

# Supplemental methods: Pairing automated mark-recapture and social network models to explore the effects of forest encroachment on hummingbird foraging patterns

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## PIT tag implantation procedure

These instructions are written for a right-handed person doing the implanting. The implant procedure is best performed last after all of the other tasks are completed (i.e., banding and measurements). The hummingbird

can be given a small amount of sugar water before the procedure, but if the bird is doing fine, it is best to wait until after the implant procedure so the enlarged crop does not interfere.

The specialized equipment for the PIT tag implantation includes a foam pad (or another soft surface) to place the hummingbird on (Figure S2). This procedure requires two people, one person to hold the hummingbird and one person to implant the PIT tag. The person holding the hummingbird on the foam pad will hold the body gently but firmly between their thumb and pointer finger so that the bird cannot wiggle or escape. With their other hand they will gently hold down the tail. The holder can also use their pointer finger on the hand that is holding down the tail to gently lift up the feathers on the upper back to expose the bare skin where the lidocaine solution will be applied (Figure S3).



Figure S1: Customized banding station setup.

Using a clean cotton applicator, the lidocaine gel ([Akorn lidocaine hydrochloride jelly USP 2%](#)) is applied to the exposed skin on the upper back of the hummingbird (Figure S4) and left to sit for a minimum of two minutes for the numbing to take effect (Figure S5). While waiting for the lidocaine gel to take effect, the PIT tag is placed in 70% ethyl alcohol for sterilization (the cap of the bottle of alcohol works well for this, see Figure S2). The needle should be placed upright (a hole or split in the foam works well for this, see Figure S2) so that the tag can be dropped into the needle and that it is easy to pick it up for inserting. Next, place the sterilized PIT tag using the sterilized forceps (sterilize them by placing them into the open bottle of alcohol, see Figure S2) into the needle. In order to facilitate the action of picking up the needle to place it onto the plunger (it needs to be screwed in to the plunger), make sure to unclip/loosen the cap of the needle, but leave the needle in the cap to keep it sterile for when it is needed.

Next, expose the skin on the back of the hummingbird (Figure S3). There is a spot on the middle/upper back that is naturally feather-free making it unnecessary to pluck any feathers. This can be done by either



Figure S2: Set-up and hummingbird hold. The bird in the photograph is a green hermit hummingbird (*Phaethornis guy*) in Costa Rica.

the person who is doing the implanting or the hummingbird holder. In Figure S3, the holder is gently lifting away the feathers on the back which then makes it easy for the implanter to access that area. The implanter will then apply the lidocaine gel to the bare skin using a clean/new cotton applicator (Figure S4). After a generous amount of the lidocaine gel is applied, you need to wait a minimum of 2 minutes for the numbing to take effect (Figure S5). While you are waiting for the skin to numb, you can do other tasks such as sterilize and prep the plunger, sterilize the tag and drop it into the needle, and make sure the cap is loosened but not removed (see the details on those procedures below). Once the lidocaine has numbed the skin where the needle will be inserted, apply a betadine antiseptic solution ([povidone-iodine, 10%](#)) with a new/clean cotton applicator to sterilize the area before implanting (Figure S6).

## Inserting the needle and implanting the PIT tag

For the entire implant procedure, it will be very important for the person holding the bird to position their hand in a way that makes room for the implanter. This can be accomplished by the holder lifting up/holding away the area of their hand between their thumb and pointer finger (it can feel awkward while still maintaining a secure hold on the hummingbird with those two fingers). Before starting the implant procedure, make sure the needle is separate/uncapped from its cover (but still sitting in it) and has the sterilized tag in it. Then with the sterilized forceps in your left hand gently lift up the skin, pick up the needle from the base with your right hand, and then insert the needle (**the bevel facing up**) just under where the forceps are lifting up the skin (Figures S7). You will be holding the needle on its own for this step (not with the plunger attached because it is too cumbersome for this part). When inserting the needle,



Figure S3: Exposing the bare spot on the back of the bird. The bird in the photograph is a green hermit hummingbird (*Phaethornis guy*) in Costa Rica.

take care to not nick/puncture the body, just the skin, which makes holding up the skin with the forceps extremely important in this step. The needle should be inserted through the skin until the bevel is fully covered (Figure S8). If the needle does not insert smoothly, making it difficult to get the bevel fully covered by the skin, you can use the forceps to help push the skin by feeding it up the needle very gently.

