- Title: Pan trap color preference across Hymenoptera in a forest clearing M.L. Buffington<sup>1</sup>, A. Garretson<sup>2</sup>, R.R. Kula<sup>1</sup>, M.W. Gates<sup>1</sup>, R. Carpenter<sup>3</sup>, D.R. Smith<sup>1</sup> and A.A.R. Kula<sup>4</sup> <sup>1</sup>Systematic Entomology Laboratory, USDA-ARS c/o National Museum of Natural History, Smithsonian Institution, 10<sup>th</sup> & Constitution Ave NW, Washington DC 20013-7012 <sup>2</sup>George Mason University, Department of Biology, 4400 University Dr, Fairfax, VA 22030, and George Mason University, School of Systems Biology, 10900 University Boulevard Manassas, Virginia 20110 <sup>3</sup>The State University of New York College at Geneseo, 1 College Cir, Geneseo, NY 14454 <sup>4</sup>Mount St. Mary's University, 16300 Old Emmitsburg Rd, Emmitsburg, MD 21727 Corresponding author: M.L. Buffington Running headline: Pan trap color preference Key words: bees, biodiversity, Mid-Atlantic, parasitoid, sawflies, species abundance, species
- 21 richness

## **ABSTRACT**

Insect biodiversity reveals much about ecosystem health and function; however, field studies of insect community composition and diversity are often unintentionally biased by the sampling methods deployed in the study area. Pan traps, particularly yellow pan traps, are a common method for passive community assessment across at a variety of taxonomic levels. Our study finds that the diversity, richness and abundance of hymenopterans in pan trapping projects are significantly impacted by the color of the pan trap deployed. Additionally, we find that individual species display significant preferences for not only yellow pan traps but also for white, fluorescent yellow, blue and fluorescent blue pans. Our data support recent studies that suggest yellow traps alone may be insufficient for sampling the true diversity of certain hymenopteran groups in a region.

### INTRODUCTION

Insect biodiversity can reveal a great deal about natural and agricultural ecosystem health and productivity (Showalter et al., 2018). The power of observations on insect biodiversity relies heavily on repeatable and statistically comparable sampling methodologies (Southwood & Henderson, 2000). Underpinning the importance of insect biodiversity research are ecosystem services (e.g., pollination, detrivory, pest control) that insects provide in excess of 57 billion USD annually to the US economy (Losey et al., 2006) and 33 trillion USD globally (Costanza et al., 1997). Recent reports on the decline of insects in general (Hallmann et al., 2017) and pollinators, in particular (Millenium Ecosystem Assessment, 2005; Beismeijer et al., 2006), suggest that insect biodiversity research is reaching a new level of priority.

Field studies of insect community composition and diversity are often unintentionally biased by the sampling methods deployed in the study area (Toler et al., 2005; Saunders & Luck, 2013; Heneberg & Bogusch, 2014; Hall, 2016). Pan traps, particularly yellow pan traps, are a common method for passive insect community assessment across a variety of taxonomic levels (Kirk, 1984; Leksono et al. 2005a, 2005b; Laubertie et al., 2006; Campbell & Hanula, 2007; Vrdoljak & Samways, 2012; Saunders & Luck, 2013; Spafford & Lortie, 2013; Harris et al., 2016; Wheelock & O'Neal, 2016; Wang et al., 2017; Bashir et al., 2019; Shrestha et al., 2019). In comparison with Malaise trapping, sweep-netting, leaf-litter sifting and direct rearing, pan trapping is readily deployable with little formal training, is very cost-efficient and produces a great deal of specimens for research. A pan trap consists of a relatively shallow vessel (average 20cm wide, 10cm deep), typically made of colored plastic, filled with soapy water, salt, propylene glycol, or any combination of these. The trap works by insects flying into the pan of soapy water and drowning; salt and propylene glycol are sometimes included as preservatives

and/or to reduce evaporative loss. As the pans deployed can be of uniform size and spaced according to a predetermined pattern, their use is conducive to sampling so that statistical comparisons can be made among treatments.

Among the colors of pan traps that can be deployed, yellow has long been considered the most effective for collecting a maximum number of species. However, published color preference studies have assessed relatively few insect taxa, with a focus on particular agroecosystems, herbivores and natural enemies (Capinera & Walmsley, 1978; Moreno et al., 1984; Trimble & Brach, 1985; Anderbrant et al., 1989; Boiteau, 1990; McClain et al., 1990; De Barro, 1991; Messing & Jang, 1992; Niwa, 1995; Kostal & Finch, 1996; Udayagiri et al., 1997; Vasquez et al., 1997; Cornelius et al., 1999; Idris et al., 2002; Showler & Armstrong, 2007; Larsen et al., 2014). Studies utilizing multiple pan trap colors to sample Hymenoptera have focused on bees (Leong & Thorpe, 1999; Cane et al., 2000; McIntyre & Hostetler, 2001; Bartholomew & Prowell, 2005; Toler et al., 2005; LeBuhn et al., 2006; Campbell & Hanula, 2007; Roulston et al.; 2007; Kwaiser & Hendrix, 2008; Westphal et al., 2008; Wilson et al., 2008; Tuell et al., 2009; Gollan et al., 2011; Ramírez-Freire et al., 2012; Gonçalves & Oliveira, 2013; Geroff et al., 2014; Hall, 2016; McCravy & Ruholl, 2017; Sircom et al., 2018). Conversely, relatively few studies have used multiple colors of pan traps to sample non-bee hymenopterans or assess color preference for those taxa. Ritzau (1998) and Skvarla et al. (2016) used multiple colors of pan traps, among other sampling methods, to determine sawfly and woodwasp diversity on dune islands in the German North Sea and oak-hickory dominated forest of Arkansas, respectively. Barker et al. (1997) assessed sawfly preference for five colors of pan traps in crop fields, mainly cereal grains, in England. Heneberg & Bogusch (2014) and Moreira

et al. (2016) assessed the use of different pan trap colors mostly for stinging aculeate wasps, in addition to bees, in various habitats in Europe and Brazil, respectively.

The largest void in knowledge of color preference for hymenopterans is for parasitoid wasps despite the extensive use of colored pan traps for sampling those taxa. Weseloh (1986) assessed parasitoid wasp preference over a two year period for six colors, as well as black and clear, using plexiglass sticky traps in eastern deciduous forest in Connecticut. Aguiar & Sharkov (1997) reported collecting the stephanid *Megischus bicolor* (Westwood) in blue pan traps but not yellow pan traps in oak-pine forest in Georgia. Abrahamczyk et al. (2010) assessed color preference for all hymenopterans, other than ants, using fluorescent yellow and fluorescent blue pan traps in Bolivian tropical and subtropical forest. While Weseloh (1986) was a comprehensive assessment of color preference for non-bee hymenopterans, specimens were sampled with sticky traps, rather than the much more commonly used water pan traps, from two sites. Thus, research on color preference for non-bee hymenopterans, sampled using water pan traps in a variety of habitats, is necessary to discern how pan trapping can most effectively sample parasitoid, predatory and plant-feeding hymenopteran diversity in the Nearctic Region.

