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Bombus impatiens (Hymenoptera: Apidae) display reduced pollen foraging behavior when marked with bee tags vs. paint

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Abstract. Numbered bee tags, developed for marking honey bees (*Apis mellifera* Linnaeus), are glued to the mesosoma of many bees to uniquely identify them. We recorded whether or not bees sonicated to collect pollen after being marked, and we compared the sonication frequency, sonication length, and wing beat frequency of *Bombus* (*Pyrobombus*) *impatiens* Cresson that were tagged with bee tags vs. marked with paint. We found that bees with tags glued to their mesosoma had no significant change in wing beat frequency, sonication frequency, or sonication length, relative to bees that were marked with paint; however, we found that the probability of collecting pollen via sonication after being marked was much lower for bees marked with bee tags vs. paint.

INTRODUCTION

Behavioral experiments on bees often require that researchers mark individuals so that they can be uniquely identified. If researchers do not know the identities of individual bees, then they risk pseudoreplication, in which they treat each observation as a unique individual when they may actually be collecting repeated measurements on the same individuals. Pseudoreplication can cause incorrect estimates of errors and lead to invalid statistical results (Hurlbert, 1984). If researchers treat many observations as independent individuals but have only several individuals, the standard errors associated with coefficients in a multiple regression may be erroneously low, leading to p-values that are also erroneously low.

To remedy this problem, individual bees can be removed from their colonies and

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placed into experimental cages (Poissonnier *et al*., 2015). An alternative method of avoiding pseudoreplication while allowing bees to continue to interact with all of their nest-mates is to mark individuals, and then collect only one observation per bee, or account for repeated measurements on the same individual (Milinski, 1997). Few studies have investigated how marking insects affects behavior (Packer, 2005; De Souza *et al*., 2012).

Common methods for marking individual bees include marking with dots of paint or attaching uniquely-numbered tags. When marking bees, numbered tags are often used (*e.g*., Osborne *et al*., 1999; Osborne & Williams, 2001) because paint comes in limited colors, and combinations of paint can quickly become complex and difficult to decipher if researchers wish to mark tens or hundreds of bees. Numbered bee tags, glued to the mesosoma, have regularly been used by beekeepers to identify honey bee queens, and are often used to mark other types of bees.

To attach the tags or apply dots of paint, some researchers narcotize bees with cold or CO_2 to keep them from moving while being marked, but Poissonnier *et al*. (2015) found that these methods affect bee behaviors — activity, brood care, foraging, aggression, and egg production — for up to four days after treatment. In addition, Wilson *et al*. (2006) found that cold narcosis affects bumble bee foraging recruitment. Because of these potential confounding factors, alternative methods may be necessary to study bee behavior.

Another method of immobilizing bees is to use a honey bee queen-marking cage (Capaldi *et al*., 2000; Reynolds *et al*., 2009), in which a bee is pressed against a mesh grid with a piece of foam. A paint dot or marker can then be placed on the bee, typically on the mesoscutum, by reaching through the grid to access the bee's body. A researcher using a queen-marking cage does not need cold or CO_2 narcosis, and thus queen-marking cages are more convenient for field-based experiments.

A variety of glues have been used to affix tags to bees. In general, scientists marking individual insects need an adhesive that is durable, non-toxic in the amount applied, easy to apply, lightweight, and quick drying (Walker & Wineriter, 1981). Many bee-tagging kits include lacquer for attachment, but tags attached with lacquer sometimes fall off after a period of time. Superglue (cyanoacrylate) meets many of the qualifications of an effective tag adhesive, and it has been used to attach tags to bees and wasps on many occasions (*e.g*., Coelho *et al*., 2007; Crall *et al*., 2015; Hagbery & Nieh, 2012; Medeiros & Araújo, 2014; Tenczar *et al*., 2014; Wilson *et al*., 2006).

Though commonly used, cyanoacrylate has been reported to affect some aspects of insect behavior. One study documented a high level of mortality when cyanoacrylate was used on the cuticle of corn rootworms, *Diabrotica* Chevrolat (Coleoptera: Chrysomelidae) — the authors suggested the softer cuticle, relative to other unaffected species, as a cause (Boiteau *et al*., 2009). Other authors describe preparation of honey bees for a flight mill, and recommend not using superglue, because "bees will quickly die" (Scheiner *et al*., 2013). However, evidence that superglue increases mortality when used on bees is scarce.

