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Darwin's finches combat introduced nest parasites with fumigated cotton

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Main text

Introduced parasites are a threat to biodiversity when naïve hosts lack effective defenses against such parasites [1]. Several parasites have recently colonized the Galápagos Islands, threatening native bird populations [2]. For example, the introduced parasitic nest fly *Philornis downsi* (Diptera: Muscidae) has been implicated in the decline of endangered species of Darwin's finches, such as the mangrove finch (*Camarhynchus heliobates*) [3]. Here, we show that Darwin's finches can be encouraged to "self-fumigate" nests with cotton fibers that have been treated with permethrin. Nests with permethrin-treated cotton had significantly fewer *P. downsi* than control nests, and nests containing at least one gram of cotton were virtually parasite-free. Nests directly fumigated with permethrin had fewer parasites and fledged more offspring than nests treated with water.

Adult *P. downsi* flies, which are not parasitic, lay their eggs in the nests of Darwin's finches and other land birds in the Galápagos. Once the eggs hatch, the fly larvae feed on the blood of nestlings and adult females when they sit on the nest. Several previous studies have shown that *P. downsi* reduces the reproductive success of Darwin's finches [reviewed in 4]. In some years, 100% of nests at a given location can fail due to *P. downsi* [4,5,6]. It is therefore critical that control measures be developed to help reduce the effect of *P. downsi* on endangered Darwin's finches and other birds [3,7].

Our study was conducted January-April, 2013 at the El Garrapatero field site on Santa Cruz island [4,5]. The study was prompted by observations of several species of Darwin's finches incorporating cotton fibers from laundry lines into their nests (Figure 1A). To determine whether finches can be encouraged to self-fumigate their nests, we placed 30 cotton dispensers (Figure 1B) at 40-meter intervals along two transects through our study site (Figure S1).

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Preliminary trials showed that finches transport cotton up to 20 meters (Supplemental information).

We used two types of (interspersed) dispensers: 1) experimental dispensers, which contained cotton treated with a 1% permethrin solution, and 2) control dispensers, which contained cotton treated with water. Processed and unprocessed cotton were used to distinguish between the treatments. The two types of cotton were similar in appearance, but could be distinguished upon close inspection. A coin toss determined which treatment was assigned to which cotton type: processed cotton was used for the experimental treatment and unprocessed cotton for the control treatment. A preliminary experiment showed that finches do not discriminate on the basis of cotton type or fumigant (Figure 1C; Supplemental information).

Over the course of the study, we searched once a week for active nests within 20 meters of each dispenser. When a nest was found, it was checked with a camera on a long pole to confirm breeding activity. After the birds finished breeding, the nests were collected and dissected to quantify the number of *P. downsi* in each nest. Cotton and natural nest materials were separated and weighed.

We located 26 active Darwin's finch nests, 22 (85%) of which contained cotton (Figure 1D). None of the nests contained more than one type of cotton. Thirteen nests had experimental (permethrin) cotton and nine nests had control (water) cotton. Nests were constructed by four species of Darwin's finches: *Geospiza fortis*, *G. fuliginosa, Camarhynchus parvulus,* and *Platyspiza crassirostris*. Nests with experimental cotton had a mean (± SE) of 14.69 ± 9.54 parasites; control nests had a mean of 29.89 ± 7.69 parasites (Mann-Whitney test: $U = 31.00$, $P = 0.03$). The effect of the experimental cotton was dose-dependent. Of the eight nests that contained at least one gram of experimental cotton, seven had no parasites and the eighth had

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only four parasites (Figure 1E). There was no relationship between cotton and parasite load among control nests (Figure 1E).

Monitoring reproductive success requires climbing to nests and banding nestlings, which could interfere with self-fumigation behavior. We therefore quantified the effect of fumigation on host reproductive success using another 37 Darwin's finch nests adjacent to the self-fumigation transects. We sprayed experimental nests with a 1% permethrin solution and control nests with water. Nestlings were banded with color bands, enabling us to confirm fledging success by identifying individual birds after they left the nest [4,5]. Once all of the nestlings in a nest had fledged or died, the nest was collected and dissected to quantify the number of parasites.