The objective of this research is to determine if species richness, composition and abundance differ with pan trap color for Hymenoptera (excluding ants and stinging wasps) in a forest clearing in the Mid-Atlantic Region of the United States. We asked the following questions: (1) How do abundance, species richness and species diversity (Shannon Weaver index and evenness) differ among colors for hymenopteran groups and specifically for bees (Anthophila clade of Apoidea)? (2) Do species show significant affinity for pan colors? (3) What colors do species choose significantly more than clear bowls? The results presented here will

help inform researchers conducting biodiversity studies on the collection biases inherent in various pan colors.

## MATERIALS AND METHODS

Site description. The study was conducted from May 7–June 6, 2007, in an approximately 100m x 1,850m forest clearing (i.e., power line right-of-way owned and operated by the Baltimore Gas & Electric Company) located in Calvert Co., MD. The clearing runs roughly north-south and is bordered to the east and west by eastern deciduous forest. A sampling area was defined within the clearing as follows. The northern border was the Route 402 access gate to the power line right-of-way (38°33'21.11"N 76°33'07.81W"); the southern border was set at 38°32'22.43"N 76°32'53.11W", as habitats within the clearing are relatively inaccessible south of this point (due to Parker Creek). The eastern and western borders were set by selecting one GPS point each at the eastern and western edges of the clearing at the same latitude as the northern and the southern borders. The eastern and western borders at the northern border were 38°33'21.11"N 76°33'10.96W", respectively; at the southern border were 38°32'22.43"N 76°32'55.53W", respectively.

Experimental design. Pan traps consisted of seven treatments: blue, fluorescent blue, yellow, fluorescent yellow, red, white and clear (control) 12 oz. Solo<sup>TM</sup> (Urbana, IL, USA) party bowls. The manufacturer's colors were used for blue, red and white. Clear bowls were painted fluorescent blue and fluorescent yellow using Krylon Fusion spray paint for plastics.

Array placement was determined by parsing the entire sampling area into fourths. Within each fourth of the field site, four arrays were positioned to ensure similar habitat in the array area and help control for plant species composition effects on the insect community. We avoided

inaccessible shrubby areas due to logistical challenges. We placed arrays so that inter-array pan distance was more than intra-array pan distance. Therefore, there was >12.74 m distance from a pan of one array to a pan of another array, and this distance is based on the diameter of each array. For each array, a pole was placed at the center, a 6.37 m nylon rope was tied to the pole, and the first pan was placed 6.37 m directly north of the pole. Additional pans were set in a clockwise fashion at 2 m intervals, 6.37 m from the center pole. Pan color sequence was randomized within groups of seven pans repeated three times for each array using Research Randomizer v3.0 (http://randomizer.org/). Each array consisted of 21 pans (i.e., three pans/treatment/array).

After pan placement was established, pans were deployed. Each pan was filled with 250 ml of water. Three drops of Liquinox® detergent (Alconox, Inc., White Plains, NY, USA) were placed in each pan to break the water surface tension. Samples were collected every two days for one month, totaling 13 sampling events. Pans were redeployed immediately after sample collection and placed back into the same location.

Sample processing. For each array, the three pans of the same color were combined during sample collection, poured through a fine mesh net, rinsed with water and transferred to a whirl pack plastic bag along with 85% ethanol for transport from the field site to the Smithsonian Institution National Museum of Natural History (USNM), Washington, DC. Thus, for each sample date, seven samples (i.e., one sample/color treatment) were collected from each array, and samples from different arrays and collection dates were kept separate pending sample processing.

Hymenopteran specimens were pulled from samples at the level of superfamily or family, dehydrated chemically following Heraty & Hawks (1998), point- or card-mounted, labeled and

sorted to morphospecies or determined to species. The groups were assigned to authors and collaborators for determinations as follows: Braconidae and Ichneumonidae, RRK; Chalcidoidea, MWG; Ceraphronoidea, Cynipoidea, Diaprioidea, Platygastroidea, MLB; "Symphyta," DRS; and Anthophila clade of Apoidea (hereafter bees), Sam Droege, USGS-Patuxent Wildlife Research Center. Representative specimens of the most abundant species were mounted and labeled. Voucher specimens of each morphospecies were deposited in the USNM.

Statistical analyses. Samples across the sampling dates were combined for analyses. The number of individuals of each species were compiled for each array and pan color combination using R with RStudio and tidyverse (R version 3.5.2, R Core Team, 2013; RStudio version 1.1.453, RStudio Team, 2015; tidyerse version 1.2.1, Wickham, 2017). For each pan color in each array, the Shannon Weaver index and its corresponding evenness measure were calculated as a measure of hymenopteran diversity (Hill, 1973). These metrics were calculated in R using the vegan package (version 2.5-4, Oksanen et al., 2019). The final dataset, therefore, included species abundance (number of individuals), species richness (number of species), Shannon Weaver diversity and evenness across 16 sampling arrays for seven pan colors.

After abundance and richness were compiled and diversity measures were calculated, ANOVA was used to test for an effect of pan color on overall abundance (number of individuals), richness (number of species) and species diversity (Shannon Weaver index, evenness). We also examined differences among means for bee abundance and richness. Significant main effects tests were followed by post hoc Tukey tests to make pairwise comparisons among colors.

To determine the differences in richness and abundance estimates using different sampling pans, we developed two statistical models for each hymenopteran group. Both models

were general linear mixed models with the color of the pan as the fixed effect and the array identity as a random factor. The first model had abundance as the response variable while the second model had richness as the response variable. In addition to the species-specific models, we used the combined dataset with all hymenopteran groups to look at more general trends. Because the response variables are count data, we used a Poisson link-log error distribution. All models treated "clear" as the comparison color that coefficients were calculated from, and all models were implemented in R using the lme4 package (version 1.1-21).

We performed an indicator species analysis because (1) they take into account both relative abundance among pan colors and occurrence in each pan color, (2) they are able to detect significant differences for rare species and (3) they can be used with data that contain a high proportion of tied zero scores, present non-normal distributions and exhibit a wide variability. To complete indicator species analysis for color affinity, individual species association with color was assessed using the group-equalized indicator species index described in Cáceres et al. (2010). This index, derived from Dufrene & Legendre (1997), is the product of two quantities: A and B. Quantity A is the positive predictive power of the species as an indicator of the color, while quantity B describes how frequently the species is found in a pan of particular color. The indicator value was calculated using the indicspecies package in R (version 1.7.6) for each color for each species, and then species with significant affinity for only one color were considered. Focusing on single-color affinity allowed us to specifically identify the species that are potentially omitted from biodiversity assessments or species sampling when a specific color of pan is not used. For example, if a particular species shows significant association with the blue pan, this species would likely not be considered in a community assessment sampled only with yellow pans. P-values were calculated for the association index using the permutation

test described in Cáceres & Legendre (2009). We considered P-values less than 0.1 to demonstrate significant affinity of a species for a color.

