exception concerns a genetic cluster well represented in both Taiwan and California (fig. S8). STRUCTURE simulations run separately on each regional NIA support the existence of two or more distinct genetic clusters within each, with individual clusters corresponding almost perfectly to single sample sites or groups of sites (Fig. 1). These NIA clusters are also well represented in the southern United States. These results suggest two or more recent invasions from the United States into each major regional NIA (*14*, *15*). In total, we identified nine putative introductions into NIAs, each represented by a distinct genetic cluster (two each for Taiwan, China, and California, and three for Australia; Fig. 1). This conclusion that NIA ants are directly derived from the United States is well supported by additional population genetic analyses (figs. S5, S9, and S10, and table S3).

Finally, we inferred the sources of the nine NIA introductions using a model-based Bayesian inference framework [approximate Bayesian computation (ABC)] that assumes complex demographic scenarios such as those characterizing colonization events (*16*). The final selected scenarios based on the cumulative results under three scenarios and various parameter settings (fig. S11) (*13*) are presented in Fig. 1. In virtually every simulation using site data, a scenario of separate introductions from the United States into each NIA was supported with very high probability relative to the alternative serial introduction scenario (table S4A). The only exception involves a putative serial introduction whereby fire ants in southern Taiwan are derived from a California population, which itself is derived from an earlier introduction from the southern United States. Results for ABC analyses using STRUCTURE-defined genetic clusters within geographic regions are similar, but in a few instances suggest more specific U.S. source locations for some of the NIA populations (Fig. 1 and table S4B).

Our study indicates that fire ants have been introduced on no fewer than nine separate occasions to California, Asia, and Australia from the southern United States, where *S. invicta* populations previously were confined for decades. We consider this a minimum estimate, because our NIA sample collections were not geographically exhaustive, and analyses of a few individuals obtained in Trinidad and New Zealand (from intercepted colonies) suggest that these ants also originated in the United States (fig. S2). Although we find little evidence for serial introductions among regional NIA populations, long-distance human-mediated transport of *S. invicta* after an initial introduction probably explains the dispersion of a single genetic cluster (China_2) across several hundred kilometers in China (Fig. 1). Such long-distance transport evidently was responsible for much of the early spread of the ant within the southern United States (*17*).

Repeated introductions of an invasive organism from a single source population which was itself established by recent invasion has been termed the invasive bridgehead effect (*18*–*20*). Although it is predicted to be common (*7*), empirical demonstrations of the effect are limited (*18*, *19*). Nonetheless, its occurrence has implications both for understanding evolutionary shifts associated with introductions and for developing effective management strategies. For instance, our finding of repeated successful introductions of *S. invicta* from the southern United States suggests that particular population traits associated with its success there may have pre-adapted these ants for ready colonization of other areas. Alternatively, the repeated introductions could reflect higher propagule pressure from the United States relative to native areas, given that the probability of introductions increases with escalating traffic flow in global transportation networks (*7*). The utility of data such as ours for identifying and modifying specific means of conveyance responsible for fire ant invasions remains unclear. This is because fire ants, like many other invasive insects, are probably transported as cargo stowaways, the invasion potential of which is defined more by the tempo and mode of transport (a proxy for propagule pressure) than by any specific attributes of the commodity (*21*). Nonetheless, because invasions tend to originate from locations with high cargo movement volume that are highly connected to the transport network, the integration of knowledge of invasion routes with details of transport networks may usefully inform the deployment of such measures as targeted surveillance and incursion response actions designed to limit the risk of spread of stowaways within transport networks (*7*).

