Report

Resurrecting Extinct Interactions with Extant Substitutes

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Summary

There is increasing evidence that restoration ecologists should be most concerned with restoring species interactions rather than species diversity per se [1]. Rewilding with taxon substitutes, the intentional introduction of exotic species to replace the ecosystem functions of recently extinct species, is one way to reverse ecosystem dysfunction following the loss of species interactions [2]. This is highly controversial [3], in part because of a lack of rigorous scientific studies [4]. Here we present the first empirical evidence of an in situ rewilding project undertaken as a hypothesis-driven ecosystem management option. On Ile aux Aigrettes, a 25-hectare island off Mauritius, the critically endangered large-fruited endemic ebony, *Diospyros egrettarum* (Ebenaceae), was seed-dispersal limited after the extinction of all native large-bodied frugivores, including giant tortoises. We introduced exotic Aldabra giant tortoises, *Aldabrachelys gigantea*, to disperse the ebony seeds. Not only did the tortoises ingest the large fruits and disperse substantial numbers of ebony seeds, but tortoise gut passage also improved seed germination, leading to the widespread, successful establishment of new ebony seedlings. Our results demonstrate that the introduction of these exotic frugivores is aiding the recovery of ebonies. We argue for more reversible rewilding experiments to investigate whether extinct species interactions can be restored.

Results and Discussion

We undertook this experiment on a small island because large vertebrates, and hence their species interactions, were lost relatively recently [5], because the simplicity of island ecosystems facilitates assessments of the impact of taxon substitutes [6], and because islands are potentially easier to restore than mainland ecosystems [7]. We studied regeneration of the endemic slow-growing hardwood *Diospyros egrettarum* (Ebenaceae), which once covered the Mauritian island of Ile aux Aigrettes, because it is critically endangered following

intensive logging for firewood. To determine the extent to which the ebony forest had been decimated, we surveyed and mapped all ebony trees in an island-wide survey in 2007. Although we located a total of 3518 adult trees, large tracts of the island that had previously been forested remained denuded, even though logging ended in the early 1980s (Figure 1).

The Dispersal-Limited Ebony Tree

Diospyros egrettarum produces large, pungent, oval fruits (mean size ± standard error: length 31.6 ± 3.0 × width 24.0 ± 1.5 mm, weight 16.6 ± 3.7 g) typically containing eight large seeds (16.9 ± 0.9 × 12.6 ± 1.0 mm, 0.86 ± 0.11 g) encased in a sticky pulp and a hard, brittle exocarp. Preanthropogenic Ile aux Aigrettes hosted populations of several endemic frugivorous vertebrates large enough to swallow these fruits and disperse the seeds; these included giant skinks (*Leiolopisma mauritiana*) and giant tortoises (*Cylindraspis* species) [8]. Since human colonization in the late 16th century, these large-bodied terrestrial frugivores have become extinct, leaving Ile aux Aigrettes with only small-bodied frugivores unable to swallow and disperse the ebony seeds. As a consequence, *D. egrettarum* had become seed-dispersal limited; since conservation and restoration work began on the island in 1986 [8], ebony seedlings have only been observed under maternal trees.

To determine whether there was a lack of abiotic dispersal, we quantified the distribution of fallen fruits for 18 trees. Only 7 of 7437 fruits were found beyond the canopy, and these were within 1 m of the canopy edge. Indeed, for 15 of the 18 trees, all fruits were found directly under the canopy. This is not surprising, because downhill movement cannot occur on this flat coralline island and so dispersal is entirely gravity derived. This is a direct parallel to the nondispersal of fruits by neotropical megafauna following the Pleistocene mammalian extinctions [9].

Can Taxon Substitutes Resurrect Extinct Interactions?

Giant tortoises were chosen as our target frugivores to replace lost seed-dispersal interactions for three reasons. First, the endemic *Cylindraspis* tortoises were among the last Mauritian megafauna to go extinct; they persisted on several offshore islands until the mid-19th century [8]. Hence, their interactions with long-lived trees like ebony probably ended within the lifetime of many of the adult trees present today. Second, extant giant tortoises have broad diets and are therefore likely to eat foods similar to those eaten by extinct tortoises [10]. Third, giant tortoises are low-risk substitutes that are easy to employ and monitor in small-scale, reversible rewilding projects [10, 11]. We chose the Aldabra giant tortoise (*Aldabrachelys gigantea*) as our taxon substitute because of its ecological and taxonomical similarities to the extinct Mauritian tortoises [11].