Here, we measure the effects of tagging vs. painting bees on their behavior and performance when collecting pollen from plants in large, outdoor enclosures. We measured differences in pollination behavior on tomato (*Solanum lycopersicum* L.) plants, which release pollen through small pores at the tips of the anthers. Bumble bees collect pollen from poricidal anthers using a behavior termed sonication, or buzz pollination (Buchmann, 1983). During sonication, bumble bees grasp the anthers of the flower and vibrate their flight muscles, without flapping the wings (King *et al*., 1996).

This vibration is transferred to the anthers, and pollen is shaken out of the pores onto the bee's body (King, 1993). Because tomato flowers produce no nectar, bees visiting these flowers could collect only pollen.

We measured the sonication frequency and sonication length of unmarked bumble bees during buzz pollination, as well as their wing beat frequency during flight, and then marked bees with either paint or bee tags. Then, we recorded whether these bees sonicated again and recorded the same sonication and flight parameters from marked bees that did resume pollination behavior. We chose to use superglue gel (cyanoacrylate) because it has been used on bees in the past (Tenczar *et al*., 2014), and because the gel formulation is less likely than liquid superglue to drip into the tegula and interfere with the wings.

MATERIAL AND METHODS

Study Organisms and Foraging Space

We purchased four, class-A, colonies of *Bombus* (*Pyrobombus*) *impatiens* Cresson from Biobest (http://www.biobestgroup.com). Two colonies arrived on 10 Sept 2015, and two colonies arrived on 22 Sept 2015. Upon receiving the colonies, we verified that queens were present and removed any males. Each colony was placed in a mesh cage that was 1.8 m long by 1.8 m tall by 0.6 m wide. These cages were placed in a pollinator-excluding greenhouse. The greenhouse had mesh walls and a plastic roof — thus the conditions inside the greenhouse were similar to the outdoor conditions. We allowed bees to acclimate to the cages for at least two days prior to starting experiments.

The colonies were insulated by placing them in styrofoam coolers with small holes cut for entry and exit. Each cage contained a nectar feeder (1.0 M sucrose) and pollen feeder to provide nectar and pollen *ad libitum*. Pollen was purchased from Koppert Biological Systems (http://www.koppert.com), ground with a mortar and pestle and placed (~2 g) in a small, plastic dish. Pollen was replaced approximately every three days.

In addition to the artificial feeders, each cage contained a potted tomato (*S. lycopersicum*). We used two varieties of cherry tomatoes, "Cherry Roma" and "Sweet 100 Hybrid". Each day that we observed the bees, we replaced the plant inside the bees' cage with a different plant that had been kept in a greenhouse that excluded pollinators — thus, we were able to constantly provide freshly-opened flowers for foraging. We observed all four of the colonies until 16 Oct 2015.

We also recorded local weather data — barometric pressure, temperature, relative humidity, and light intensity — at the time of every observation, using a weather station inside the greenhouse.

Marking Foragers and Collecting Audio Recordings

During each observation day, we placed a plant with freshly-opened flowers inside a cage, and waited outside the cage, observing bees foraging on the flowers of *S. lycopersicum*. When a forager landed on a flower, we reached into the cage with a shotgun microphone (SGM-1X, Azden, Tokyo, Japan), and collected an audio recording that included both sonication and flight behavior (after the bee took off) with a digital recorder (DR-100mkII, Tascam, Montebello, California). After recording an individual bee, we captured it with an insect vacuum (2820GA, Bioquip, Rancho Dominguez,

Figure 1. Individuals of *Bombus* (*Pyrobombus*) *impatiens* Cresson marked with bee tags (left) and paint (right). Scale bar = 1 cm.

California) and transferred the bee from the aspirator tube into a queen-marking cage with a plunger (The Bee Works, Oro-Medonte, Ontario, Canada). We gently pressed

the bee against the mesh at the top of the tube to immobilize her while we marked her mesosoma.