As new species are recorded from additional sampling during a survey, species accumulation curves are an increasingly precise assessment of the species richness of a community. As additional samples are pooled, if the species richness curve stabilizes, then the observed species richness can be considered a good estimate of the community species. However, different sampling approaches may lead to different measures of richness or lead to different levels of effort required to stabilize the richness estimates. Species accumulation curves are useful to determine at what sampling effort no new species are added to the dataset. Here, the curves were plotted for each of the pan colors across all transects in R using the vegan package (version 2.5-4). A multiplier of two was used to generate confidence intervals for the species accumulation curves from the standard deviation. The curves were computed using the exact method, which finds the expected accumulation curve using the Mao Tau estimate, a sample-based rarefaction method (Chiarucci et al., 2008; Colwell et al., 2012).

### **RESULTS**

Across all transects and treatments, we collected a total of 21,458 hymenopteran individuals representing 420 species. The mean number of hymenopteran individuals per sample across all 112 samples (seven colors per array x 16 replicate arrays) was  $191.58\pm159.15$  (mean  $\pm$  1 standard deviation), and the mean number of species per sample was  $48.08\pm25.55$ .

Our results demonstrate that pan color was significantly associated with both the number of hymenopteran species ( $F_{6,16}$ =136.49, P < 0.0001) and the number of hymenopteran individuals ( $F_{6,16}$ =41.81, P < 0.0001). The pan color with the highest overall species and

individuals was yellow, and the lowest richness and abundance were found in red and clear pans (Figure 1A & 1B). Within bees there was a significantly lower number of individuals collected in red and clear pans, but there was no significant difference between the number of individuals collected in yellow, fluorescent yellow, white, blue and fluorescent blue pans (Figure 2A). Similarly, though red and clear had the lowest number of species collected; fluorescent blue, blue, white and fluorescent yellow performed similarly; and yellow sampled a significantly higher number of species than all other pans except fluorescent yellow (Figure 2B).

Biodiversity measures followed a similar trend to the overall hymenopteran richness and abundance results, with the highest Shannon Weaver diversity for hymenopterans occurring in yellow pans and the lowest diversity occurring in red and clear pans (Figure 3A & 3B). The trend was reversed for the evenness measure, with yellow demonstrating the lowest evenness and red and clear demonstrating the highest. This was likely influenced by the low abundance and richness numbers in red and clear pans (Figure 3A & 3B).

The results of the generalized linear mixed models show that, when compared to clear pans, all colors (except red) significantly increased in both richness and abundance, and this result holds across all hymenopteran groups (Table 1). The model fits for species richness in all hymenopteran groupings (except bees and Chalcidoidea) were singular because the variance attributed to the random factor (array) was close to zero meaning that the variation between transects was low. In "Symphyta," the model fit was nearly unidentifiable because no "Symphyta" species were recovered in 93 bowls (83%). While the results of these models are presented in Table 1, the coefficient estimates are likely unstable for this group.

For overall richness, red pans sampled significantly worse than clear pans, though the effect size was small (estimate = -0.23, P = 0.0066). Red pans also significantly negatively

affected abundance and richness within Chalcidoidea and abundance of bees compared to clear pans. For all hymenopteran groups except sawflies, yellow significantly positively impacted both abundance and richness. Fluorescent yellow performed similarly, with the abundance of all groups except sawflies and the richness of all groups except sawflies and Chalcidoidea significantly positively affected. White pans positively impacted the abundance of Ichneumonidae and Platygastroidea, as well as both the richness and abundance of bees. The abundance and richness of bees were also positively affected by blue pans, along with the abundance of Ceraphronoidea and Platygastroidea (Table 1).

The results of the indicator species analysis revealed that 112 species (out of 420 collected) demonstrated affinity for only one pan color. The majority of these species (n=63) demonstrated affinity for yellow pans; no recorded species demonstrated significant affinity for red or clear pans (Figure 4). Of the 63 species that demonstrated affinity for yellow pans, 26.9% (17) were bees, but species in all nine assessed hymenopteran groups had at least one species with a demonstrated affinity for yellow pans (Figure 5). Additionally, eight species across three hymenopteran groups—Braconidae (2), Chalcidoidea (2) and bees (4)—demonstrated affinity for fluorescent yellow (Figure 5). The only hymenopteran group with significant affinity for fluorescent blue or blue was bees, and bees and Platygastroidea were the only two groups with at least one species that demonstrated affinity for white pans. A full list of the results of the indicator species analysis with all A and B values and P-values for each species are available in Supplemental Table 1.

The fitted species accumulation curves for the overall data and the hymenopteran groups appeared to approach, but not reach, asymptotes. The curve fitted for all Hymenoptera data shows a distinct break in the fitted species accumulations for the clear and red pans compared to

the white, blue, yellow, fluorescent yellow and fluorescent blue pans (Figure 6). However, additional patterns emerge when the species accumulation curves are broken down by hymenopteran group (Figure 7, A-F). Bees (Figure 7A) show little difference in accumulation curves for colors aside from red and clear, whereas braconids (Figure 7C), ichneumonids (Figure 7D) and cynipoids (Figure 7F) all show a break between yellow and fluorescent yellow versus all other colors. For cynipoids, yellow and fluorescent yellow appear to perform similarly; for braconids and ichneumonids, yellow estimates a higher species richness. In the case of platygastroids (Figure 7B), yellow pans outperform the other colors, but this particular group appears to be relatively evenly attracted by all non-yellow pans. Due to low numbers of collected specimens (due low abundance or species localized and recovered in only one or a few arrays), diapriids, ceraphronoids and sawflies generated curves that were difficult to interpret because they did not accurately represent an actual accumulation of species.

## 274 DISCUSSION

Our findings suggest that pan color used to sample hymenopterans significantly impacts richness, abundance and diversity estimates of species sampled. All colors resulted in significantly higher abundance and richness than red and clear; similarly, all colors had a significantly higher Shannon Weaver index than red and clear (Figure 3). Yellow pans yielded significantly higher hymenopteran abundance and richness than any of the other colors (Figure 1), a pattern Weseloh (1986) found in most instances where preference for one color was observed, although that study did not include either bees or sawflies and had low or no representation for several parasitoid wasp groups. Our results confirm that biodiversity estimates can be biased by sampling method deployed (Cane et al., 2000; Heneberg & Bogusch, 2014;

Hall, 2016) and underscore the necessity of controlling for sampling method in comparative analyses and analyses that synthesize results from multiple studies (Roulston et al., 2007; Ptasznik, 2015). These impacts are likely more profound in taxa with species that displayed preferences for non-yellow pan colors and for taxa with species where the generalized models showed significantly positive impacts from pan colors other than yellow, aspects which were observed in our study.