Aldabrachelys gigantea as Taxon Substitutes on Ile aux Aigrettes

The first four animals were introduced in 2000. Initially, they were kept in a 1-hectare pen, where preliminary surveys indicated no significant detrimental impact on the indigenous

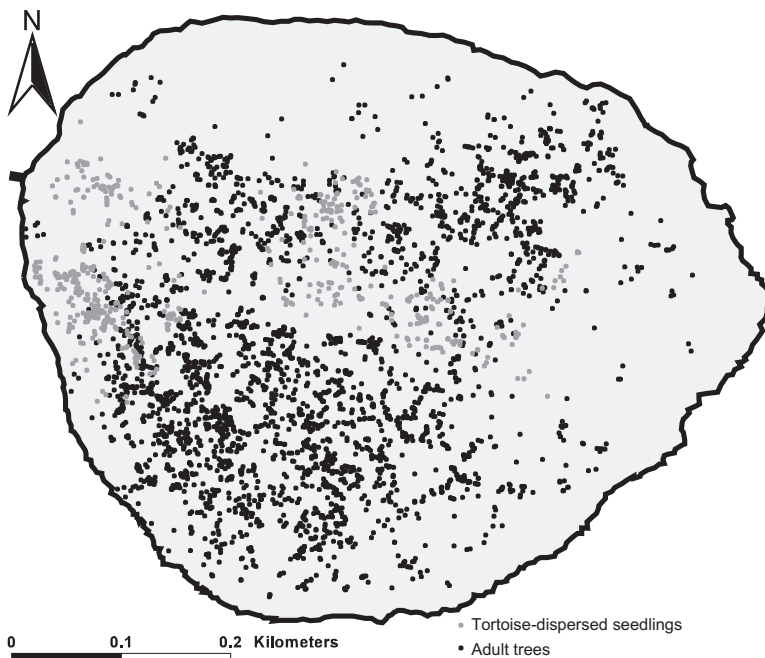*Correspondence: christine.griffiths@bristol.ac.uk

Figure 1. Tortoise Dispersal of Ebony Seeds on Ile aux Aigrettes

Location of adult *D. egrettarum* trees (n = 3518) and tortoise-dispersed seedling patches more than 1 m from the canopy of an adult ebony tree (n = 535) are shown. See also Figure S1.

- Tortoise-dispersed seedlings
- Adult trees

plant community. Several additional tortoises were added to the pens without adverse effects on the vegetation becoming apparent at the higher density, and so 11 tortoises were allowed to roam freely on the island in late 2005. By 2009, after additional introductions, there was a total population of 19 adults. Since then, few ripe ebony fruits have remained under fruiting trees as the tortoises consumed large quantities, resulting in the development of dense seedling patches beyond the gravity-derived seed shadow of maternal trees (Figure 1, see also Figure S1 available online). These dense seedling patches away from adult trees could only be due to tortoise ingestion and defecation, because there were no scatter-hoarding or seed-caching animals on the island that could produce similar seedling patches.

Tortoise-dispersed seedling patches, i.e. those found beyond the canopy of adult trees, were mapped at the same time as the island-wide survey of adult trees. We found over 500 tortoise-dispersed seedling patches, most commonly in the areas most heavily used by tortoises (Figure 1). The lack of non-tortoise-dispersed seedlings and saplings, either isolated or occurring in low-density clumps beyond the seed shadow, confirmed that seed dispersal was absent prior to releasing the Aldabra tortoises.

