Electronic Supplementary Methods

Avian husbandry

We obtained adult male and female zebra finches (*Taeniopygia guttata*) from commercial suppliers across the Tampa Bay area, Florida (2012 and 2014). Finches were maintained at the University of South Florida, College of Medicine, in 15 -18 free-flight cages in separate sex groups of ~8-10 individuals until experiments began. Finches were fed a standard diet of seed (ABBA 1900 exotic finch food and millet, ABBA Products Corp., Hillside, NJ). Photoperiod was maintained at 13h of light and 11h of dark for the study duration (on at 0600 and off at 1900), and finches were maintained at an average temperature of ~70-72°F and ~50% relative humidity. Finches were randomly selected from these cage groups for experiments to minimize biasing treatments with cage-mates. Procedures were carried out under institutional animal care protocols at the University of South Florida (IACUC protocol #0396).

Mosquito husbandry

A laboratory colony of *Culex quinquefasciatus* was established using a previous colony (generation > F75) from Indian River County, FL [1]. Larvae were reared at 28°C and maintained under a 14:10 (light : dark) cycle. Three to four egg rafts were placed in each of 12-15 rearing pans (45.7 cm × 53.3 cm × 7.62 cm) containing approximately 3L water. Larvae were fed daily with (20 mg/mL) of 1:1 Brewer's yeast and lactalbumen. Pupae were transferred to containers with ~250 mL of clean water, and placed into cages (30.48 cm3) for emergence. Adults were provided 20% sucrose *ad libitum*. Twenty-four hours prior to bioassays, sucrose was removed, and adult females were transferred to one-liter cardboard holding cages with mesh screen tops where they were held until introduction to bird cages. Females of uniform age were used in all trials.

Assessment of Implant Effects on Circulating CORT

To assess implant effects, we measured levels of corticosterone in finch plasma with enzyme immunoassay (EIA) kits (Arbor Assays, Ann Arbor, MI, product # K014-H5) 3 days post-surgery. We sampled all individuals immediately following the mosquito challenge. However, a notable difference between blood sampling for experiment 1 and experiment 2 was that we were only able to carry out standard sampling procedures for baseline CORT levels in experiment 1. That is, we were able to bleed finches within 3 minutes of capture, and performed captures and bleeding in a separate room from the main housing area for finches, thereby minimizing the magnitude and duration of stress at sampling. Logistical constraints prevented us from sampling quickly and in a separate space for experiment 2; instead sampling was carried out over a 1-2 hour period in the observatory room. This phenomenon is reflected in figure S1, where maximum CORT concentration levels are greater from some individuals in experiment 2, compared to experiment 1, although methods were otherwise identical. Our surgical approach, however, affected corticosterone as expected: control birds had significantly lower corticosterone than CORT-manipulated birds, and CORT in CORT-implanted birds varied in a dose-dependent manner (electronic supplementary material, figure S1).

Microsatellite genotyping protocol for Experiment 1 host blood meal identification

Blood meals were extracted from the mosquito abdomens with a DNAzol-based procedure. Briefly, mosquitoes were ground with individual RNase-free disposable pestles (Fisher Scientific, Pittsburgh, PA, product # 12-141-364) in 1.5 ml centrifuge tubes with 200 ul DNAzole BD (Molecular Research Center, Inc., Cincinnati, OH, product # DN 129) and then incubated at room temperature for 5-10 minutes. Tubes were centrifuged at 8000 rpm for 10 minutes and supernatants were transferred to new 1.5 ml tubes. DNA was precipitated by adding

80 ul isopropyl alcohol to tubes, followed by centrifugation at 6000 rpm for 10 minutes and washing (2X) with 75% ethanol. After drying, DNA pellets were resuspended in 50 ul TE8 buffer. Extracted DNA was allowed to sit overnight to maximize pellet rehydration before multiplex PCR of microsatellite loci (Pdoμ1, Pdoμ2, Pdoμ3, Pdoμ5, Pdo10, Indigo; [2-6]). We selected these loci based on their high polymorphism and reliability in scoring. The Georgia Genomics Facility at the University of Georgia performed fragment analysis, resultant data were analyzed with PEAKSCANNER v 1.0 (Applied Biosystems), and birds were discriminated within cages based on microsatellite genotype. For 13 cages, we were able to confidently discriminate individual birds from the insect blood meal DNA with one microsatellite locus; 4 cages required a combination of two loci to identify non-overlapping bird genotypes. Results for Experiment 1 are displayed as percent of vectors that fed on each type of host in multiply-housed bird cages.

Further description of behavioral assessments in Experiment 2

Although we collected 2 hours of video, we observed and scored behavior in exactly 10 minutes of pre-mosquito challenge and 10 minutes of post-mosquito challenge video. Scoring of pre-challenge segments allowed us to establish the presence of any CORT effects on behavior before interactions with vectors. In pre-mosquito (control) behavioral videos, the "vector-directed" behaviors that we counted in the absence of vectors included the same physical activities (head, tail and body shakes, and feather fluffing, picking and preening) because the birds often display many of these behaviors irrespective of vector presence. For post-exposure behavior, we scored video segments beginning approximately 2 minutes after addition of vectors into cages. We chose to score this time period because we observed the greatest total amount of activity among

birds and mosquitoes during this time. Though it was not quantified, we observed a drastic decrease in hopping and vector-directed behaviors by the last 1h of video. We acknowledge that behaviors exhibited by birds throughout the night and over the remaining hours until collection of vectors (~11-12 hours) may have affected vector-feeding success. However, we did not record behavior throughout these evening hours. As with Experiment 1, blood samples to assess circulating corticosterone levels were obtained from birds at 0600 the following morning, just prior to mosquito recovery.