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Supporting Online Material

www.sciencemag.org/cgi/content/full/331/6020/1066/DC1
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Cascading Effects of Bird Functional Extinction Reduce Pollination and Plant Density

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Reductions in bird numbers could hamper ecosystem services such as pollination, but experimental proof is lacking. We show that functional extinction of bird pollinators has reduced pollination, seed production, and plant density in the shrub *Rhabdothamnus solandri* (Gesneriaceae) on the North Island ("mainland") of New Zealand but not on three nearby island bird sanctuaries where birds remain abundant. Pollen limitation of fruit set is strong [pollen limitation index (PLI) = 0.69] and significant on the mainland but small (PLI = 0.15) and nonsignificant on islands. Seed production per flower on the mainland is reduced 84%. Mainland sites have similar adult densities, but 55% fewer juvenile plants per adult, than island sites. Seed addition experiments near adult *R. solandri* plants on the mainland found strong seed limitation 5 years after sowing for *R. solandri* but not for two other co-occurring woody species. This demonstrates a terrestrial trophic cascade.

Bird species have declined in range and density worldwide, raising concerns that the ecological services they provide, such as pollination and dispersal, may fail (*1*–*5*), with cascading impacts on biodiversity. However, there are few documented cases where failure of

dispersal mutualisms has caused a plant population to decline (*1*, *6*, *7*) and no known cases from pollination failure (*8*).

The possible failure of ecological services provided by birds is a particular concern for Oceania, where avifaunas have suffered extensive extinctions and range reductions from human impact (*9*), and bird pollination is important (*3*, *10*). New Zealand has lost 49% of its land bird species (*11*, *12*), leaving it with only "the wreckage of an avifauna" (*13*) and raising concern about whether bird pollination and dispersal are adequate (*10*, *14*–*16*). Although all three important and previously widespread endemic bird pollinators (*17*) are still extant, only one (the tui, *Prosthemadera novaeseelandiae*, Meliphagidae) is still common on the upper North Island. The other two important pollinators [bellbird, *Anthornis melanura*, Meliphagidae; and stitchbird, *Notiomystis cincta*, Notiomystidae (*18*)] were extirpated from the mainland of the upper North Island around 1870 when mammalian predators arrived, but both species survive on small adjacent northern islands (*19*), and bellbirds also occur further south on the mainland (New Zealand's large extensively modified North and South Islands). Hence, there is an opportunity to test the consequences of functional extinction on bird-pollinated native plants by comparing replicated, paired mainland and island sites. The island sites are nature reserves free of introduced mammalian predators, so still approximate the prehuman avian community (*17*), whereas only 4 to 20 km away the mainland has many mammalian pests and a depleted avifauna (*20*).

We studied *Rhabdothamnus solandri* (Gesneriaceae), a bird-pollinated endemic New Zealand shrub that grows in the forest understory throughout the upper half of the North Island (*21*, *22*). *R. solandri* has orange ornithophilous flowers with a 10-mm-long tube (Fig. 1). The flowers are visited only by the three endemic specialist pollinators—bellbirds, tui, and stitchbirds (*23*, *24*)—plus native silvereyes (*Zosterops lateralis*, Zosteropidae) (*25*). Silvereyes are generalists with relatively short tongues and usually rob nectar from *R. solandri* by ripping the corolla tube, so they may not be effective pollinators.

Pollination success of *R. solandri* was measured in two replicate regions (Auckland and Whangarei) on three island nature reserves (Little Barrier and Tiritiri Matangi near Auckland and Lady Alice near Whangarei) and at five adjacent mainland sites [two near Auckland and three near Whangarei (*26*)]. The islands have abundant tui, bellbirds, and (on Little Barrier and Tiritiri Matangi only) stitchbirds, plus low numbers of silvereyes. In contrast, the mainland sites have similar numbers of tui, no bellbirds, no stitchbirds, and abundant silvereyes [differences in 5-min bird counts were significant for all species except tui (table S4)]. In both island and mainland locations, bagged (pollinator-excluded) flowers showed very low fruit set, and hand-cross-pollinated flowers showed high fruit set (Fig. 2A), but there was a highly significant effect of location on natural fruit set [significant location by treatment interaction (table S1)]. On islands, natural (bird-accessible) flowers had equally high fruit set as hand-pollinated flowers ($P = 0.44$, post hoc means comparison), showing extremely good natural pollination, whereas on the mainland natural flowers set far fewer fruits than hand-crossed flowers ($P < 0.001$), showing strong pollination failure. Overall, the pollen limitation index (PLI) (*27*) showed no significant difference between the two regions but significant effects of location (table S2): On the islands, the PLI averaged 0.15, compared with 0.69 on the mainland (i.e., on the mainland, 69% of fruits that could have been set were lost through inadequate pollination).