We alternated between marking captured bees with paint or bee tags (Fig. 1), to randomize the age distribution among bees with each type of mark. In total, we marked 100 bees with paint and 112 bees with tags. We excluded one individual marked with a bee tag from statistical analyses because we later determined that it was a newly emerged queen. We did not use all of the marked individuals for all analyses because we were not able to obtain all types of data for all individuals. We used Sharpie oil-based paint pens (Sharpie, Oak Brook, Illinois), after finding that water-based paints wore off too quickly in preliminary experiments. We used unique colors or combinations of two colors on each individual. After placing small dots of paint on the dorsal part of the bee's mesosoma, we used the output vent from the insect vacuum to blow air onto the paint for 30 s to dry, before releasing it back into the cage.

For marking bees with tags, we used queen-marking tags, which are small, colored plastic discs (~3 mm diameter, ~1.5 mg) that are numbered 1–99 with a variety of background colors (queen marking kit, Abelo, Full Sutton, York, United Kingdom). To apply a tag, we pressed the bee gently into the mesh at the top of the queen-marking cage and applied a small dot of superglue gel (cyanoacrylate, Gel Control, Locktite, Henkel Corporation, Westlake, Ohio). We attempted to apply glue only to the mesoscutum but sometimes covered other areas, especially if the bee was very small. We then pressed the bee tag onto the glue and used the output vent from the insect vacuum as indicated above. We released bees back into the cage by letting them fly out of the queen marking cage, and thus confirmed that at the time of release they were able to flap all of their wings.

Whenever we observed previously-marked individuals foraging for pollen on *S. lycopersicum* plants, we again collected audio recordings of their sonication and flight behavior, for comparison with the recordings we made before marking. We observed 118/212 bees engaging in sonication behavior after being marked. Of these, 40 were marked with bee tags and 78 were marked with paint. We did not observe each cage every day due to poor weather conditions on some days. Rain hitting the top of the greenhouse or heavy wind shaking the greenhouse interfered with audio recordings by increasing background noise.

At the end of the experiment (16 Oct 2015), we collected all of the bees from the colonies, recorded whether or not they were alive, and used digital calipers to measure their intertegular (IT) span, the minimum distance between the inner margins of the tegulae (wing bases). We were unable to collect IT span measurements for all marked bees, as the marks sometimes wore off of the bees before the end of the experiment. We excluded these individuals from our analysis, because we have no evidence that either paint or bee tags were more likely to wear off (paint = 17/100 bees missing at the end of the experiment; bee tag = 17/112 bees missing).

Extracting Data from Audio Recordings

We used R (R Core Team, 2015), with the packages seewave (Sueur *et al*., 2008) and tuneR (Ligges *et al*., 2013), to extract sonication and wing beat frequencies from the audio recordings. We first listened to the recordings to identify the loudest, longest sonication sound. We analyzed only the loudest, longest sonication because during observations we noticed that bees often performed shorter, higher-frequency buzzes on the petals of the flowers. In an effort to compare the same type of sonication (*i.e*.,

pollen-collecting buzzes) among all bees, we excluded these short "petal buzzes" from analysis. We classified a bout of buzzing as a single sonication if there were no audible breaks of ~0.2 s or more in the buzzing. After selecting the loudest, longest sonication, we determined the length of the sonication buzz, and used the "spec" function from the seewave package to calculate the power spectral density, using a hanning window of 2048 points (Sueur *et al*., 2008). To identify the sonication frequency (the dominant frequency at which the bee was vibrating), we selected the highest peak on the spectrum between 195 Hz and 400 Hz. We chose this range based on results from De Luca *et al*. (2013), Switzer *et al*. (2016), and preliminary observations on commercial colonies of *B. impatiens*, all of which suggest that sonication buzzes of *B. impatiens* fall within this range of frequencies.

