Supplemental Material

Standardized protocol for collecting community-level bee data

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ADDITIONAL PASSIVE TRAPPING TECHNIQUES TO COLLECT BEES

In the main text, we provide two methods that are most widely used to document bee communities–active hand-netting and passive trapping using bowl traps filled with soapy water. Here, we discuss additional passive trapping techniques that have been employed to collect bees, often used in more specialized contexts.

Bowl traps with propylene glycol solution (glycol traps): This approach allows the traps to be left out for weeks at a time (see Droege *et al*. (2016) for a monitoring protocol using this method). Propylene glycol evaporates much more slowly compared to soapy water, and acts as a preservative against decomposition. Thus, this can be a useful method for collecting bees particularly when sites are difficult to access or there is limited time for repeated visits, particularly in hot, dry, and windy environments. This method also allows for the detection of rare species in unproductive environments where bee densities are expected to be low (*e.g.*, Packer & Graham, 2020). We have chosen not to include this method in our main protocols because it can increase processing times of collected specimens (bees soaked in glycol are more difficult to clean to the extent that hairs are not matted to occlude morphological structures); and there are concerns that propylene glycol can limit downstream molecular analysis depending on the conditions (Nakamura *et al*., 2020; Martoni *et al*., 2021). See López-Uribe *et al*. (2024) for more guidance on molecular analyses. Leaving traps unattended for long periods also increases the possibility of overharvesting bees and other non-target taxa since trapping cannot be stopped in real-time if too many individuals are being captured (especially those belonging to species of conservation concern). Additionally, in some locations, wildlife may also trample or otherwise tamper with traps.

Blue vane traps: Blue vane traps are often employed in a similar fashion as bowl traps, and have the advantage of being amenable to long-term deployment. Blue vane traps can be highly effective in capturing both a large number and high diversity of bee species (Turley *et al*., 2022; Campbell *et al*., 2023), including large-bodied bees infrequently captured in bowl traps. However, there are concerns that this sampling method can oversample some taxa (Stephen & Rao, 2005; Stephen & Rao, 2007; Gibbs *et al*., 2017), particularly collecting higher numbers of bumble bees, and specifically bumble bee queens (Kimoto *et al*., 2012), compared to other methods. Although more data on how this sampling method truly impacts the populations of these taxa are needed, we have chosen to exclude this method from our main protocols due to the fact that many bumble bee species have been listed or petitioned for listing under the Endangered Species Act of 1973. Furthermore, collection results are sensitive to manufacturing specifications, such as what pigments are used (pers. comm.).

Malaise and flight-intercept traps: Malaise and flight-intercept traps are unlike bowl and vane traps in that they do not attract bees; rather, bees are intercepted and funneled into collection vessels in mid-flight when they collide with the traps. This non-attracting property provides some advantages, such as having capture rates that reflect bee traffic rather than how attractive the traps are against a backdrop of competing flower blooms, and being able to document the direction in which bees are flying when intercepted. However, the lack of attraction may also lead to these traps having much lower capture rates compared to bowl traps (*e.g*., Bartholomew & Prowell, 2005, Bashir *et al*., 2013). The non-selective nature of these traps also generally leads to capture of large numbers of non-bee arthropods (Missa *et al*., 2009; Hallmann *et al*., 2021), requiring a robust system to appropriately handle the high volume of bycatch; the traps are also expensive to obtain, time-consuming to set up, and liable to damage by wildlife and the elements when left out over long periods of time due to their relatively large size.

Emergence traps: Emergence traps are placed over patches of ground (bare dirt, lawns, etc.) to capture bees as they emerge from their natal or under-construction nests (Sardiñas & Kremen, 2014). Thus, these traps are ideal for documenting nesting habits of ground-nesting bees. However, since the dispersion of ground-nesting bee nests tends to be highly patchy, capture rates by emergence traps tend to be low (Pane & Harmon-Threatt, 2017). Thus, a large number of emergence traps deployed over long periods may be necessary to capture a significant subset of the ground-nesting bee community.