Effect of Tortoise Gut Passage

We also investigated the effect of tortoise gut passage on germination time and on the percentage of ebony seeds germinating, because passage through the gut of a seed disperser can alter the capacity of seeds to germinate [12]. We found that tortoise gut passage enhanced the germination of ebony seeds, with gut-passed fruit much more likely to germinate ($Z = 9.277$, $p < 0.0001$) and germinating faster ($t = -16.98$, $p_{\text{Markov chain Monte Carlo}} = 0.001$) than seeds left encased in whole fruit (Figure 2). We compared this result to germination of seeds from a manually depulped treatment and found that the enhanced germination was associated with the removal of the fruit pulp as a consequence of gut passage (Figure S2). Had we not been forced to stop the

experiment 105 days after the first seeds germinated (see Experimental Procedures), it is likely that more seeds from the whole-fruit treatment would have germinated, as indicated by the growth curve (Figure 2). Nevertheless, early germination as a consequence of gut passage may be more important than whether gut passage significantly enhances the percentage of seeds germinating, because tree seedlings that emerge early in the growing season can have significant growth and fecundity advantages, especially in competitive situations [13]. Furthermore, increasing the distribution of ebony is important. Our study demonstrates that these substitutes are a useful restoration tool to spread endemic seeds, such as ebony, which once would have been dispersed by several extinct species. The restoration of degraded forests on mainland Mauritius, where many endangered large-seeded species suffer from a lack of dispersal, could also benefit by the introduction of such taxon substitutes.

The Longer-Term Perspective

Although we have presented strong evidence that taxon substitutes can restore an extinct endemic seed-dispersal interaction, longer-term research is needed to determine whether the tortoise-dispersed ebony seedlings will develop into reproductive adult trees, the effects of the tortoises on other native biota, and how the tortoises interact with exotic and invasive plant species. Although there is an immediate risk of tortoises dispersing seeds of exotic plants, this needs to be viewed in the context of cost-benefit analyses. For instance, although *A. gigantea* potentially could spread exotic seeds on Ile aux Aigrettes, so too could the endemic *Cylindraspis* tortoises, had they survived. In fact, continual grazing pressure could suppress the reproductive output of exotic plants, and there have been no significant detrimental impacts on the fauna and flora of Ile aux Aigrettes observed in the six years following the first *A. gigantea* releases, despite continuous monitoring. However, should negative impacts appear in the longer term, the tortoises can easily be removed, or their population size, age, and sex structure, density, and movements can be manipulated to maximize the desired outcomes and minimize any negative impacts.

Conclusion

The concept of taxon substitution conflicts with traditional management options adopted in ecological restoration projects, where the norm is to remove rather than introduce exotic species [14]. Concerns about the perils of introducing exotic species as taxon substitutes need to be weighed against the risks of ignoring the effects of functional gaps left behind by recent extinctions on ecosystem dynamics and resilience. In an era of ever-increasing species extinctions and biodiversity loss [15], restoration ecologists need novel
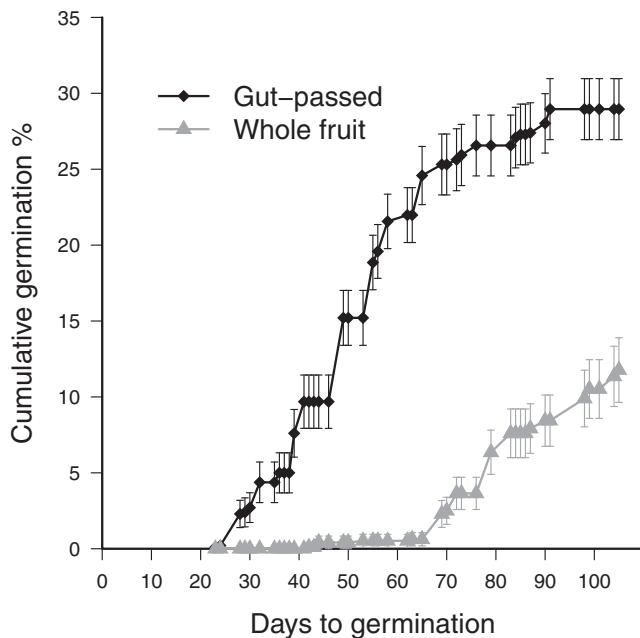
Figure 2. Mean Cumulative Germination Percentage for Whole Fruit and Gut-Passed Fruit of *D. egrettarum*

Error bars represent standard errors. Gut-passed seeds were on average twice as likely to germinate as whole fruit (mean ± standard error germination percentage: 29.0% ± 2.0% versus 11.8% ± 2.1%) and germinated more quickly (52.3 ± 1.0 days versus 82.3 ± 1.4 days). See also Figure S2.

solutions to help restore ecosystem functions and increase resilience of communities to future perturbations. Reversible and small-scale rewilding projects such as ours, with specific a priori hypotheses and explicit aims that can be rigorously quantified, provide much-needed empirical evidence on the potential benefits of taxon substitution.