To check the accuracy of the frequency identified as the highest peak in the spectrum, we generated a sine wave at this frequency, and C.M.S. aurally compared the sound of the sine wave to the audio recording of sonication by listening to the two sounds, played in close succession. Sometimes the frequency identified as the highest peak in the spectrum sounded very different in pitch from the raw audio recording; this often occurred when the recording had a great deal of background noise. In these cases, we used Audacity (Audacity, 2015) to identify the sonication frequency. Within Audacity, we selected the sonication portion of the audio recording and plotted the spectrum (hanning window, 2048 points). We then generated sine waves at each of the frequencies corresponding to the peaks in the spectrum. C.M.S. compared each of these sine waves to the recording, aurally, and chose the peak that corresponded most closely in frequency to the audio recording of the sonication.

We used the same process to quantify wing beat frequency during flight — selecting a portion of the recording that contained the bee flying, plotting a spectrum, and selecting the highest peak. We changed the range to 120 Hz to 220 Hz for selecting the peak — based on Switzer *et al*. (2016) and preliminary data collected from similar commercial colonies — and checked all wing beat frequencies aurally in the same way as for sonication frequency.

Statistical Analysis

To determine whether the two marking methods affected sonication frequency, sonication length, or wing beat frequency, we subtracted the value of each variable recorded after marking bees from the value recorded before marking. Thus, if bees had the same value for these variables before and after marking, the change in behavior would be zero. If a bee had a lower value after marking, then the difference in behavior would be negative.

We performed multivariate multiple regression to determine if there were significant changes to the bees' behaviors — wing beat frequency, sonication frequency, and sonication length. We were able to make comparisons only on bees that performed sonication behavior again after being marked ($n_{tag} = 30$, $n_{paint} = 62$). Since we suspected that environmental variables such as temperature might affect some of these behaviors (Unwin & Corbet, 1984), we initially included the following weather covariates in our models: temperature, pressure, light intensity, and relative humidity. We also included the following variables: mark type, IT span, tomato variety, number of days between initial recording and post-mark recording, and colony number (since we used four colonies). We used the "vif" (Variance Inflation Factor) function from the car package to check for multicollinearity, and found no problems with our data (Fox &

Weisberg, 2011). We used the "mStep" function from the qtlmt package in R to drop terms from the model sequentially, using Akaike Information Criterion (AIC) (Cheng, 2013). We conducted stepwise procedures for backward stepwise regression, starting with all of the covariates listed above and the interaction of mark type * intertegular span. We included this interaction because, prior to collecting data, we suspected that bee tags might affect smaller bees more severely. We had no prior reasons to include any other interactions. We forced all of the models to contain mark type as a covariate. We report the model with the lowest AIC from the stepwise procedure.

To determine if the marking method affected whether or not bees continued foraging for pollen from tomato plants after being marked, we used survival analysis techniques from the R package, survival (Therneau & Grambsch, 2000). This type of analysis is often used in clinical studies that are right-censored. The data recorded includes the amount of time since diagnosis, and whether or not an event (often death) occurs. The data are right-censored because the event does not occur for all participants in the study. Survival analysis can be performed with many events. For instance, it has been used to model the amount of time until seeds germinate (Manso *et al*., 2013). Seed germination time is right-censored because some of the seeds may die, whereas others are not dead, but do not germinate by the end of the study. Here we use "collecting pollen from *S. lycopersicum* after being marked" as our event. Our data are right-censored because some of the marked bees died, whereas others stayed alive, but were never observed sonicating on *S. lycopersicum* after being marked, within the time limits of the study.

We used Cox proportional hazards regression to determine if there was a significant difference between the two mark types in the probability of bees sonicating after being marked. We used Cox regression so we could include IT span and colony number as covariates. We centered the IT span variable before modeling to make interpretation easier. We also suspected an interaction between mark type and IT span, so we included an interaction: IT span * mark type. We used a likelihood ratio test to determine whether including colony number in the model made it significantly better.

We report no p-value corrections to account for multiple comparisons because available correction methods would not change our results (*i.e*., the significant results we report regarding the effects of mark type on behavior are all with p-value $<< 0.05$). We used the R packages, ggplot2 (Wickham, 2009) and ggfortify (Horikoshi & Tang, 2015) to make figure 2.

RESULTS

Does Marking Affect Flight or Sonication Mechanics?