Trap nests: Trap nests are artificial cavities placed into the field by researchers to mimic naturally occurring cavities in which a subset of the bee community nests. They have been used to document bee communities in many contexts (*e.g*., MacIvor, 2015; Staab *et al*., 2018), and have the advantage of being amenable to non-lethal sampling if researchers obtain photographs or genetic samples of nesting females or resultant offspring reared in the laboratory and released (Satyshur *et al*., 2023). Additionally, trap nests provide the opportunity to examine the pollen and other nesting resources used by the occupying bees (Dürrbaum *et al*., 2022). However, as the name indicates, trap nests will only capture bee species that nest in cavities above ground, which, in many environments, constitute a minor proportion of the total bee community (Danforth *et al*., 2019). In addition, there is increased labor to monitor and process individuals emerging from the trap nests. Colonization patterns of trap nests also depend on where and when they are deployed; and in some cases, they may be primarily colonized by wasps (Taki *et al*., 2008; de Araújo *et al*., 2018).

Table S1. Summary of the *core*, *recommended*, and *optional* practices for following the community-level bee data protocol, specifically regarding site set up and data recording. Specific goals of each project should be used to make decisions on specific measurements to be used and additional data to collect.

Protocol Component	*Core* Practices	*Recommended* Practices	*Optional* Practices
Sample scheme	• Use a sample plot or transect.		
Sample transect	• In one ecotone, within one habitat type, within one sampling site. • Use a belt transect, sampling within 1 meter on any side. Report length and width. • At least one transect for *survey* and *monitoring*. • Sample the entire transect during sampling period.	• A minimum of 25 meters in length for small plots and 200 meters for large plots (see below). • At least one transect for *inventory*. • Transect separation of 2 km is typically sufficient, but seek guidance from experts.	• Record sampling area with GPS.
Sample plot	• In one ecotone, within one habitat type, within one sampling site. • May use a meandering path. Report exact plot area used. • At least one plot for *survey* and *monitoring*. • Sample the entire plot during sampling period.	• Establish a small (ranging from 0.01–0.24 hectare, *e.g.*, 10 x 10 m to 50 x 50 m) or large (0.25–1.0 hectare, *e.g.*, 50 x 50 m to 100 x 100 m) plot. • At least one plot for *inventory*. • Plot separation of 2 km is typically sufficient, but seek guidance from experts.	• Record sampling area with GPS.
Data recording	• Follow *The Wild Bee Data Standard* (Du Clos *et al*., 2024), see Table 2.		• Record additional data beyond this protocol (see Table 4).

Table S1 (Continuation). Summary of the *core*, *recommended*, and *optional* practices for following the community-level bee data protocol, specifically regarding sample collection. Specific goals of each project should be used to make decisions on set ups and numbers of bowl traps to be used.

Protocol Component	*Core* Practices	*Recommended* Practices	*Optional* Practices
Sample collection	• Report sample method used. • Prior to collecting, make a plan for sample processing.	• Use bowl traps and net collecting. • Deploy bowl traps along a single line or crossing in an "X".	
Bowl traps	• Report the number of bowl traps used, including null data. • Use an equal number of each of the three colors of bowl traps (fluorescent blue, fluorescent yellow, and white bowls). • Use a minimum of 9 bowls and a maximum of 30 bowls.	• Place bowls 5 meters apart. • Alternate among fluorescent blue, fluorescent yellow, and white bowls. • Place bowls in the open, not under brush or in significant shade. • Space bowls apart, do not cluster.	• If elevating bowls, place them just above the densest part of the vegetation.
Net collecting	• Cover the entire plot or transect length during the sampling period. • Collect all bees, of any species, during the sampling period, including honey bees. • For *survey* and *monitoring*, document sampling events when no bees are collected.	• Search and collect all bees foraging on flowers, in the air, and on the ground. • For *inventories*, document sampling events when no bees are collected. • For *inventories*, can also collect opportunistically and haphazardly.	

Table S1 (Continuation). Summary of the *core*, *recommended*, and *optional* practices for following the community-level bee data protocol, specifically regarding sample collection. Specific goals of each project should be used to make decisions on set ups and numbers of bowl traps to be used.