As well as reducing fruit set, pollination limitation also reduced fruit size, which is a significant predictor of seed number per fruit (*26*), thereby reducing total seed production further. In the Auckland area, estimated seeds per fruit (Fig. 2B) showed the same pattern as fruit set, with significant effects of island versus mainland location ($F_{1,239} = 25.1$, $P < 0.001$), pollination treatment ($F_{2,\ 239} = 52.4$, $P < 0.001$), and location by treatment ($F_{2,\ 239} = 11.4$, $P < 0.001$). Just as with fruit set, on islands naturally pollinated fruits had as many seeds as hand-pollinated fruits ($P = 0.48$), whereas on the mainland naturally pollinated fruits had significantly fewer seeds ($P < 0.001$). Combining the proportion of fruit set and seeds per fruit, naturally pollinated flowers averaged $0.582 \times 398 = 232$ seeds per flower on islands but only $0.215 \times 168 = 37.0$ seeds per flower on the mainland, a reduction of 84% on the mainland.

In addition to the comparison of pollination treatments at matched island and mainland sites, there is other direct evidence that fruit set was reduced because of inadequate bird visitation. Flowers were scored for evidence that they had been visited by birds [rubbing on the pollen disc (Fig. 1), caused when birds insert their beaks to feed on nectar]. There was a much higher percentage of bird-visited flowers on islands (78.8%) than on the mainland (25.2%), and the difference was highly significant [binomial generalized linear model (GLM), location chi-square = 257.9, df = 1, $P < 0.001$; region was nonsignificant, chi-square = 3.27, df = 1, $P = 0.07$]. Flowers caged in the Auckland region with wire mesh to exclude birds, but not pollinating insects, had very low fruit set (comparable to bagged flowers), both on Tiritiri Matangi island (6.8%, $n = 4$ plants) and on the adjacent mainland (mean 3.1%, $n = 4$ plants), showing that birds are essential for pollination. Observations of *R. solandri* flowers in the Whangarei region showed regular visits by bellbirds on Lady Alice island (mean visitation rate 0.239 s of bird visit per flower per hour), but on the mainland zero visits by bellbirds and only rare visits by silvereyes. Similarly, in the Auckland region flowers on islands were visited by stitchbirds (Little Barrier and Tiritiri) and bellbirds (Tiritiri), whereas on the mainland the only visitors seen were silvereyes. Nectar robbing by silvereyes, revealed by slit corolla tubes, was always rare on islands (means 3.2% of flowers near Whangarei and 4.3% near Auckland) compared with the mainland [14.1 and 79.2% in Whangarei and Auckland regions, respectively; significant effects of island versus mainland location (binomial GLM, Chisq = 160.0, df = 1, $P < 0.001$), region (Chisq = 165.6, $P < 0.001$), and location by region (Chisq = 12.3, $P < 0.001$)]. These data reinforce the conclusion that a shortage of visits by endemic bird pollinators on the mainland is the cause of the failure of seed production and that recently self-introduced silvereyes (*19*) are not effective substitute pollinators.

Interestingly, this pattern of few bird visits to flowers on the mainland and frequent nectar robbing by silvereyes was already established by 1902 (*21*). If, as we believe likely, pollination failure began around 1870 when bellbirds and stitchbirds vanished from the upper North Island, there should have been time for reduced regeneration of *R. solandri* to become evident. This appears to be the case, with plot surveys in forested areas containing *R. solandri* showing similar densities of adult plants (≥30 cm tall) on mainland versus island sites (Fig. 3; $F_{1,52} = 0.347$, $P = 0.558$) but significantly lower densities of juveniles (<30

Fig. 1. A flower of *R. solandri* viewed from underneath, showing the narrow 10-mm-long corolla tube and the ridged fused pollen disc that is marked if the flower has been visited by a bird. The stigma elongates after pollen presentation is finished, so the flowers require bird visitors for successful fruit set. [Credit: M. Walters, University of Canterbury]