The twenty experimental nests sprayed with permethrin had no parasites, while the 17 control nests sprayed with water had a mean of 17.00 ± 3.89 parasites (Mann-Whitney test, $U = 20.00$, $P < 0.0001$). Nineteen of the twenty experimental nests (95%) fledged at least one offspring, while only 11 of the 17 control nests (65%) fledged any offspring (Fisher's Exact, $P = 0.03$). Overall, 50 of 60 nestlings (83%) fledged from experimental nests, compared to just 29 of 54 nestlings (54%) from control nests (Figure 1F).

Our study shows that Darwin's finches can control *P. downsi* with permethrin-treated cotton, and that fumigation increases fledging success. There are currently no other effective methods for controlling *P. downsi*. Self-fumigation may thus be a viable approach for combatting *P. downsi* in the nests of Darwin's finches. The mangrove finch is the most critically endangered species of Darwin's finch, with a population of less than 100 individuals restricted to a home range of less than 1km^2 on Isabela Island [3]. Sixty cotton dispensers could treat this entire population. Self-fumigation may be a particularly efficient approach because mangrove finches often build their nests high in mangrove trees, where they are relatively inaccessible [3].

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Our study is the first to demonstrate the effectiveness of self-fumigation against parasites. This approach has been tried previously where mice were encouraged to incorporate fumigated cotton into their nests to kill ticks that vector Lyme Disease; however, the effectiveness of the method is not clear [8]. Self-fumigation might also be useful for controlling the fleas that vector plague, which can contribute to the local extinction of black-tailed prairie dogs (*Cynomys ludovicianus*) [9]. Because prairie dogs incorporate plant fibers into their burrows, it might be possible to encourage them to use fumigated materials. Self-fumigation also has potential for the control of parasites in other threatened and endangered bird species. For example, it might be useful for combating explosive increases in lice that appear to have contributed to the decline of the Hawaiian endemic akepa honeycreeper (*Loxops cocineus cocctneus*) [10].

Supplemental Information

Supplemental Information including experimental procedures and one figure can be found with this article online at http://dx.doi.org/10.1016/j.cub.2014.03.058.

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Figure Legend

Figure 1. Incorporation of permethrin-treated cotton into nests by Darwin's finches.

(A) Female medium ground finch (*Geospiza fortis*) removing fibers from a cotton laundry line at the Charles Darwin Research Station, Galápagos. (B) Cotton dispenser at the field site; cotton has been removed from the lower half by finches. (C) Small ground finch (*G. fuliginosa*) removing cotton from a dispenser in a preliminary experiment. (D) Finch nest containing about one gram of cotton. (E) Parasite abundance was negatively correlated with the mass of experimental cotton (Spearman rank correlation: $r_s = -0.62$, $P = 0.03$), but not with the mass of control cotton ($r_s = 0.22$, $P = 0.58$). (F) Experimental nests treated with permethrin fledged more offspring than control nests treated with water (Fisher's Exact test: $P = 0.001$). Orange bars are the total number of nestlings monitored; green bars are the total number of nestlings that fledged.

