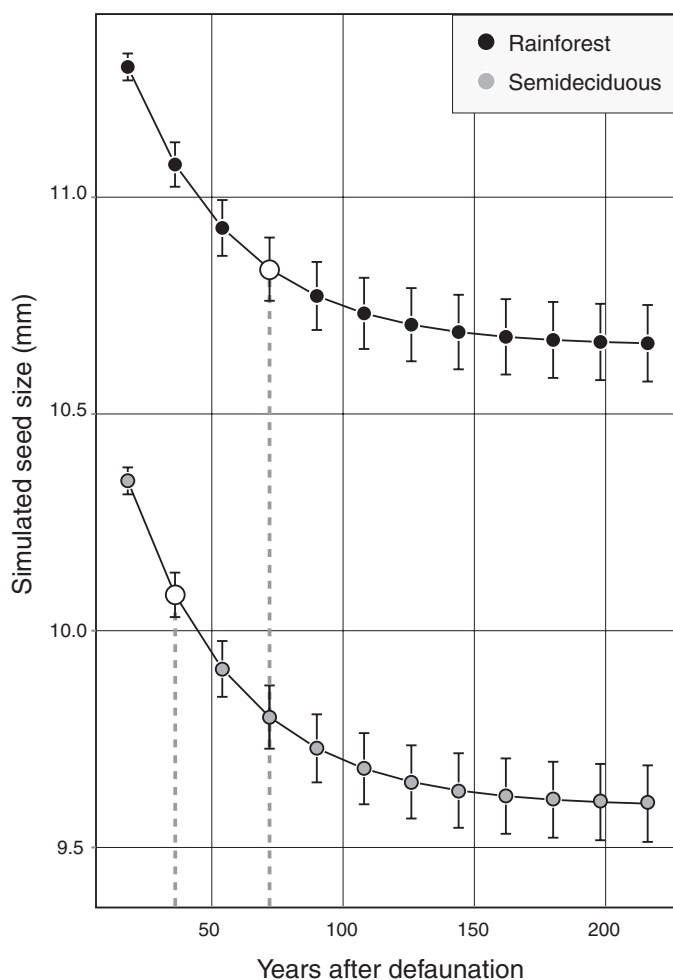seedling establishment of this threatened palm species.

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).

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.

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

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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 ± 12% of the workers (percentage ± SD across the six colonies) (Fig. 1A), whereas the second group represented 31 ± 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 ± 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 ± 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

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