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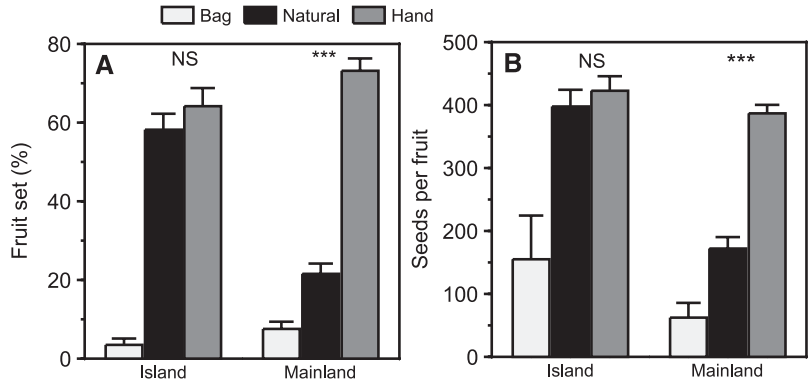

Fig. 2. Evidence for strong pollen limitation for the bird-pollinated shrub *R. solandri* on the New Zealand mainland, where its bird pollinators are functionally extinct, but not on adjacent offshore islands, where bird densities remain high. (**A**) Pollination success (mean percentage fruit set ± SEM). (**B**) Seeds per fruit (mean and SEM, estimated from fruit size). In both cases, natural (unmanipulated) flowers did significantly worse than hand-pollinated flowers on the mainland, but not on islands (post hoc tests).

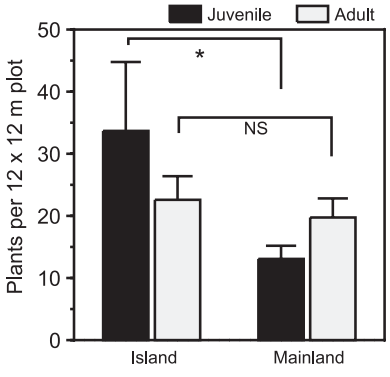

Fig. 3. Evidence for regeneration failure of *Rhabdothamnus solandri* on the mainland: densities of adult (≥30 cm tall) and juvenile (<30 cm) *R. solandri* plants (mean ± SEM) in plots centered on *R. solandri* adults on islands (high bird densities) and on the mainland (low bird densities). Island and mainland sites had similar adult densities ($P = 0.56$), but there were significantly fewer juveniles on the mainland ($P = 0.020$).

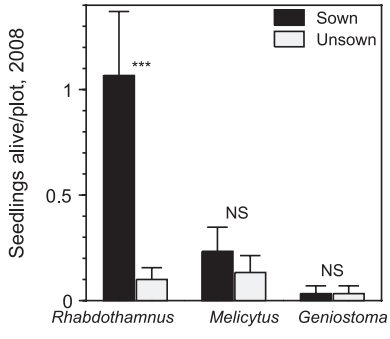

Fig. 4. Evidence for seed limitation of *R. solandri*: densities of seedlings in October 2008 (mean ± SEM) from three species sown into 10- by 20-cm seed augmentation plots on the mainland in April 2003, compared with adjacent unsown plots. Seedling density after 5 years was significantly higher in sown plots for *R. solandri*, but not for *M. ramiflorus* or *G. ligustrifolium*.

cm tall) on the mainland ($F_{1, 52} = 5.73$, $P = 0.020$). Islands had an excess of juveniles compared with adults, as predicted for a continuously regenerating population, whereas the mainland had fewer juveniles than adults, consistent with historical recruitment failure. The number of juveniles per adult was 1.490 on islands but only 0.662 on the mainland, a reduction of 55%. There are no data on how long *R. solandri* plants take to reach 30 cm tall in the field; two-year-old seedlings in fertilized pots in a glasshouse averaged 26 cm tall, but field growth rates are likely to be very much slower.