The final model for sonication frequency, sonication length, and wing beat frequency included only mark type and the number of days between observations as explanatory variables — none of the other covariates significantly improved the model (*i.e*., no other covariates reduced AIC). Our overall model showed no significant differences in wing beat frequency, sonication frequency, or sonication length depending on the mark type (MANOVA; Pillai test stat = 0.133; approx. $F_{(6,91)} = 2.09$, p-value = 0.056). This model included only bees that sonicated after being marked ($n_{tag} = 30$, $n_{paint} = 62$). Generally, when the overall model is not significant, researchers do not investigate further comparisons (Hsu, 1996). However, because of the nearly significant p-value (0.056) for the overall model, we chose to skeptically investigate the separate multiple

Table 1. Mean and standard deviation of change in bee behaviors, stratified by the type of mark.

Behavior	Mark type	Mean Diff.	Std. Dev.
Sonication freq. (Hz)	Paint	-12.85	31.51
	Bee tag	-13.56	27.83
Sonication length (sec)	Paint	-0.01	1.06
	Bee tag	-0.35	0.75
Wingbeat freq. (Hz)	Paint	-1.53	7.28
	Bee tag	-5.44	9.69

regressions. When we investigated the coefficients of separate multiple regressions for wing beat frequency, sonication frequency, and sonication length with mark type and days between observation as independent variables, we found no significant effects of bee tags vs. paint marks on any of the variables. However, the regression for wing beat frequency may warrant further investigation ($\beta_{(\text{mark = tag})} = -3.2$; $t_{(89)} = 1.76$; p-value = 0.081), with tagged bees displaying a slightly lower (~5 Hz) wing beat frequency than painted bees. See table 1 for mean and standard deviations for each of these behaviors.

Does Marking Affect the Likelihood of Engaging in Further Sonication?

Figure 2 shows the cumulative percentage of bees that were observed sonicating on *S. lycopersicum* after being marked with paint or a bee tag, out of the total number of marked bees that were recovered by the end of the study ($n_{tag} = 94$; $n_{paint} = 83$; $n_{missing} = 34$). We started with a model that included colony number as a covariate, but removed this based on the results of a likelihood ratio test. Our final model is based on the following significant covariates: mark type, IT span, and the interaction between these two variables (Likelihood Ratio Test: $\chi^2_{(3)} = 59.05$, p-value < 0.001). The hazard ratio for being marked with a bee tag is 0.25, which means that for a fixed point in time, individuals of average IT span that were marked with bee tags were about one fourth as likely to engage in further sonication behavior, as compared to bees that were marked with paint ($\beta_{(\text{Mark: Bee tag})} = -1.37$; z = 5.97; p-value < 0.001).

Though IT span was not a significant predictor of whether bees would engage in further sonication for bees marked with paint ($\beta_{(\text{IT Span})} = 1.73$; z = 1.83, p-value = 0.067), we included it in the model because there was a significant interaction of IT span * mark type. The interaction term suggests that the effects of different types of marks vary depending on bee size. In particular, for bees marked with bee tags, a larger IT span has a larger an effect on the probability of bees engaging in further sonication behavior than it does for bees marked with paint; for bees marked with tags, a one-mm increase in IT span corresponds to being 2.77 times more likely to engage in further sonication behavior ($\beta_{(\text{Mark: Bee tag * IT Span})} = 1.02$; Hazard ratio = 2.77; z = 2.12; p-value = 0.034). We discuss possible explanations for the interaction below.

Bee Mortality

After collecting all bees on 16 Oct 2015, we classified them as either dead or alive. Out of all the bees we marked, about half (118/212) were observed engaging in further sonication behavior. These were used in another study and thus excluded from the mortality results. Of the bees that did not sonicate after marking (94/212), 47 were