We found, through our indicator species analyses, that bees had the least specific preference for any single pan color, meaning that bees had the most colors for which species had a significant affinity (Figure 2). While most other groups only had species primarily displaying preferences for fluorescent yellow and yellow, bees had species displaying preferences for blue, fluorescent blue and white pans, in addition to yellow and fluorescent yellow pans. However, red and clear were significantly less effective for sampling bees. This result has also been recovered in other research on pan color preference for bees (Toler et al., 2005; Wilson et al., 2008; Tuell et al., 2009); however, these results differ from Cane et al. (2000), Campbell & Hanula (2007) and Geroff et al. (2014) in which bees preferred blue in most cases, as well as Kwaiser & Hendrix (2008), Gollan et al. (2011) and Ramírez-Freire (2012) in which bees preferred yellow in most cases. These results also echo previous research suggesting that multiple pan trap colors should be deployed to obtain the most accurate species richness estimate for bees (Cane et al., 2000; Stephen & Rao, 2005; Toler et al., 2005; Roulston et al., 2007; Wilson et al., 2008).

The indicator species analyses show species-level patterns. While the ANOVA and generalized linear models indicated that among yellow, fluorescent yellow, white, blue and fluorescent blue, there is similarity in the abundance of individuals or the number of species collected. The indicator species analyses allow a closer examination to see that there are species

with affinity for one of the five colors making those colors the best choice for collecting those particular species. Some species have significant affinity for multiple colors, but those species are not examined here and can be found in Supplemental Table 1.

For non-bee hymenopterans studied here, we found a significant individual-level preference for yellow across all sampled species groups (Table 1). Our results of sawfly preference for yellow support the results of Ritzau (1988) and Barker et al. (1997), although the latter study found one sawfly species each that preferred white and black pans over other colors, including yellow. Our results also are congruent with Weseloh (1986) in that species of Ichneumonidae, Ceraphronoidea, Cynipoidea and Diapriidae preferred yellow over all other colors except ichneumonids also preferred orange over all colors other than yellow (note: orange not tested here). However, in addition to yellow, both braconids and chalcidoids showed some preference for fluorescent yellow (Table 1) in our study, a treatment not considered in Weseloh (1986). Further, while we found chalcidoids showed preference for yellow and fluorescent yellow, chalcidoid color preference was indistinct in Weseloh (1986). Color preference was not analyzed for Chalcidoidea collectively in Weseloh (1986) but rather for five families separately. Yellow was preferred over all other colors in only two of eight instances analyzed, and none of the five families exhibited preference for a single color in both sampling years. Rather unexpectedly, our research showed platygastroids with preference for white in addition to the more frequently observed preference for yellow (Table 1). Thus, our results are congruent with platygastrid preference for yellow found in Weseloh (1986) but differ in that Weseloh (1986) found white no more attractive to platygastroids than blue, clear, or red depending on sampling year.

The results here suggest that if sampling to measure species richness for a broad range of Hymenoptera, some sampling bias due to color will occur (Figure 1B). While significantly more hymenopteran species showed affinity for yellow over the other treatments in this research, not all species are biased in the same way (Table 1). Thus, sampling bias can be addressed in the field by deploying various colors simultaneously, thereby increasing the likelihood of sampling species with preference other than yellow. Further, our data show that other pan trap colors are attractive to hymenopterans compared with clear pan traps (Table 1; thus, using colors other than yellow might be less effective for sampling most species, but those other colors are still attractive to hymenopterans while increasing the likelihood of sampling species with color preference other than yellow.

Species accumulation curves (Figures 6 & 7) are another way to examine pan color preferences across Hymenoptera. When all Hymenoptera are calculated in the same curves (Figure 6), there is a distinct break between clear/red and white/blue/yellow/fluorescent yellow/fluorescent blue. Extrapolating from these curves, the overall species richness estimates between these colors varies significantly (e.g., 300 species at 15 sites for yellow and 150 species at 15 sites for blue). This indicates the color choice can either double, or halve, the total estimated number of Hymenoptera measured in a given area.

The taxon-specific accumulation curves provide more insight into the taxonomic differences in the community structure measured by different sampling regimes. Because there is little difference between the species accumulation curves for the non-clear and non-red pans for bees (Figure 7) and relatively even measurements of species accumulation across all colors for platygastroids (Figure 7B), while individual species may be missed, the overall diversity estimates for these taxonomic groups may not be impacted significantly by pan colors used to

sample an area. However, the significant break in the species accumulation curves at yellow/fluorescent yellow for braconids (Figure 7C), ichneumonids (Figure 7D) and cynipoids (Figure 7F) suggests that failing to use yellow pans may substantially underestimate the true diversity of these taxonomic groups in biodiversity studies. Additionally, while for cynipoids yellow and fluorescent yellow appear to perform similarly, yellow estimates a higher species-richness for braconids and ichneumonids. Using yellow pans, instead of fluorescent yellow, is necessary for accurately estimating braconid and ichneumonid diversity in a region.

## Conclusions

The pan trap is a well-established method of collecting Hymenoptera. To this point, most studies on color preference in Hymenoptera focused on bees; this research is unique in that we assessed color preference for all hymenopterans except ants and stinging wasps. Here we have determined that for most hymenopteran groups examined, yellow and fluorescent yellow are indeed the most effective color for collecting Hymenoptera in an eastern deciduous forest clearing; for some other taxa, other colors are just as effective. Our results also highlight that while yellow and fluorescent yellow may give similar overall diversity measures, some species, specifically some bees, demonstrate affinity for non-yellow pans. Consequently, it may be less likely to sample those species when omitting blue, fluorescent blue or white pans. Appreciating those differences is critical to accurately estimate hymenopteran diversity using pan traps.

As is the case with this research, most studies of pan trap color preference in Hymenoptera have focused on a particular habitat in a narrowly defined geographic area due to the logistical challenges of specimen processing and identification. Future research on pan trap color preference should focus on sampling hymenopterans in a variety of habitats, geographic

locations, elevations and time of year. Many factors could influence pan color preference and the efficacy of pan traps at a site, such as plant diversity; seasonal background flora, including extent of floral bloom; host insect diversity; host seasonal phenology; microclimate; elevation; height at which pan traps are set; and density of foliage at a site. We did not explore the efficacy of pan traps relative to other methods commonly used to sample hymenopteran diversity, notably aerial/sweep netting and Malaise trapping. Thus, additional research is necessary to establish a consensus on which pan trap colors provide the most accurate estimate of diversity in particular ecological scenarios and also how pan trapping compares to other methods in those scenarios.