Protocol Component	*Core* Practices	*Recommended* Practices	*Optional* Practices
Time and Duration of sampling event	• Collect using a net for a minimum of 10 minutes. • When netting, pause the timer while transferring specimens into a collecting vessel. • For *survey* and *monitoring*, record start and stop time, regardless of sampling method.	• Collect between 09:00–16:00 hours. • Deploy bowl traps for a minimum of 6 hours and a maximum of 24 hours. • For *inventories*, record start and stop time, regardless of sampling method.	• Sample at a location in both the morning and afternoon.
Sample frequency	• Conduct at least one sampling event for *inventory* and *survey*. • For *monitoring*, decide sample frequency based on project goals.	• Conduct at least two sampling events for *inventory* and *monitoring* at distinct periods of time within peak bee flight.	• Sample every 2–3 weeks.
Sample conditions	• Record conditions during sampling.	• Sample in warm temperatures: above 50° F (10° C), below 110° F (~43° C). • Sample with clear skies: no precipitation occurring and no more than lightly overcast. • Sample with no or minimal wind: less than 12 kph or 3 on the Beaufort scale. • Sample with minimal smoke haze: conditions no more than the equivalent of lightly overcast.	

REFERENCES

Bartholomew, C.S. & D. Prowell. 2005. Pan compared to malaise trapping for bees (Hymenoptera: Apoidea) in a longleaf pine savanna. *Journal of the Kansas Entomological Society* 78: 390–392.

Bashir, M.A., S. Saeed, & A. Sajjad. 2013. Monitoring Hymenoptera and Diptera pollinators in a sub-tropical forest of southern Punjab, Pakistan. *Pakistan Journal of Agricultural Research* 50: 359–366.

Campbell, J.W., A. Abbate, N.M. West, L. Straub, & G.R. Williams. 2023. Comparing three collection methods for pollinating insects within electric transmission right-of-ways. *Journal of Insect Conservation* 27: 377–387. https://doi.org/10.1007/s10841-023-00460-4.

Danforth, B.N., R.L. Minckley, & J.L. Neff. 2019. The Solitary Bees: Biology, Evolution, Conservation. Princeton University Press, Oxford, UK. https://doi.org/10.2307/j.ctvd1c929.

de Araújo, G.J., G.F. Monteiro, M.C.T.B. Messias, & Y. Antonini. 2018. Restore it, and they will come: trap-nesting bee and wasp communities (Hymenoptera: Aculeata) are recovered by restoration of riparian forests. *Journal of Insect Conservation* 22, 245–256. https://doi.org/10.1007/s10841-018-0058-8.

Droege, S., J. Engler, E. Sellers, & L. O'Brien. 2016. National protocol framework for the inventory and monitoring of bees. *US Fish and Wildlife Service*, Fort Collins, Colorado.

Dürrbaum, E., F. Fornoff, C. Scherber, E.J. Vesterinen, & B. Eitzinger. 2022. Metabarcoding of trap nests reveals differential impact of urbanization on cavity-nesting bee and wasp communities. *Molecular Ecology* 32: 6449–6460. https://doi.org/10.1111/mec.16818.

Gibbs, J., N.K. Joshi, J.K. Wilson, N.L. Rothwell, K. Powers, M. Haas, L. Gut, D.J. Biddinger, & R. Isaacs. 2017. Does passive sampling accurately reflect the bee (Apoidea: Anthophila) communities pollinating apple and sour cherry orchards? *Environmental Entomology* 46(3): 579–588. https://doi.org/10.1093/ee/nvx069.

Hallmann, C.A., A. Ssymank, M. Sorg, H. de Kroon, & E. Jongejans. 2021. Insect biomass decline scaled to species diversity: General patterns derived from a hoverfly community. *PNAS* 118(2): e2002554117. https://doi.org/10.1073/pnas.2022554117.

Kimoto, C., S.J. DeBano, R.W. Thorp, S. Rao, & W.P. Stephen. 2012. Investigating temporal patterns of a native bee community in a remnant North American bunchgrass prairie using blue vane traps. *Journal of Insect Science* 12(1): 108. https://doi.org/10.1673/031.012.10801.