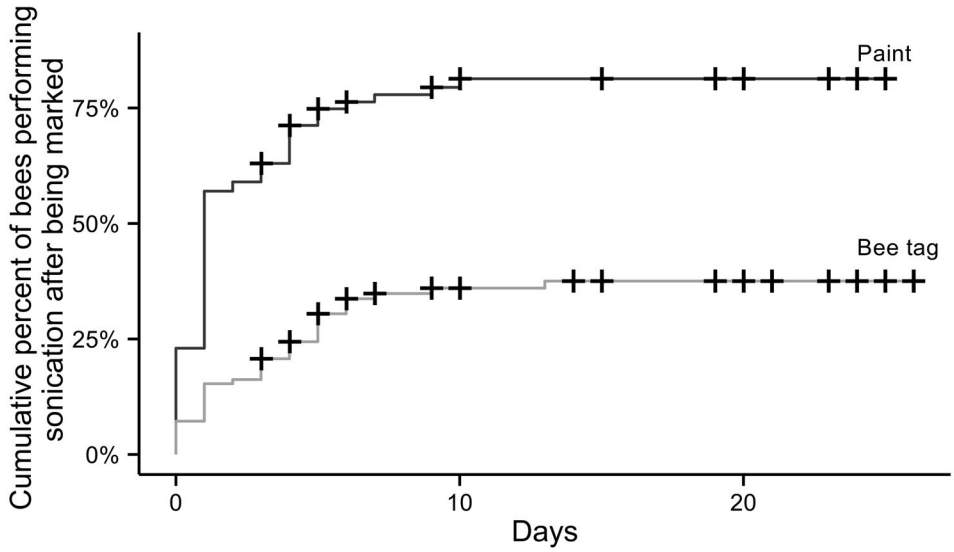

Figure 2. Curves showing the cumulative percentage of bees that performed sonication on *Solanum lycopersicum* L. after being marked with paint vs. bee tags, out of the total number of marked bees recovered by the end of the experiment (n_{paint} = 83; n_{tag} = 94; $n_{missing}$ = 34). The "+" symbols indicate censored data — bees that never were observed collecting pollen after being marked, within the time constraints of the experiment.

alive, 31 were dead, and 16 no longer had a mark. Of the painted bees that did not engage in further sonication behavior after being marked (22/94), we found 5 dead and 14 alive, and we were unable to recover 3 (23% dead, 64% alive, 13% N/A). Of the bees marked with bee tags that did not engage in further sonication behavior (72/94), we found 26 dead and 33 alive, and we were unable to recover 13 (36% dead, 46% alive, 18% N/A). However, because bees that engaged in sonication behavior again after being marked were excluded from the mortality analysis, a formal test for differential mortality is inappropriate in this case.

DISCUSSION

We investigated how two common methods of marking individual bees — paint and bee tags — affected the sonication behavior of bumble bees. For bees that engaged in further sonication behavior after being marked we found that tagging resulted in no significant differences in wing beat frequency, sonication frequency, or sonication length, relative to paint markings. We found a trend, though not a statistically significant one, suggesting that bee tags may result in a decreased wing beat frequency, relative to marking with paint. Thus, future experiments aimed at testing the effects of mark type on wing beat frequency are warranted; these tests would likely require a larger sample size of wing beat frequencies collected before and after marking than we were able to collect in the current study (n_{tag}= 30, n_{paint} = 62), to determine whether results are significant.

Though we found no significant differences in the mechanical behavior of bees that engaged in further sonication after being marked, we did find that the probability of observing bees engaging in further sonication behavior on *S. lycopersicum* after be-

ing marked was greatly affected by the mark type (Fig. 2). Bees with bee tags were much less likely to sonicate on *S. lycopersicum* again after being marked.

We did not have enough evidence to determine whether bees marked with bee tags are more likely to die than those marked with paint, and we acknowledge that our sample may be biased, because we were only able to quantify mortality in bees that were not observed sonicating again after being marked. Our sample sizes for recording mortality differed greatly between mark types, with 26 dead of 72 individuals marked with bee tags and 5 dead of 22 individuals marked with paint. Our results do not provide evidence to link mortality with the mark type. We also did not compare our marked bees to bees that are completely unmarked, because we needed to mark bees in some way to be able to identify them throughout the experiment. We report our mortality results, however, because they may suggest follow-up studies to determine whether different marking methods affect mortality.