### Preparing the plunger

It is important to make sure that the plunger is in a non-plunged position (i.e., ready to be plunged) *before* inserting the needle into the hummingbird. Before placing it into that position, it must be sterilized by dipping it into the alcohol a couple of times and opening and closing the plunger to clean it thoroughly. After sterilizing, then leave it in the non-plunged position, but make sure that the tip of the metal plunger is slightly inserted into the track, otherwise it can move around and not plunge when you need it to.

### Attaching the plunger and inserting the tag

Attaching the plunger to the needle (**that is already inserted into the hummingbird**) is a very delicate step and will require very steady hands and complete focus. The implanter will gently switch their hold of the **inserted needle** from the right to the left hand while keeping it in place. Maintaining a steady and gentle hold on the needle with the left hand, pick up the (sterilized and not-plunged) plunger and gently screw it into the needle while holding the needle very steady so that it does not go in any further or come out and expose the bevel (Figure S9). Make sure the plunger is fully screwed into the needle. Then slowly and gently shift your grip on the plunger with your right hand in the position of pushing down the plunger.





Figure S4: Applying lidocaine gel to exposed skin.



Figure S5: With lidocaine gel applied, wait at least 2 minutes for the gel to take effect.

While gently holding the needle in place with the forceps (Figure [S10](#)), slowly and steadily push the plunger in. You will see the tag appear at the tip of the needle then continue inserting the plunger until the tag is fully expelled from the needle and under the hummingbird's skin. Once the tag is in the hummingbird and out of the needle, slowly remove the needle/plunger while continuing to gently hold the skin at the base of the entrance to keep the skin from being pulled (and tag expelled) when the needle is being removed. The tag should then be visible under the skin, and ideally you would have a little space between the needle hole and the inserted tag (Figure [S11](#)) which will make closing the hole easier.



Figure S6: Sterilizing the area with betadine antiseptic solution.



Figure S7: Inserting the needle, using forceps to lift the loose skin.

## Closing the needle hole

Now that the tag is inserted and under the skin, the next step is closing the needle hole. This is done using forceps and [Vetbond tissue adhesive](#) (Figure [S12](#)). Carefully pinch the hole closed with the forceps and apply a couple of drops of vetbond on the hole. Be careful not to apply too much as it can get on the feathers and make them hard. Once the hole stays closed on its own, release the forceps. It is best to do this while the vetbond is still a little tacky and not stuck to the forceps. Then gently blow on the area to promote drying. Once the vetbond is dry, use the tip of the plunger to arrange the feathers to cover the exposed area (the lidocaine gel already applied to the bird helps with this and keeps the feathers in place). Check the location of the tag in the neck by blowing the feathers in that area. Read the tag now that it is in the bird using the



Figure S8: Needle inserted under the skin.



Figure S9: Attaching the plunger by screwing it onto the needle while keeping the needle still.

reader and antennae to confirm the tag ID and that it is reading properly.

## List of materials

- Tags: 8mm Passive Integrated Transponder tag (weight 0.034 g) from Biomark, [MiniHPT8 8.4 mm × 1.4 mm, 134.2 kHz ISO FDX-B](#). Further documentation at <https://www.biomark.com/pub/media/HP T8.pdf>.
- [N165 Needles](#)
- [MK165 Implanter](#)





Figure S10: Inserting the PIT tag while holding onto the needle with forceps to keep it in place.



Figure S11: PIT tag inserted under the skin. The needle hole is still visible.