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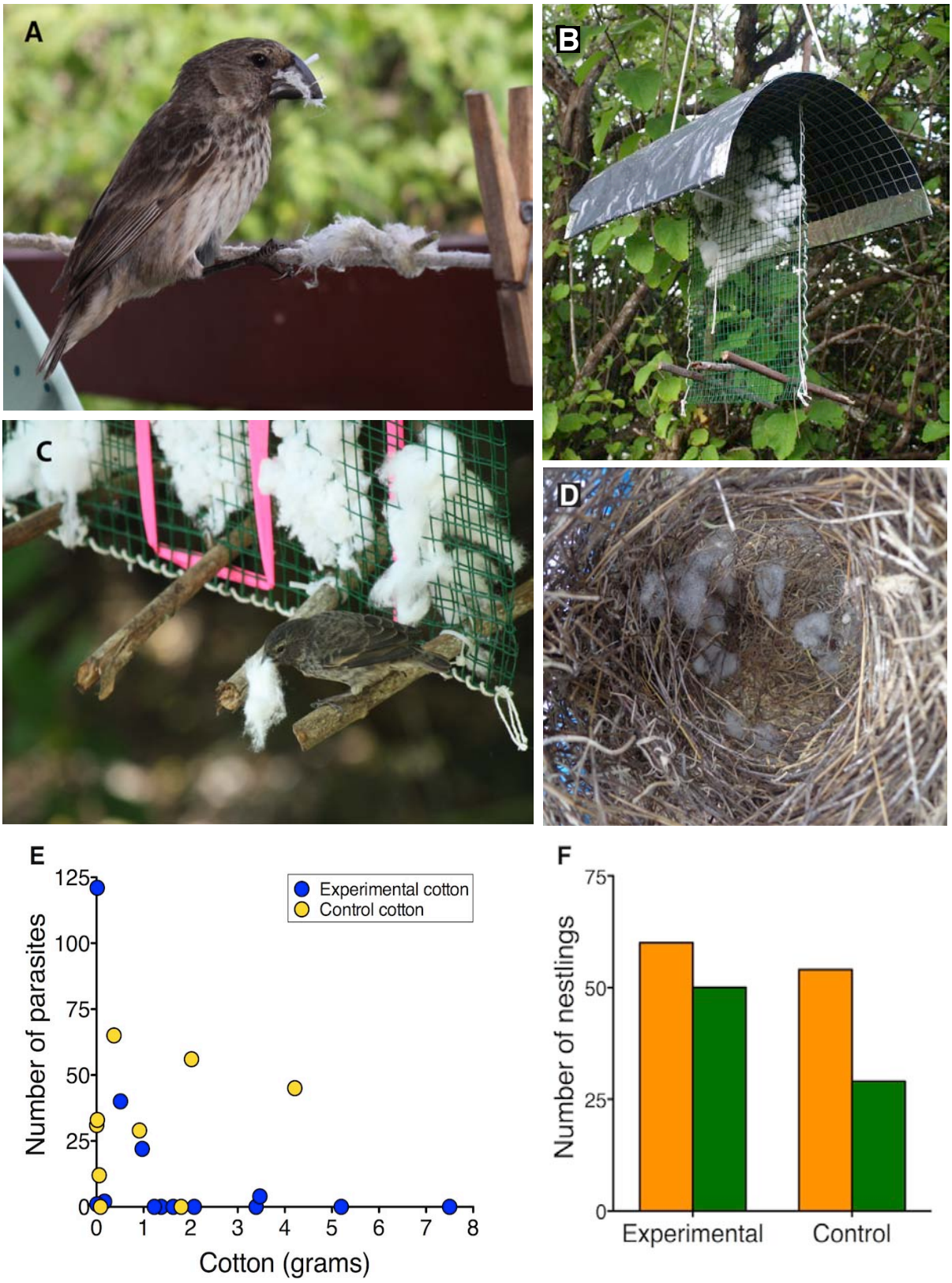
Figure 1