Many studies have glued markers and other devices to bees, but the effects of these manipulations have rarely been examined. Hagen *et al*. (2011) glued radio transmitters (200 mg) to *Bombus* spp. to track their foraging behavior, and they reported a significant behavioral change due to the transmitter. They suggested that the large mass (about 100 times more than a bee tag) may be the cause of the behavioral change, but they did not directly test this hypothesis.

Bee tags have also frequently been used in mark-recapture studies (*e.g*., Eltz *et al*., 1999). In our study, we would have overestimated the population size if we had been using bee tags as markers, because far fewer of the bees marked with tags were "recaptured" (*i.e*., observed engaging in further sonication behavior), relative to painted bees.

We do not know why applying bee tags had such a dramatic effect on the likelihood of bees engaging in further sonication, but we can speculate about several potential explanations. First, due to the difficulty of immobilizing a bee in a queen-marking cage, errors in marking are common — smeared glue or paint, off-center tag placement, *&c*. These errors are likely to be more problematic with glue than with paint, as glue transferred to other parts of the body may have more negative effects. Sometimes the bee can reach the tag with her legs while in the queen cage, before the glue is dry. Glue smeared accidentally onto other body parts could be a cause of some bees changing their behavior after being tagged. Future studies could test for the effect of superglue alone by marking bees with superglue (perhaps colored) without a bee tag to determine if the glue or the tag is more problematic.

A second potential source of the behavioral differences in our experiments is the length of time we allowed for the glue to dry before releasing bees from the queen cage. We chose to dry the glue for 30 s before releasing the bee because we were trying to minimize handling time — but drying the glue for a longer period of time may have helped prevent any potential glue smearing. Third, the size and solid shape of the bee tags themselves, relative to the paint markings, could have contributed to the behavioral change. Though we did not quantify the amount of time bees spent trying to clean their dorsal mesosoma after being marked, we noticed that bees that had been marked with bee tags tended to spend a lot of time using their middle legs to try to remove the tag. This agrees with past research. De Souza *et al*. (2012) found that marking social wasps with water-based ink caused an increase in short-term grooming behavior. Finally, the difference in whether bees engaged in further foraging/sonication behavior could potentially be related to colony dynamics. Are the bees inside the colony excluding or acting aggressively toward bees that have bee tags, as compared

to those with paint markings? Packer (2005) found that marking solitary and semisocial halictid bees on the top of the head with paint affects their interactions with conspecifics, in terms of aggression and cooperation. Future, in-colony observations may provide an answer to this question.

We can also propose a few potential explanations for the significant interaction term between mark type and IT span in the Cox regression. One is that the bee tag is proportionally larger for smaller bees — in the smallest individuals, sometimes the bee tag is wider than the intertegular span — and this relatively larger tag may lead to greater behavioral changes in smaller bees. Second, smaller bees may be more likely to get glue smeared onto their wings. This could happen because of operator error: tagging small bees is more difficult than tagging large bees, because they are more difficult to hold in the queen-marking cage. Since the wing bases are closer together, the researcher may be more likely to place glue onto them than with a large bee. The same type of operator error may have happened with paint, but the consequences for the bee may be less severe when the bee is marked with paint, rather than glue.

This work has several implications for future experiments that involve marking bees. First, since we found no significant mechanical differences (sonication frequency, sonication length, or wing beat frequency) in bees that engaged in further sonication behavior after being marked, we can justify using tags or paint to mark bees for experiments aimed at measuring these variables. Furthermore, although CO_2 and cold narcosis have been shown to cause behavioral changes in activity level, brood care, foraging, aggression, and egg production (Poissonnier *et al*., 2015), these methods may still be preferable to immobilizing bees with queen cages when applying bee tags. If bees are narcotized with cold or CO_2, then tag position is more precise and the glue has longer to dry, so the risk of bees smearing glue into the tegula should be decreased. However, based on our current results showing that bee tags decrease the probability that a bee will engage in further sonication, researchers studying this behavior may get better returns if they mark bees with paint, rather than bee tags. Most importantly, we suggest that experiments be carried out one or two days after tagging bees, so that researchers perform experimental treatments only on marked bees that have resumed normal behavior following the marking treatment.

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