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FIGURES.

Figure 1. Mean number of hymenopteran individuals (abundance) (A) and mean number of hymenopteran species (richness) (B) for each pan color (Y, yellow; FY, florescent yellow; W, white; B, blue; FB, florescent blue; R, red; C, clear) within the 16 circular arrays. Jittered data points from each sampling array are shown. For boxplots, median abundance or richness across sampling arrays is indicated by the horizontal bar within the box, lower and upper limits of the boxes correspond to the first and third quartiles, and whiskers extend to the largest and smallest data point (but only up to 1.5 times from within the interquartile range limits). Different lowercase letters above each boxplot indicate statistically significant group differences among pan colors resulting from a Tukey's HSD.

Figure 2. Mean number of bee individuals (abundance) (A) and mean number of bee species (richness) (B) for each pan color (Y, yellow; FY, florescent yellow; W, white; B, blue; FB, florescent blue; R, red; C, clear) within the 16 sampling arrays. Jittered points and boxplots as in Figure 1. Different lowercase letters above each boxplot indicate statistically significant group differences among pan colors resulting from a Tukey's HSD.

Figure 3. Hymenopteran species diversity calculated for each color: Shannon Weaver diversity index (A) and evenness (B) for each pan color (Y, yellow; FY, florescent yellow; W, white; B, blue; FB, florescent blue; R, red; C, clear) within the 16 sampling arrays. Jittered points and boxplots as in Figure 1. Different lowercase letters above each boxplot indicate statistically significant group differences among pan colors resulting from a Tukey's HSD.

Figure 4. Number of hymenopteran species with significant affinity for a single pan color (Y, yellow; FY, florescent yellow; W, white; B, blue; FB, florescent blue) over all the hymenopteran groups. Individual species association with color was assessed using the group-equalized indicator species index. No species had significant affinity for red or clear pans. Species with significant affinity for more than one color combination are not shown.

Figure 5. Number of species with significant affinity for a single pan color (Y, yellow; FY, florescent yellow; W, white; B, blue; FB, fluorescent blue) within hymenopteran groups. Individual species association with color was assessed using the group-equalized indicator species index. All hymenopteran groups included species with significant affinity for yellow pans. No hymenopteran groups included species with significant affinity for red or clear pans.

Species with significant affinity for more than one color are not shown.

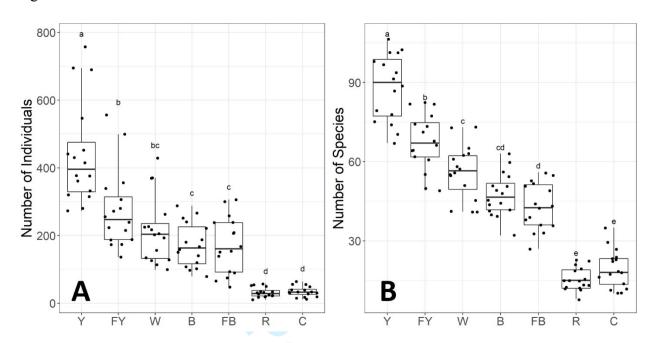
Figure 6. Species accumulation curve for all Hymenoptera data combined. Rarefaction curves were computed using the exact method using the Mao Tau estimate. Colored regions around best-fit curves indicate a confidence interval from the standard deviation with a multiplier of two and are colored by the pan color: yellow pans = yellow curve, blue pan = blue curve, clear pan = wheat curve, florescent blue pan = turquoise curve, florescent yellow pan = green curve, red pan = red curve, white pan = white curve.

Figure 7. Species accumulation curves by group. A. Anthophila; B, Platygastroidea; C,
 Braconidae; D, Ichneumonidae; E, Chalcidoidea; F, Cynipoidea. Other groups are not included
 here because of low species abundance and occurrence across arrays. Rarefaction curves were

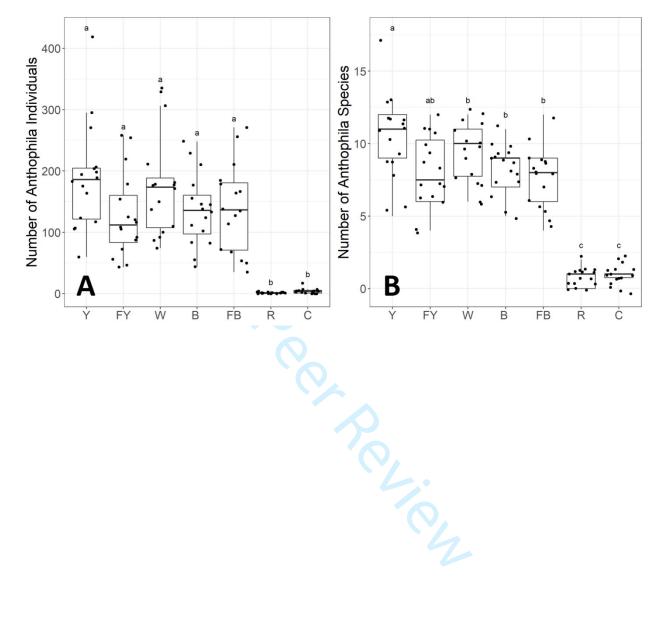
computed using the exact method using the Mao Tau estimate. Colored regions around best-fit curves indicate a confidence interval from the standard deviation with a multiplier of two and are colored by the pan color: yellow pans = yellow curve, blue pan = blue curve, clear pan = wheat curve, florescent blue pan = turquoise curve, florescent yellow pan = green curve, red pan = red curve, white pan = white curve.



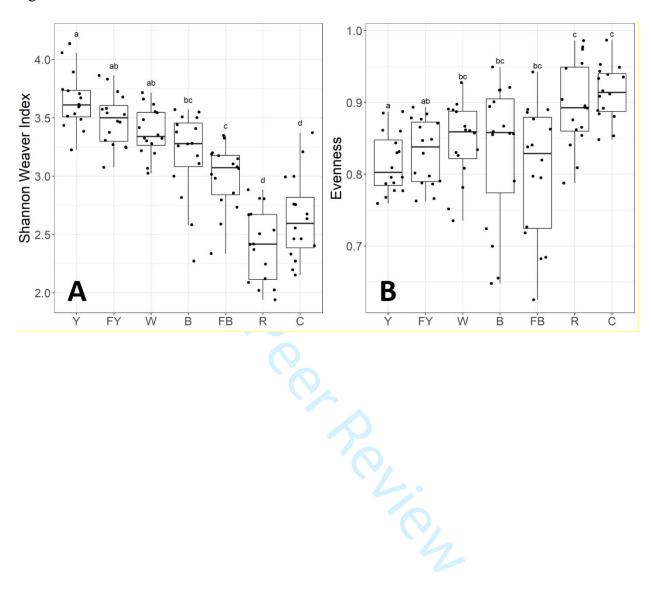
718 Figure 1.