López-Uribe, M.M., J.P. Strange, L. Whiteman, B.N. Danforth, S. Jha, M. Branstetter, J.B.U. Koch, H.K. Levenson, B. Du Clos, & S.H. Woodard. 2024. Standardized protocols for collecting bee samples for genetic or genomic data. *Journal of Melittology* 123(7): 1–x.

MacIvor, J.S. 2015. Building height matters: nesting activity of bees and wasps on vegetated roofs. *Israel Journal of Ecology & Evolution* 62(1–2): 88–96. https://doi.org/10.1080/15659801.2015.1052635.

Martoni, F., E. Nogarotto, A.M. Piper, R. Mann, I. Valenzuela, L. Eow, L. Rako, B.C. Rodoni, & M.J. Blacket. 2021. Propylene Glycol and Non-Destructive DNA Extractions Enable Preservation and Isolation of Insect and Hosted Bacterial DNA. *Agriculture* 11(77). https://doi.org/10.3390/agriculture11010077.

Missa, O., Y. Basset, A. Alonso, S.E. Miller, G. Curletti, M. De Meyer, C. Eardley, M.W. Mansell, & T. Wagner. 2009. Monitoring arthropods in a tropical landscape: relative effects of sampling methods and habitat types on trap catches. *Journal of Insect Conservation* 13: 103–118. http://dx.doi.org/10.1007/s10841-007-9130-5.

Nakamura, S., S. Tamura, H. Tki, & E. Shoda-Kagaya. 2020. Propylene glycol: a promising preservative for insects, comparable to ethanol, from trapping to DNA analysis. *Entomologia Experimentalis et Applicata*. https://doi.org/10.1111/eea.12876.

Packer, L. & L. Graham. 2020. Four new species of Isepeolini (Hymenoptera: Apidae) from northern Chile. *BMC Zoology* 5(3). https://doi.org/10.1186/s40850-020-00052-8.

Pane, A.M. & A.N. Harmon-Threatt. 2017. An assessment of the efficacy and peak catch rates of emergence tents for measuring bee nesting. *Applications in Plant Sciences* 5. https://doi.org/10.3732/apps.1700007.

Sardiñas H.S. & C. Kremen. 2014. Evaluating nesting microhabitat for ground-nesting bees using emergence traps. *Basic and Applied Ecology* 15:161–168. https://doi.org/10.1016/j.baae.2014.02.004.

Satyshur, C.D., E.C. Evans, B.M. Forsberg, T.A. Evans, & R. Blair. 2023. Determining Minnesota bee species' distributions and phenologies with the help of participatory science. *PeerJ* 11:e16146. https://doi.org/10.7717/peerj.16146.

Staab, M., G. Pufal, T. Tscharntke, & A.M. Klein. 2018. Trap nests for bees and wasps to analyse trophic interactions in changing environments-A systematic overview and user guide. *Methods in Ecology and Evolution* 9: 2226–2239. https://doi.org/10.1111/2041-210X.13070.

Stephen, W.P. & S. Rao. 2005. Unscented color traps for non-*Apis* bees (Hymenoptera: Apiformes). *Journal of the Kansas Entomological Society* 78(4): 373–380. https://www.jstor.org/stable/25086288.

Stephen, W.P. & S. Rao. 2007. Sampling native bees in proximity to a highly competitive food resource (Hymenoptera: Apiformes). *Journal of the Kansas Entomological Society* 80(4): 369–376. https://doi.org/10.2317/0022-8567(2007)80[369:SNBIPT]2.0.CO;2.

Taki, H., B.F. Viana, P.G. Kevan, F.O. Silva, & M. Buck. 2008. Does forest loss affect the communities of trapnesting wasps (Hymenoptera: Aculeata) in forests? Landscape vs. local habitat conditions. *Journal of Insect Conservation* 12: 15–21. https://doi.org/10.1007/s10841-006-9058-1.

Turley, N.E., D.J. Biddinger, N.K. Joshi, & M.M. López-Uribe. 2022. Six years of wild bee monitoring shows changes in biodiversity within and across years and declines in abundance. *Ecology and Evolution* 12: e9190. https://doi.org/10.1002/ece3.9190.