Details about blood metrics for Experiment 3

Blood glucose was measured with a glucometer (CVS brand Advanced Glucose Meter, AgaMatrix, Inc., Salem, NH), but as avian blood glucose levels are high compared to humans, maximal readings from the glucometer were conservatively scored as 1 point higher than the highest measureable score (562 mg/dL). Body temperature was measured with a BAT-12 microprobe thermometer and RET5 probe (Physitemp Instruments, Inc., Cliffton, NJ, product #BAT7001H) with tip diameter of 0.053 cm, which was inserted into the cloaca. Body mass was measured with a spring-loaded scale, and hematocrit was measured by spinning ~ 50 ul of blood in a heparinized microhematocrit tube and comparing the volume of the packed red blood cell layer to the total volume of blood in the tube (expressed as a percentage).

Vector survival and productivity trial

While moving vectors, one by one, from bird cages to plastic containers, we visually inspected their abdomens, to ensure they were fully engorged, and not partially fed or unfed. Domiciles were lined with wet paper towels to maintain high humidity and small plastic cups were filled with oviposition substrate (tap water with a 1 ml aliquot 1% 1:1 yeast: sugar solution; [7]).

Additionally, holes were drilled into the side of mosquito domiciles and fitted with open-ended 1.5 ml centrifuge tubes, which allowed feeding of nectar (honey) to adult mosquitoes without disruption or removal from the containers. Adults were fed fresh organic honey *ad libitum* every other day. Mosquitoes were kept at 27°C, 75% relative humidity, and a 13h light: 11h of dark photoperiod in a closed environmental chamber for 30 d after feeding on birds. We checked mosquito container for egg rafts daily. As soon as egg rafts were observed, egg counts were performed. Adults were gently aspirated and egg rafts were carefully removed from cups to glass microscope slides with water. Two separate counts of eggs in each mass, with the observer blind to sample identity, were made on a light microscope (40X-2000X Biological Compound Microscope, AmScope, Irvine, CA) and counts averaged for each mosquito. After egg counts, females were returned to domiciles and the environmental chamber. Survival of adult mosquitoes was also monitored for 20 d.

Data analysis

Because vector preference, vector feeding success and host behaviors (for number of hops and number of vector-directed behaviors) represented count-structured data, we specified a Poisson distribution in our generalized linear mixed models. We also included an offset term in the vector preference and vector feeding success models to account for the total number of blood fed vectors collected at the end of the 12h challenge (in the case of the vector preference study – experiment 1) or to account for the total blood fed plus unfed vectors recovered from the cage after the 12h challenge (in the case of the vector feeding success study – experiment 2). The offset term accounted for the fact that, although virtually identical numbers of mosquitoes were added to each cage, the number of vectors recovered varied across cages. This difference was

due to the fact that birds ate some of the mosquitoes or crushed them during the 12h challenge. We could not discriminate mosquitoes crushed physically or due to failed consumption so we do not report those data here. Again though, this number was a small proportion of the total mosquitoes introduced to cages. Thus, our models took into account the fixed effect of treatment (control, CORT+ or CORT++), the random effect of cage (for experiment 1) or cohort (for experiment 2), as well as an offset term for total vector counts in order to predict responses about vector feeding preference and feeding success. No offset term was used in the models for host behavior, because it did not apply. Because the two host behaviors we quantified were highly correlated but biologically distinct, we present them as separate analyses. The factors, sex and body mass, were not included as factors in any final models, because they were non-significant in all cases. Individual bird ID was used as a random effect when appropriate to allow for comparisons of pre- and post-mosquito challenge behavior (hopping and vector-directed).

For experiment 3 analyses, we used Cox Mixed Effect Proportional Hazards Models for vector (1) rate of survival and (2) rate of egg laying. We used cage ID (or "bird") as a random effect, as multiple mosquitoes were collected from each cage, and treatment as the main predictor of rate of survival or reproduction in each model. We used linear mixed models to examine the relationship between clutch size and time to egg laying as a function of treatment with host individual on which mosquitoes fed as a random effect. We limited our analysis of reproductive success of mosquitoes (clutch size and day of egg laying) to 1 week after blood-feeding, twice the length of the typical gonotrophic cycle of *C. quinquefasciatus* [8]; 70% of vectors collected from bird cages laid eggs within this period of time, 18% laid eggs after day 6 and 12% did not lay clutches at all or died before laying eggs). Because time could potentially be viewed as a continuous or discrete predictor, we investigated both models and found suggestive

but not definitive support for a significant difference in the slope describing clutch size over time in the CORT++ treatment. We provide extensive explanation in figure S6A-B and table S7A-B. To further investigate this trend, we provide effect size estimates (eta squared and partial eta squared) and their confidence intervals (table S7A-B) for interaction terms in both models. These effect size estimates are an "r family" correlational metrics, indicating the amount of variance accounted for by model factors. Effect sizes were calculated from a linear fixed effect model. Effects of corticosterone on mosquito survival were assessed over the full 20 d trial.

SI Literature Cited

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