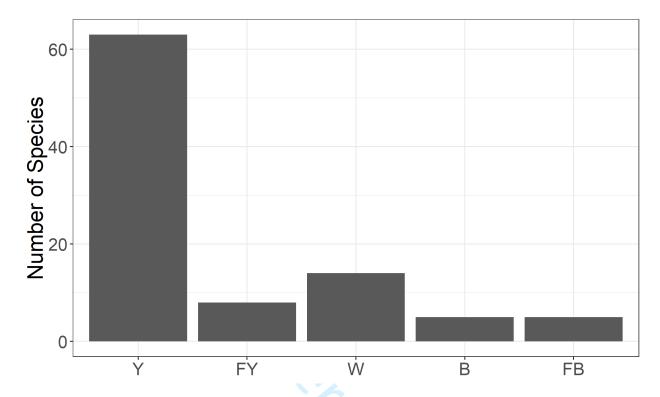
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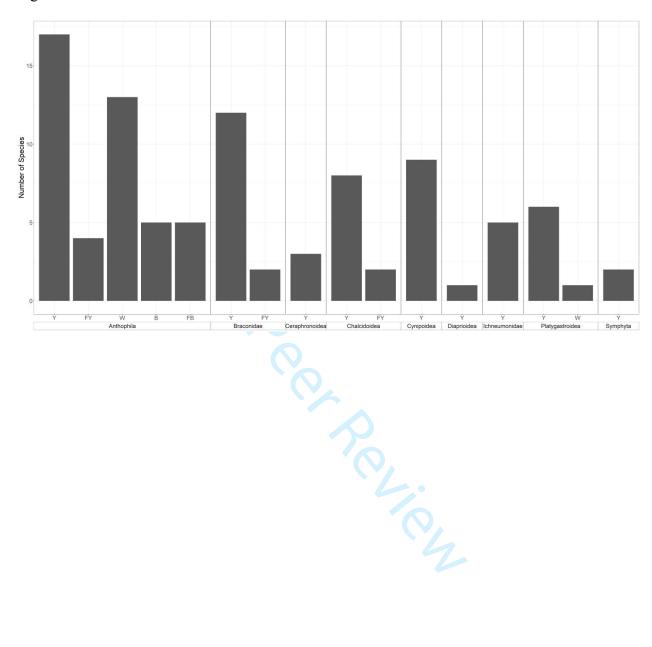
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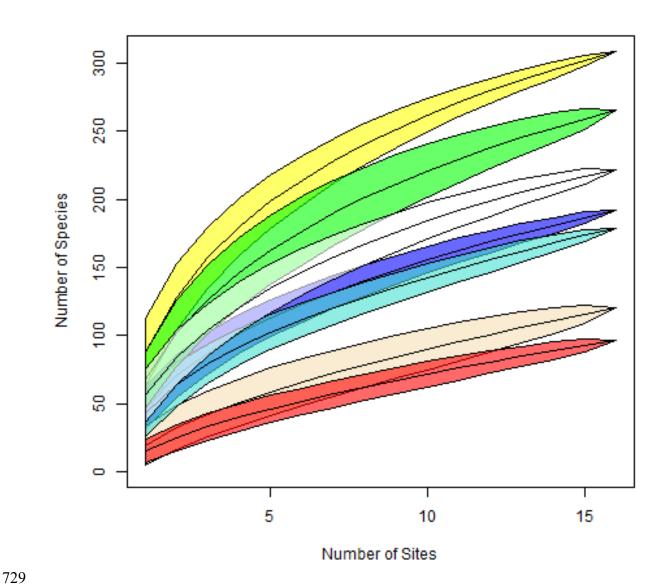
724 Figure 4.



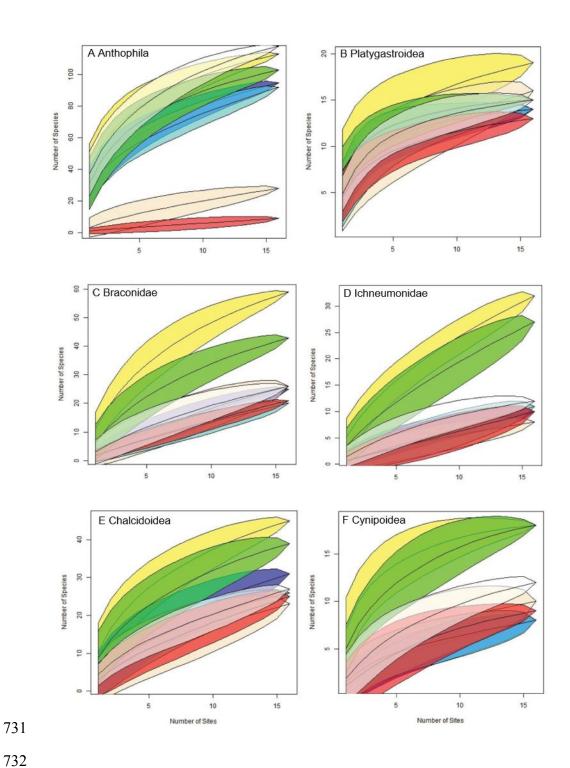
726 Figure 5.



728 Figure 6.



730 Figure 7.



**TABLES** 

Table 1. Results of the general linear mixed models with abundance and richness as outcome variables, pan color as a fixed effect and transect as a random factor. All comparisons with color were made to "clear" as the baseline. Parameter estimates, standard error, t-statistic and P-values are presented for each hymenopteran grouping and the full dataset of all combined species. Positive coefficients indicate significantly more individuals or species were found in that color compared to clear pans, whereas negative coefficients indicate significantly fewer individuals or species were found in that color compared to clear pans. \* indicates richness models with singular fits, due to a variance of 0 in the "array" random factor, \*\* and italicized text indicates an unidentifiable model of "Symphyta" richness. 

Supplemental Table 1. Full results of the indicator species analyses for all species that showed significant affinity for one color. Individual species association with color was assessed using the group-equalized indicator species index (= product of A and B). Quantity A is the positive predictive power of the species as an indicator of the color, while quantity B describes how frequently the species is found in a pan of particular color. P-values were calculated for the association index using the permutation test. We considered P-values less than 0.1 to demonstrate significant affinity of a species for a color.