The plot surveys strongly suggest regeneration failure, but even replicated observational data are vulnerable to confounding factors. We carried out a manipulative experiment to test for regeneration failure. If *R. solandri* is declining on the mainland because of inadequate pollination, seed addition should result in increased densities of seedlings, compared with adjacent unsown plots. We set up experimental seed additions in 2003 at three mainland sites in the Whangarei region, adding seed of *R. solandri* and also of two locally common small trees that were not believed to be pollen limited (*Melicytus ramiflorus* and *Geniostoma ligustrifolium*). Our prediction was that all three species would show higher numbers of seedlings germinating immediately in sown plots but that only for *R. solandri* would these seedlings persist long-term, because the other two species were predicted to be microsite-limited not seed-limited. This was exactly what was found. The number of surviving seedlings per plot after one year (February 2004) was significantly higher in sown than unsown plots for *R. solandri* (7.5 versus 0.4 seedlings per 10- by 20-cm plot, $F_{1, 52} = 25.1$, $P < 0.001$), *M. ramiflorus* (1.4 versus 0.10, $F_{1, 52} = 23.2$, $P < 0.001$), and *G. ligustrifolium* (0.35 versus 0, $F_{1, 52} = 19.5$, $P < 0.001$). However after 5 years (October 2008), only *R. solandri* still showed significantly elevated seedling densities in the sown plots (Fig. 4 and table S3), consistent with only that species being pollen-limited and seed-limited. This result is especially noteworthy because the seed-sowing plots were all placed within a few meters of adult *R. solandri* plants, where natural seed rain should already be at a maximum, but were not necessarily near adult *M. ramiflorus* or *G. ligustrifolium*.

All the data therefore point to the same conclusion: *R. solandri* is now strongly pollen-limited on the New Zealand mainland because of local functional extinction of the three endemic birds that are its only competent pollinators. Meanwhile, less than 20 km away on predator-free offshore islands where the endemic pollinators persist, the pollination mutualism is still functioning. Mutualism failure has occurred despite the fact that none of the three birds is extinct, and one of them still occurs on the mainland but now largely feeds higher in the canopy (perhaps to avoid predators) and on more nectar-rich exotic plants. On the mainland, the plant has experienced an 84% reduction in seed output, has 55% fewer juveniles per adult, and is strongly seed-limited even in close proximity to adult plants. However, this decline could very easily have escaped notice, because it is so gradual. Individuals of *R. solandri* are slow-growing and may be long lived, so it could take many decades for the plant to disappear from an area even if regeneration was completely halted. Therefore, we have documented not a crisis but a gradual cascading effect of bird declines on the plant community. It may be that similar slow plant declines as a result of failing ecological interactions have begun elsewhere, but the relevant studies have not been done to detect them. It would be important to do so, because early conservation action is much more effective while species are still widespread and genetic diversity has not been lost. Proof that bird losses negatively affect ecological services like pollination could help to support stronger action to protect and enhance bird densities. As Janzen (*2*) stressed, we must conserve not only the component parts (species) but also the workings (ecological interactions) of our biotic communities.

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Supporting Online Material

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Global Tissue Revolutions in a Morphogenetic Movement Controlling Elongation

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Polarized cell behaviors drive axis elongation in animal embryos, but the mechanisms underlying elongation of many tissues remain unknown. Eggs of *Drosophila* undergo elongation from a sphere to an ellipsoid during oogenesis. We used live imaging of follicles (developing eggs) to elucidate the cellular basis of egg elongation. We find that elongating follicles undergo repeated rounds of circumferential rotation around their long axes. Follicle epithelia mutant for integrin or collagen IV fail to rotate and elongate, which results in round eggs. We present evidence that polarized rotation is required to build a polarized, fibrillar extracellular matrix (ECM) that constrains tissue shape. Thus, global tissue rotation is a morphogenetic behavior that uses planar polarity information in the ECM to control tissue elongation.