- [Akorn Lidocaine hydrochloride jelly USP 2%](#)
- [Betadine antiseptic solution \(povidone-iodine, 10%\)](#)
- Foam pad on which to perform the procedure
- Hand sanitizer
- Forceps - flat, no ridges, with blunted ends
- 70% ethyl alcohol
- Cotton swabs





Figure S12: Applying Vetbond to the needle hole and closing it with forceps.



Figure S13: Needle insertion hole closed with Vetbond, waiting for glue to dry.

- RFID reader and antennae (see Figure 1 of the main text)

## Narrated video

A link to a narrated video of the procedure can be found at <https://drive.google.com/file/d/0B3rFpStTFy5HTW85ektRcElJMzg/view?usp=sharing>.



Figure S14: Recaptured bird with implanted PIT tag (green hermit hummingbird in Costa Rica).

## Statistical methods

### Background

We compiled records of bird relocations from four years in which we maintained RFID-equipped hummingbird feeders and extracted movement information by tallying occasions on which an individual was recorded at feeder  $i$  at time  $t$  and again at feeder  $j$ ,  $j \neq i$ , at time  $t'$ ,  $t' > t$ , within the same day. We chose to limit our focus to movement that occurred within the same day to gain insight into hummingbird movements that may be relevant to pollination. Additionally, we chose to sum the movements over the year in order to get multiple measurements of movement between two feeders (one per year) that can more reasonably be treated as exchangeable observations. If instead we sum over shorter periods of time, yielding multiple measurements in a given year, measurements within the same year are unlikely to be exchangeable with measurements of a different year, requiring a more complicated model. Instead, we effectively average movements over the flowering period of the primary nectar-producing plants. Because we only consider movements during the flowering period, this approach should still reflect the potential for pollen flow among locations in the landscape.

We treated each feeder as a node in a graph (network) and modeled the edge weight (degree of connectivity between two nodes) of each edge in the graph. We denote  $\lambda_{ij}$  as the weight of the directed edge (in directed graphs,  $\lambda_{ij} \neq \lambda_{ji}$ ) connecting node  $i$  to  $j$  and assume that the observed number of movements between two feeders in a given year,  $y_{ijk}$ , where  $k = 1, 2, \dots, K$  indexes the year, was a random draw from a Poisson distribution with rate parameter  $\lambda_{ij}$ . Thus, in year  $k$ , when  $r_k$  out of the total  $R$  feeders were positioned and maintained on the landscape, there was a total of  $n_k = r_k(r_k - 1)$  possible movements (because  $\lambda_{ii}$  is not

defined), yielding  $N = \sum_{k=1}^K n_k$  total observations.

## Model description

Let  $y_{ijk} \in \mathbb{N}$  be the number of movements detected between readers  $i$  and  $j$  by hummingbirds in year  $k$ , where  $i \neq j = 1, 2, \dots, R$ , and  $R$  is the total number of feeders used (20 in total). Following Hoff (2005), we model the number of movements in a bilinear model where

$$\log(\lambda_{ijk}) = \mathbf{x}'_{ij}\boldsymbol{\beta} + u_i + w_j + \gamma_{ij} + \log(b_k) + \log(d_k)$$

where  $\boldsymbol{\beta}$  is a vector of dyad specific effects with  $\mathbf{x}_{ij}$  the vector of dyad-specific regressors for dyad  $\{i, j\}$  and  $'$  denotes the matrix transpose. The effects  $u_i$  and  $w_i$  are the average effects of reader  $i$  as a “sender” (movements originating at feeder  $i$ ) and “receiver” (movements ending at feeder  $i$ ), respectively, and the term  $\gamma_{ij}$  is an average effect on movement for the pair of feeders  $\{i, j\}$ . Finally,  $b_k$  and  $d_k$  are offsets for the cumulative number of birds that had been implanted with RFID tags in year  $k$  and the number of days the readers were maintained in year  $k$  (respectively).