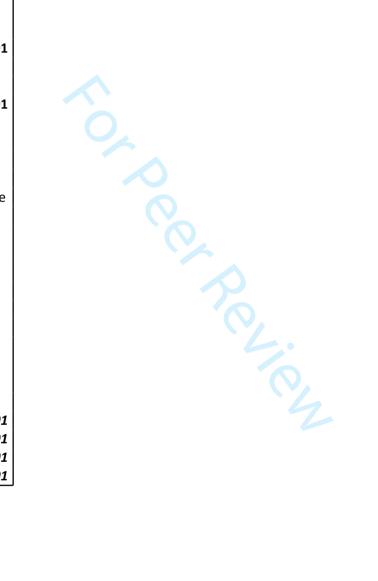
	Full				Anthophila							
	Abundance				Abundance							
Treatment	Estimate	St. Error	Test Statistic	p-value	Estimate	St. Error	Test Statistic	p-value				
Clear	-	-	-	-	-	-	-	-				
Blue	1.61	0.047	34.51	<0.0001	3.56	0.13	27.66	<0.0001				
Florescent Blue	1.60	0.047	34.34	<0.0001	3.58	0.13	27.81	<0.0001				
Florescent Yellow	2.08	0.045	45.97	<0.0001	3.49	0.13	27.08	<0.0001				
Red	-0.089	0.062	-1.45	0.15	-1.29	0.27	-4.73	<0.0001				
White	1.84	0.046	40.04	<0.0001	3.82	0.13	29.73	<0.0001				
Yellow	2.55	0.044	57.67	<0.0001	3.88	0.13	30.27	<0.0001				
		Ri	chness			Rich	nness					
Clear	-	-	-	-	-	-	-	-				
Blue	0.90	0.067	13.32	<0.0001	2.17	0.27	7.97	<0.0001				
Florescent Blue	0.80	0.068	11.67	<0.0001	2.10	0.27	7.71	<0.0001				
Florescent Yellow	1.25	0.064	19.41	<0.0001	2.14	0.27	7.87	<0.0001				
Red	-0.23	0.086	-2.71	0.0066	-0.31	0.40	-0.78	0.43				
White	1.07	0.066	16.17	<0.0001	2.30	0.27	8.49	<0.0001				
Yellow	1.52	0.063	24.17	<0.0001	2.42	0.27	8.98	<0.0001				
		-	ipoidea			Diaprioidea						
			undance		Abundance Estimate St. Error Test Statistic p-value							
Class		St. Error	Test Statistic	p-value	Estimate	St. Error	lest Statistic	p-value				
Clear	- 0.26	-	1.00	-	-	-	-	-				
Blue	-0.26	0.24	-1.08	0.28	-0.41	0.23	-1.79	0.07				
Florescent Blue	-0.17	0.24	-0.71	0.48	0.00	0.20	0.00	1.00				
Florescent Yellow	2.07	0.17	12.19	<0.0001	0.93	0.17	5.51	<0.0001				
Red	-0.41	0.25	-1.61	0.11	0.27	0.19	1.43	0.15				
White	0.21	0.22	0.97	0.33	0.33	0.19	1.77	0.08				
Yellow	3.07	0.16	18.78	<0.0001	1.58	0.16	10.02	<0.0001				
Class	Richness*					RICH	ness*					
Clear	0.12	- 0.25	- 0.25	- 0.72	0.074	- 0.2052	- 0.10	- 0.05				
Blue Florescent Blue	-0.13	0.35	-0.35	0.72 0.72	0.074 -5.26E-16	0.3852	0.19	0.85				
	-0.13	0.35	-0.35			0.3922	0.00	1.00				
Florescent Yellow	1.10	0.28	<b>3.92</b>	<0.0001	0.69	0.3397	<b>2.04</b>	0.041				
Red	-0.19	0.36	-0.54	0.59	-0.17	0.4097	-0.41 0.10	0.68				
White	0.21	0.33	0.65 5.17	0.52	0.074	0.3852	0.19	0.85				
Yellow	1.40	0.27	5.17	<0.0001	0.73	0.3376	2.17	0.030				

Braconidae				Cerapl	hronoidea			Chal	
Abundance			Abu	ndance			Abu		
Estimat	e St. Error	Test Statistic	p-value	Estimate	St. Error	Test Statistic	p-value	Estimate	St. Error
-	-	-	-	-	-	-	-	-	-
0.29	0.20	1.48	0.14	0.37	0.12	3.03	0.0024	0.08	0.14
-0.35	0.23	-1.50	0.13	-0.11	0.14	-0.76	0.45	0.25	0.13
1.32	0.17	7.83	<0.0001	1.06	0.11	9.59	<0.0001	2.14	0.11
-0.35	0.23	-1.50	0.13	0.11	0.13	0.85	0.39	-0.37	0.16
0.22	0.20	1.11	0.27	0.25	0.13	1.96	0.050	0.19	0.13
1.91	0.16	11.87	<0.0001	1.67	0.10	16.10	<0.0001	2.33	0.10
	Ric	hness*			Ric	hness*			Ric
-	-	-	-	-	-	-	-	-	-
0.06	0.34	0.17	0.87	0.12	0.25	0.49	0.62	-0.036	0.27
-0.13	0.35	-0.35	0.72	-2.60E-15		0.00	1.00	-0.036	0.27
0.72	0.30	2.44	0.015	0.23	0.24	0.95	0.34	0.87	0.22
0.00	0.34	0.00	1.00	-0.067	0.26	-0.26	0.80	-0.44	0.30
0.11	0.33	0.33	0.74	0.092	0.25	0.37	0.71	0.13	0.26
1.06	0.28	3.76	0.0001	0.50	0.23	2.19	0.029	0.87	0.22
				·					
		umonidae				astroidea			Syr
		ındance				ndance			Abu
		Test Statistic	p-value			Test Statistic	•		St. Error
- 0.11	-	- 0.24	- 0.74	0.24	- 0.13	-	-	- 0.63	-
0.11	0.33	0.34	0.74	0.24	0.12	2.04	0.04	0.62 0.60	8479.04
-0.19	0.36	-0.54	0.59	0.20		1.66	0.10	1	8506.27
2.04	<b>0.26</b> 0.37	<b>7.98</b>	<0.0001	1.20	<b>0.10</b> 0.12	<b>11.99</b> 1.82	<b>&lt;0.0001</b> 0.07	1	6832.80
-0.35 0.63	0.37	-0.93 2.12	0.35 0.03	0.22 0.31	0.12	2.68	0.07	17.65 17.65	6832.80
2.61	<b>0.30</b>	10.48	< <b>0.001</b>	1.73	0.12	18.19	<0.001	1	6832.80 6832.80
2.61		hness*	<0.0001	1./3		hness*	<0.0001	22.24	Rich
_	-	-	_	_	-	-	_	_	-
0.29	0.44	0.65	0.51	0.19	0.22	0.86	0.39	-1.25	652.90
1.77E-1		0.00	1	0.14	0.22	0.65	0.51	-0.24	551.80
1.20	0.38	3.17	0.00154	0.58	0.20	2.93	0.0034	21.56	0.010
-0.12	0.49	-0.24	0.81	0.05	0.22	0.22	0.82	19.36	0.010
0.44	0.43	1.03	0.30	0.10	0.22	0.44	0.66	19.36	0.010
1.30	0.38	3.46	0.00055	0.83	0.19	4.30	<0.0001	21.66	0.010