Elongation of a tissue along a major body axis is a central and conserved feature of animal development (*1*, *2*), and defects in this process cause human developmental abnormalities (*3*). Studies of elongating tissues have uncovered morphogenetic behaviors such as convergent extension, part of a small repertoire of cell movements known to shape animal body plans (*4*). However, for many tissues, the mechanism underlying their elongation is unknown.

The development of the ellipsoid *Drosophila* egg is an elegant case of tissue elongation. *Drosophila* eggs develop from individual follicles, each consisting of a somatic follicle cell epithelium that surrounds the germline. Follicles are initially spherical and grow isotropically but acquire anisotropic growth along the antero-posterior (A-P) axis from stage 4 of oogenesis to form a mature (stage 14) egg (Fig. 1, A and B) (*5*, *6*). Seventy-four percent of this 2.5-fold elongation is achieved in 20 hours between stages 5 and 9 (Fig. 1B). How the developing follicle breaks symmetry to channel a 24-fold increase in volume during these stages (*7*), from a sphere to an ellipsoid, is poorly understood. Evidence indicates that egg shape requires activities within the follicle epithelium (*8*), specifically from proteins linking intracellular actin to the extracellular matrix (ECM) (*6*, *9*–*14*), but how follicle cells confer egg shape has remained elusive.

Our analysis of fixed samples suggested that polarized cell divisions and cell shape changes, which are associated with elongation of other tissues (*15*–*18*), are not readily apparent in elongating follicles. To determine whether dynamic cell behaviors are involved, we used live imaging of elongating follicles (fig. S1) (*19*–*21*). This analysis revealed a morphogenetic behavior (fig. S1 and movie S1). The entire follicle epithelium undergoes a dramatic migration, in a circumferential direction around the elongating A-P axis, which leads to global rotation of this geometrically continuous tissue (Fig. 1C and movie S2).

Polarized rotation is observed in >95% of wild-type (WT) follicles (n >100) with a velocity of either 0.26 or 0.78 μm/min and both left- and right-handed chirality (Fig. 1C and movie S2). Polarized rotation is developmentally regulated and occurs predominantly between stages 5 and 9, which parallels the major phase of follicle elongation (Fig. 1B). The data suggest that a developing follicle undergoes approximately three revolutions during elongation.

Visualization of germline nuclei revealed rotation in concert with follicle cells, both in direction and angular velocity (Fig. 1C and movie S2). By contrast, follicle cells move across static collagen IV fibrils (Fig. 1D and movie S3), which demonstrates active rotation over an ECM substrate. "Follicle rotation" therefore involves global polarized revolutions of each developing egg within the basement membrane that encases each follicle.

The strong correlation between the phases of follicle rotation and egg elongation suggests that this behavior might play a role in morphogenesis. We analyzed follicles mosaic for null mutants in the integrin β_{PS} subunit (*myospheroid*; *mys*), which is required for egg elongation (*10*, *22*). *mys* mosaic follicles are significantly rounder than WT controls from stage 5 (Fig. 2, B and D, and fig. S2), the time when follicle rotation normally occurs. Live imaging of round *mys* mutant follicles revealed failure to rotate or off-axis rotation in most samples (Fig. 2, F and H; fig. S3; and movies S4 to S6).

The requirement for β_{PS} integrin in follicle shape and rotation suggests that cell-ECM interactions link both processes. Unique among ECM components, collagen IV forms circumferentially planar polarized fibrils around the follicle during the entire elongation phase (Fig. 3, A to E, and fig. S4). This led us to hypothesize that collagen IV may control egg shape, as do laminin and perlecan (*6*, *14*). Indeed, follicles with epithelia entirely mutant for collagen IV α2 (*viking; vkg*) deviate in shape at stage 8 (Fig. 2, C and D, and fig. S2) and ultimately form round eggs. Live imaging revealed polarized rotation until stage 7, when there is a notable breakdown in rotation (Fig. 2, G and H, and movies S7 and S8). These data demonstrate that mutations in genes that block follicle rotation also block elongation in a similar time frame, which suggests that these two processes are tightly coupled.

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Cascading Effects of Bird Functional Extinction Reduce Pollination and Plant Density

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