Experimental Procedures

The study was conducted on Ile aux Aigrettes (57°73′05″E, 20°42′03″S) between October 2007 and February 2009. Fruit and seed morphometrics were measured on 30 fresh fruits from 14 trees.

Abiotic Dispersal

In November 2007 (the fruiting season), we investigated potential abiotic dispersal by counting the number of fallen fruits within concentric 0.5 m-wide annuli from the trunks of 18 isolated *D. egrettarum* trees in areas where sharp coral rocks formed natural barriers, making the trees inaccessible to *A. gigantea*. Searching ceased when no fruits were found in four consecutive annuli. Fruits were assumed to be from the nearest fruiting tree. Each annulus was classified as either "under canopy," if the whole or part of the annulus was below the canopy, or "beyond canopy" if no part of the annulus was below the canopy.

Quantifying Tortoise-Mediated Dispersal of Ebony

Using previously established permanent 12.5 × 12.5 m grid squares as guidelines, the whole island was searched systematically and the locations of adult *D. egrettarum* trees, i.e. those which were fruiting or had postfruiting or flowering scars on the branches, were recorded using a GPS. All adult trees were mapped because *D. egrettarum* exhibits low rates of leaky dioecy [16] and distinguishing between the different sexes (male, female, and hermaphrodite) is impossible when a tree is not fruiting. Consequently, the number of potentially fruit-producing adult trees was overestimated. In contrast, the number of tortoise-dispersed seedling patches was underestimated, because we ignored seedling patches under parent trees: we were only interested in the dispersal of patches beyond the gravity-derived seed shadow, taken to be 1 m from the canopy edge. We also searched

for non-tortoise-dispersed seedlings and saplings, either isolated or occurring in low-density clumps, beyond the gravity-derived seed shadow. We mapped the distribution of adult trees, saplings, and seedlings with ArcView 3.3 GIS (ESRI).

Germination Experiment

To determine the effect of tortoise gut passage, we compared the percentage of total seeds sown that germinated (seed germination percentage) and number of days taken to germinate (germination time, determined by the emergence of a seedling) of gut-passed seeds versus untreated whole fruits. Freshly fallen fruits were collected from beneath 15 trees. To obtain gut-passed seeds, we fed ebony fruits ad libitum to eight subadult (30–40 kg) *A. gigantea* over four consecutive days at La Vanille Réserve des Mascareignes, Rivière des Anguilles, Mauritius. On average, seeds passed through the tortoises in 1–2 weeks, and most appeared undamaged to the naked eye (smooth brown endocarp; Figure S1); these were collected and used for the germination experiment.

The germination experiment was undertaken in the conservation plant nursery on Ile aux Aigrettes between November 2008 and mid-February 2009. Using 30 trays divided into 10 × 10 cm compartments, we placed four whole fruits and the equivalent of 32 gut-passed seeds in each compartment in a randomized block design so that each tray contained both treatments. Seed quantity was chosen to reflect the high density of seeds commonly found in tortoise feces (Figure S1). The propagules were placed on a minimum of 4 cm deep soil and covered with 1 cm of soil to ensure that both seeds and fruits were sown at similar depths. Trays were rotated weekly to avoid microclimate effects and watered when the soil was dry. Seed germination was checked daily. The experiment had to be terminated 105 days after the first seeds germinated because of an unforeseen shortage in the water supply.

Linear and generalized linear mixed-effect models with treatment as a fixed factor and tray as a random factor were fitted to determine whether gut passage affected the germination time and percentage. Gaussian and binomial error distributions were used for germination time and percentage, respectively. We performed all analyses with R 2.9.2 software [17] and the package lme4 [18]. Model fit was assessed by first testing the residuals for normality and homoscedasticity and then plotting them against the predicted values. p values for Gaussian error distributions were estimated by comparison to a probability distribution obtained by 1000 Markov chain Monte Carlo simulations (mcmc function in lme4 package [19]).

Supplemental Information

Supplemental Information includes two figures and Supplemental Experimental Procedures and can be found with this article online at doi:10.1016/j.cub.2011.03.042.

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