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Supplemental Information

Supplemental Figure

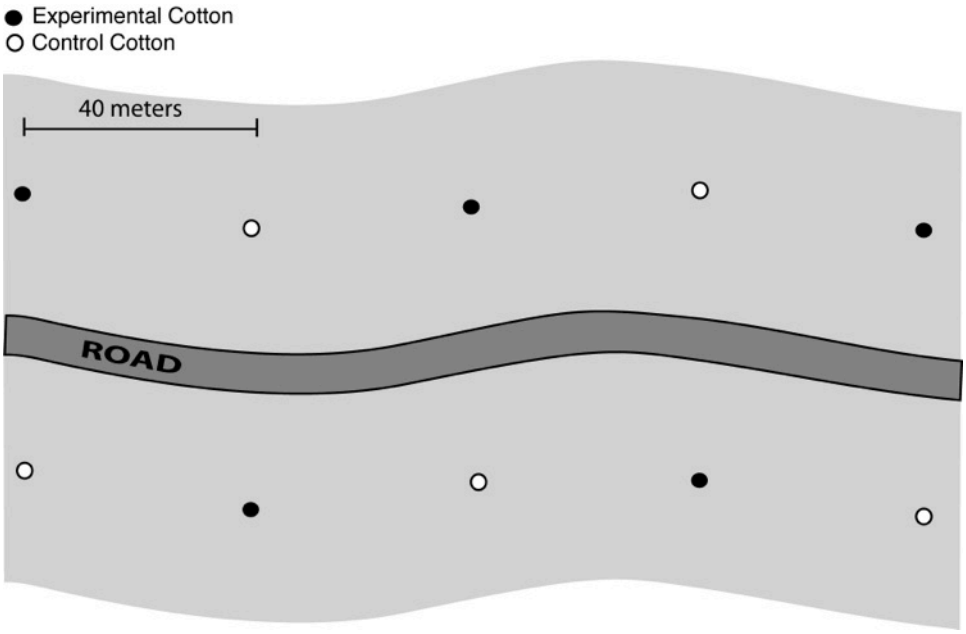

Figure S1. A partial representation of the field site with cotton dispensers. Light gray area corresponds to the area searched for nests. The experiment had a total of 30 dispensers, with 15 along each side of the road in the pattern shown here.

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Supplemental Experimental Procedures

Study system and field site

Our field site, El Garrapatero, is a 5km x 1.5km area in the southeastern arid coastal zone of Santa Cruz Island, Galápagos. Several species of Darwin's finches are abundant at this site [S1], including the medium ground finch (*Geospiza fortis*), small ground finch (*G. fuliginosa*), small tree finch (*Camarhynchus parvulus*), and vegetarian finch (*Platyspiza crassirostris*). Finch nests are dome-shaped and constructed mainly of plant fibers. Finches build their nests one to five meters above the ground at this site in endemic tree cacti (*Opuntia echios gigantea*) or *Acacia* trees. All procedures in our study were approved by the University of Utah IACUC (protocol #10-07003).

Cotton dispensers

Dispensers were made from 19-gauge hardware cloth, which held cotton in place (Figure 1B). A piece of hardware cloth was folded in half with each side bound together by cotton string along the edges. Two wooden perches were placed approximately 4cm from the bottom of the dispenser. A black plastic roof was attached to the top of each dispenser to slow the degradation of permethrin from exposure to sunlight and rain. Processed and unprocessed cotton were used to distinguish between the experimental and control treatments. Both types of cotton were obtained from U.S. Cotton™. The only difference between the cotton types is that processed cotton is combed to align the fibers.

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Discrimination test

Prior to our main study, we tested whether finches discriminate against cotton type and/or permethrin. We placed four dispensers at 100m intervals at the Charles Darwin Research Station. Each dispenser was loaded with 3g of each type of cotton and treatment: permethrin-treated processed and unprocessed cotton and water-treated processed and unprocessed cotton (Figure 1C). After 14 days, the cotton was weighed to the nearest 0.001g to determine how much of each type was removed from the dispensers. Over the course of two weeks, there was no significant difference in the type of cotton birds removed from the dispensers: finches removed a mean (± SE) of 0.83 ± 0.46g processed permethrin cotton, 1.10 ± 0.64g unprocessed permethrin cotton, 0.90 ± 0.70g processed water cotton, and 0.95 ± 0.60g unprocessed water cotton (Kruskal Wallis, $H = 1.027$, $P = 0.80$).

