Appendix S1: Floral shape predicts bee–parasite transmission potential. Mario S. Pinilla-Gallego, Wee-Hao Ng, Victoria E. Amaral, and Rebecca E. Irwin. Ecology.

Section S1: Methods

Colony maintenance

We used *B. impatiens* commercial colonies from Koppert Biological Systems (Howell, MI, USA), keeping them in a dark room at approx. 27 ºC and 50% RH, and provided sugar-water (30% sucrose) and honey bee collected pollen *ad libitum*. Upon arrival, we screened colonies for *C. bombi* infection by taking a random sample of four workers per colony, collecting fecal samples, and examining them under a compound microscope at 400x for *C. bombi* cells.

Inoculum preparation

We dissected the guts of 5-10 workers from a source colony and homogenized each intestine in 300 µl of dH_2O. We let the samples settle for 3-4 h to allow the *C. bombi* cells to swim up into the supernatant and the gut debris to sink to the bottom of the tube. We took a 10 µl sample from each tube and estimated the number of *C. bombi* cells per microliter in a Neubauer chamber with a compound microscope at 400x magnification. We took 200 µl of clean supernatant from the samples with the highest *C. bombi* concentration and mixed them together. Then we determined the new concentration of *C. bombi* cells, and if needed, we used dH_2O to dilute the inoculum to 1200 cells/µl.

Experiment 1, Deposition:

Experiment 2, Survival: Mechanism of pathogen mortality

Different mechanisms have been proposed to explain the mortality of *C. bombi* cells in fecal droplets, such as exposure to UV light, or desiccation after the droplets have evaporated. Hence, as an additional analysis to evaluate the relative roles of these mechanisms, we generated separate histograms of cell counts in droplets that persisted (i.e., the liquid matrix remained present) and those that did not (e.g., due to evaporation), at each of the time intervals, pooled across all plant species.

Predicting droplet lifetimes using the Cox proportional hazards

The lifetime of a fecal droplet is given by the area under the survival curve. However, since the survival curves were censored at 3 hours, it is impossible to estimate the area without assuming a parametric form of the survival curve. At the same time, the assumption of proportional hazards in the Cox model implies that once we know the survival curve $S_0(t)$ for droplets on a plant with risk score r_0, we will automatically know the survival curve $S(t)$ for droplets on any plants with risk score r, since $S(t) = S_0(t) \wedge (r/r_0)$. This motivated the following procedure.

First, we generated empirical droplet survival curves for each plant species. Barring uncertainties in the estimated proportions, the survival curves appeared to be concave for slowly-decaying species, linear for intermediate species, and convex for rapidly-decaying species. In the proportional hazards framework, this suggested that a linear parametric form $S_0(t) = 1 - a \cdot t$ at some intermediate (but for now unknown) risk score r_0 could be appropriate, since then $S(t) = (1 - a \cdot t) \wedge (r/r_0)$ would be convex for species with $r > r_0$ (rapid), linear for species with $r = r_0$ (intermediate), and concave for species with $r < r_0$ (slow), in agreement with the empirical curves.

Next, we calculated the risk score of each species using the best trait-based Cox model. We then constructed a dataset, where each "observation" contained the time elapsed t, the risk score r_i for some species i, and the surviving proportion of droplets S_{it} for that species at time t. We then fitted the dataset using the nonlinear least squares model $S_{it} = (1 - a \cdot t) \wedge (r_i/r_0) + \varepsilon_{it}$ to estimate the unknown parameters a and r_0. With a and r_0, we could then estimate the lifetime of a droplet on any plant with risk score r using the area under the corresponding parametric survival curve, which turned out to have the very simple form $1 / [a \cdot (r/r_0 + 1)]$.

Appendix S3: Fig. S9 shows for each plant species, (a) the empirical survival curve, (b) the parametric survival curve estimated using the above procedure but with risk scores from a species-based Cox model, and (c) the parametric survival curve from a trait-based Cox model. The purpose of this figure is to show that the procedure can indeed generate reasonable-looking parametric survival curves, especially for the species-based Cox model where there are enough model degrees of freedom for a much closer fit of the species risk scores to the data.

Section S2: Results

Experiment 1: deposition of feces on flowers

Species-based models

Initial number of bees in the cage was a significant predictor in the species-based model for all response variables except number of droplets inside the corolla (Table 2). All variables increased with the number of bees in the cage.

Trait-based models

The length of the trial was a significant predictor for the total number of droplets on flowers, number of droplets outside the corolla and the number of flowers with droplets, with all variables increasing as the length of the trial increased.

Average bee size was a significant predictor of all variables except the number of droplets outside the corolla. The total number of droplets on flowers and the number of flowers with droplets decreased as the average bee size increased, and the number of droplets inside the corolla and on the calix increased as the average bee size increased.

The average intensity of infection was a significant predictor of the total number of droplets on flowers, number of droplets outside the corolla and the number of flowers with droplets per cage, with all variables decreasing as the average intensity of infection increased.

The start time was a significant predictor of the number of droplets inside the corolla, with fewer droplets inside the corolla as the start time increased. The number of bees in the cage and plant area were significant predictors of the number of droplets inside the corolla. An increase in these factors decreased the number of droplets outside the corolla.

Experiment 2: Survival on flowers

Species-based models

The time elapsed between inoculum preparation and the beginning of the trial was a marginally significant predictor ($P = 0.065$). As the time elapsed increased, the hazard that each plant represented for *C. bombi* increased, and therefore the survival on flowers decreased.

Mechanism of *C. bombi* mortality on flowers

Among droplets that did not persist, the majority of them had zero cell counts except at 0 min (0 min: 0/5 = 0%; 30 min: 33/58 = 57%; 1 h: 78/139 = 56%; 3 h: 303/385 = 79%). In contrast, almost none of the droplets that persisted had zero cell counts (0 min: 0/453 = 0%; 30 min: 5/398 = 1.3%; 1 h: 4/318 = 1.3%; 1/73 = 1.4%). However, even then, the median cell counts in the droplets that persisted decreased with time (0 min: 16000; 30 min: 8900; 1 h: 6400; 3 h: 5400). See Appendix S3: Fig. S6 for details. Together, they suggest that two processes were occurring: a gradual reduction in cell counts while in the liquid matrix (possibly due to exposure to UV or phytochemicals), and a complete loss of all cells that occurred very rapidly after the liquid matrix was lost (possibly due to desiccation).The gradual loss also appeared to slow down with time; for example, median cell counts in droplets that persisted fell by 44% (16000 to 8900) from t = 0 min to t = 30 min, but only by 16% (6400 to 5400) from t = 1 h to t = 3 h. This slowdown could be an artifact of pooling across plant species; Appendix S3: Fig. S7 suggests that plant species that allow longer droplet persistence also had slower gradual decay, so at later times, droplets that persisted mostly came from these species, leading to the apparent slowdown. This in turn suggests that floral traits (or weather conditions, since they were connected to flower species via phenology) that affected droplet persistence could also affect gradual cell loss.

Experiment 3: acquisition on flowers

Trait-based models: time elapsed since inoculum preparation was a significant predictor of the intensity of infection ($X^2_1 = 1.95$, $P = 0.047$), with higher intensity of infections as time elapsed increased.