We assume there are multiple levels of dependence in these data. For example, movements coming from another location to feeder  $i$  may be correlated, and those leaving feeder  $i$  may also be correlated. Finally, movements within a dyad ( $\{i, j\}$  pair) may be correlated. To induce dependence among observations that involve reader  $i$ , we assume

$$\begin{bmatrix} u_i \\ w_i \end{bmatrix} \sim \mathcal{N}(\mathbf{0}, \boldsymbol{\Sigma}_{uw}),$$

$$\boldsymbol{\Sigma}_{uw} = \begin{bmatrix} \sigma_u^2 & \sigma_{uw} \\ \sigma_{uw} & \sigma_w^2 \end{bmatrix},$$

such that observations that have a common sender or receiver may be correlated. Importantly, this allows for the potential that “good senders” may be “poor receivers”, so negative correlation is a possibility. While unlikely in our landscape scale study, this can allow for source-sink dynamics in the movements (Pulliam 1988). Finally, let

$$\begin{bmatrix} \gamma_{i,j} \\ \gamma_{j,i} \end{bmatrix} \sim \mathcal{N}(\mathbf{0}, \mathbf{\Sigma}_\gamma),$$

$$\mathbf{\Sigma}_\gamma = \begin{bmatrix} \sigma_\gamma^2 & \rho\sigma_\gamma^2 \\ \rho\sigma_\gamma^2 & \sigma_\gamma^2 \end{bmatrix},$$

where  $\rho$  is the correlation between the rate of movement from  $i \rightarrow j$  and  $j \rightarrow i$ . These differ from standard random effects models because they allow for negative correlation of the observations within a dyad. For our purposes, this flexibility may help elucidate *traplining* foraging behaviors in which birds forage in a regular circuit among food sources (Feinsinger 1976). This could result in negative correlations within dyads if circuits tend to be directional (e.g., birds visit a circuit of meadows in a clockwise fashion).

## Priors

We use weakly informative,  $\mathcal{N}(0, 1)$  priors for all regression coefficients and utilize a decomposition of the covariance matrices  $\mathbf{\Sigma}_\gamma$  and  $\mathbf{\Sigma}_{uw}$  in order to simplify prior specification. In particular, let  $\mathbf{\Omega}$  be a correlation matrix and  $\mathbf{L}$  be the lower triangular Cholesky factor of  $\mathbf{\Omega}$  such that  $\mathbf{L}\mathbf{L}' = \mathbf{\Omega}$ . We put a *Cholesky LKJ correlation* prior (Lewandowski, Kurowicka, and Joe 2009) on the matrices  $\mathbf{L}_\gamma$  and  $\mathbf{L}_{uw}$  (subscript notation following from above) following recommendations by the **Stan** developers team (Carpenter et al. 2017) for computational efficiency. For a  $K \times K$  lower triangular Cholesky factor  $\mathbf{L}$ , the density  $\pi(\mathbf{L} \mid \eta)$  for the prior is

$$\pi(\mathbf{L} \mid \eta) \propto \prod_{k=2}^K \mathbf{L}_{kk}^{K-k+2\eta-2}.$$

If  $\eta = 1$ , then the density is uniform over all correlation matrices of order  $K$ . We let  $\eta = 5$  for both  $\mathbf{L}_\gamma$  and  $\mathbf{L}_{uw}$ , which forms a peak at the identity matrix (no correlation). This peak gets sharper as  $\eta \rightarrow \infty$ .

By putting a prior on the (Cholesky factor of the) correlation matrix, this allows a separate prior specification for the scale parameters for the multivariate normal distribution of the vectors  $\begin{bmatrix} u_i & w_i \end{bmatrix}^T$ ,  $i = 1, 2, \dots, R$  and  $\begin{bmatrix} \gamma_{ij} & \gamma_{ji} \end{bmatrix}^T$ ,  $i = 1, 2, \dots, R$ . For the scale parameters, we chose half-Normal(0,2) priors in order to maintain some flexibility while also constraining the parameter space to reasonable values (e.g., a log-effect size of 100 for a given feeder's random effect is not very reasonable). The movement data and code for fitting this model can be found in the github repository [RUHU-movements](https://github.com/ruhu-movements).



## References

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