Distance traveled for cotton

We also tested how far finches will transport cotton to their nests. We placed a dispenser with cotton in the field at a location away from our main study site. About four weeks later, we collected nests within 200 meters of the dispenser after birds were finished using the nests (Darwin's finches do not re-use the same nests [S2]). We dissected each nest to determine whether it contained any cotton. Two nests closest to the dispenser (7 and 17 meters) had cotton, but ten more distant nests (all >25 meters away) contained no cotton. Thus, we concluded that Darwin's finches at this site will transport cotton up to about 20 meters.

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Self-fumigation experiment

Based on the preference test, 30 cotton dispensers were hung from trees 40 meters apart (approximately 2 meters above the ground) along two transects through our field site (Figure S1). Experimental dispensers contained processed cotton treated with a 1% permethrin solution; control dispersers contained unprocessed cotton treated with water. Thirty-five grams of experimental or control cotton were placed evenly over the bottom three-quarters of each dispenser. The cotton was re-sprayed with permethrin or water every 8-10 days.

We searched for active Darwin's finch nests once a week for approximately 2 months after the dispensers were placed in the field. Once a nest was found, breeding activity (eggs or nestlings) was confirmed by checking the nest with a fiber optic camera (31mm in diameter, 36 mm in length; Sony®, Tokyo, Japan) attached to a 4m collapsible pole. During this visit, we also identified the species of Darwin's finch associated with each nest by briefly observing nest activity with binoculars from at least 5m away. Six of the experimental nests were built by *G. fortis*, five by *G. fuliginosa*, one by *C. parvulus*, and one by an unidentified finch species. Two of the control nests were built by *G. fortis*, one by *G. fuliginosa*, two by *C. parvulus*, one by *P. crassirostris*, and three by unidentified finch species.

Once nestlings had died or fledged, each nest was collected and sealed in a plastic bag. The nest was dissected within eight hours and any *P. downsi* larvae, pupae, and eclosed pupal cases were counted. First instar larvae can burrow subcutaneously into nestlings, making them impossible to quantify reliably [S3]. Therefore, as in previous studies [S3], total parasite abundance was the sum of all second and third instar larvae, pupae, and eclosed pupal cases in the nest material. Larvae and pupae removed from nests were reared to confirm their identification as *P. downsi* [S4].

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All cotton was removed from nests and weighed to the nearest 0.001g. Non-cotton nest material was also weighed to the nearest 0.001g. The amount of cotton used in nests did not differ significantly by treatment. Thirteen experimental nests had a mean (± SE) of 2.12 ± 0.62g cotton; nine control nests had a mean of 1.04 ± 0.47g cotton (Mann-Whitney test: $U = 40.00$, $P = 0.23$). The percent of nest material comprised of cotton did not differ significantly by treatment (experimental nests were 5.61 ± 1.87% cotton; control nests were 2.50 ± 1.23% cotton; Mann-Whitney test: $U = 39.00$, $P = 0.20$). Four nests did not contain any cotton; these nests had a mean of 48.25 ± 16.68 parasites.

Effect of fumigation on fledging

Active nests were visited every other day between 0600 and 1100h to record the number of eggs and nestlings present. Nests were randomly assigned to the experimental or control group. Experimental nests were sprayed with a 1% permethrin solution; control nests were sprayed with water. Nests were treated when the first nestling hatched, and again 4 days later. Nestlings, eggs and a thin layer of material from the bottom of the nest were removed before the nests were treated. Parents were quick to return to their nests following treatment, and there were no cases of nest abandonment due to treatment. Nestlings were marked shortly after hatching by coloring one toenail with a permanent marker. At ~8 days of age, nestlings were banded with a numbered monel metal band and three color bands. Banded nestlings were then re-sighted within seven days of leaving the nest to confirm fledging success. After the nest failed or all nestlings had fledged, the nest was collected and sealed in a plastic bag to quantify *P. downsi,* as described above. Three nests in the control treatment were overrun by fire ants (*Solenopsis geminata*) and therefore excluded from the analyses.

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