cidoidea	
ındance	
Test Statistic	p-value
-	-
0.55	0.58
1.91	0.057
20.37	<0.0001
-2.36	0.018
1.41	0.16
22.38	<0.0001
chness	
-	-
-0.14	0.89
-0.14	0.89
3.90	<0.0001
-1.47	0.14
0.52	0.60
3.90	<0.0001
mphyta	
ındance	
• •	p-value
ındance Test Statistic -	-
Indance Test Statistic - 0.00	1.00
Indance Test Statistic - 0.00 0.00	1.00 1.00
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ndance Test Statistic  0.00 0.00 0.00 0.00 0.00 0.00	1.00 1.00 1.00 1.00 1.00
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ndance Test Statistic  0.00 0.00 0.00 0.00 0.00 0.00	1.00 1.00 1.00 1.00 1.00
Indance Test Statistic	1.00 1.00 1.00 1.00 1.00 1.00
ndance Test Statistic  - 0.00 0.00 0.00 0.00 0.00 0.00 hness** - 0.00	1.00 1.00 1.00 1.00 1.00 1.00
ndance Test Statistic	1.00 1.00 1.00 1.00 1.00 1.00
ndance Test Statistic  0.00 0.00 0.00 0.00 0.00 0.00 hness**  - 0.00 0.00 2226.45	1.00 1.00 1.00 1.00 1.00 1.00 - 1.00 1.00
ndance Test Statistic	1.00 1.00 1.00 1.00 1.00 1.00

2237.26

<0.0001



Group	Species	Α	В	P value	Color
Andrenidae	Panurginus_potentillae	0.5714	0.5625	0.001	Yellow
Anthophila	Andrena_confederata	0.8846	1	0.001	Yellow
Anthophila	Andrena_erigeniae	0.7317	0.8125	0.001	White
Anthophila	Andrena_macra	0.4091	0.375	0.036	Yellow
Anthophila	Andrena_morrisonella	0.45	0.9375	0.001	Yellow
Anthophila	Andrena_nasonii	0.4139	0.9375	0.001	Yellow
Anthophila	Andrena_neonana	0.3168	0.5625	0.074	Yellow
Anthophila	Andrena_perplexa	0.5454	0.3125	0.019	Yellow
Anthophila	Andrena_personata_both	0.2793	1	0.017	Yellow
Anthophila	Andrena_personata_female	0.2917	0.9375	0.017	White
Anthophila	Andrena_personata_male	0.4808	0.5625	0.002	Yellow
Anthophila	Augochlora_pura	0.625	0.25	0.049	Yellow
Anthophila	Augochlorella_aurata	0.2988	1	0.003	FB
Anthophila	Augochloropsis_metallica	0.5556	0.5	0.002	Yellow
Anthophila	Calliopsis_andreniformis	0.4118	0.5	0.009	White
Anthophila	Ceratina dupla	0.4032	0.625	0.002	Blue
Anthophila	Ceratina_calcarata	0.6323	0.8125	0.001	White
Anthophila	Ceratina_dupla	0.3226	0.875	0.001	White
Anthophila	Ceratina_strenua_both	0.3016	0.6875	0.019	White
Anthophila	Ceratina_strenua_female	0.3302	0.6875	0.009	White
Anthophila	Ceratina_strenua_male	0.6	0.25	0.035	Yellow
Anthophila	Coelioxys_sayi	1	0.1875	0.017	White
Anthophila	Colletes_brevicornis	0.4167	0.3125	0.097	FB
Anthophila	Epeolus astralis	0.5	0.375	0.006	Blue
Anthophila	Eucera hamata	0.4762	0.6875	0.001	Blue
Anthophila	Eucera rosae	0.6667	0.1875	0.090	Blue
Anthophila	Halictus_ligatus.poeyi	0.3725	1	0.001	Yellow
Anthophila	Holcopasites_calliopsidis	0.625	0.25	0.031	White
Anthophila	Hoplitis_pilosifrons	0.4051	0.875	0.001	White
Anthophila	Hoplitis_producta	0.4615	0.3125	0.042	White
Anthophila	Hylaeus_affinus.modestus	0.5758	0.5	0.002	Yellow
Anthophila	Lasioglossum illinoese	0.7222	0.375	0.001	Blue
Anthophila	Lasioglossum trochangers?	0.5	0.25	0.056	Blue
Anthophila	Lasioglossum_callidum	0.4	0.25	0.098	FB
Anthophila	Lasioglossum_coreopsis	0.4792	0.9375	0.001	FB
Anthophila	Lasioglossum_imitatum	0.5	0.375	0.009	Yellow
Anthophila	Lasioglossum_pectorale	0.3028	1	0.002	FB
Anthophila	Lasioglossum_tegulare	0.2768	0.875	0.007	FY
Anthophila	Lasioglossum_versatum	0.2463	1	0.022	FB
Anthophila	Lasioglossum_vierecki	0.3375	0.5625	0.034	FB
Anthophila	Megachile brevis	0.625	0.3125	0.006	Blue
Anthophila	Nomada_bidentate_sp1	0.7143	0.3125	0.008	FY
Anthophila	Nomada_fragariae	0.4333	0.3125	0.060	FY
Anthophila	Nomada_luteola	1 0.4162	0.1875	0.017	Yellow Yellow
Anthophila Anthophila	Nomada_parva_both Nomada_parva_female	0.4162	0.6875 0.6875	0.003 0.003	Yellow
Anthophila	Nomada_parva_male	0.3930	0.0873	0.003	FY
Anthophila	Osmia_atriventris	0.4706	0.375	0.011	White
Anthophila	Osmia pumila	0.4700	0.375	0.013	White
Anthophila	Specodes_carolinus	0.4396	0.9375	0.001	FY
Anthophila	Stelis_lateralis_both	0.7273	0.1875	0.001	White
Braconidae	Aphaereta_sp	0.3793	0.1873	0.094	Yellow
Braconidae	Ascogaster_sp3	0.3793	0.3125	0.009	FY
Braconidae	Dinotrema_sp1	0.4737	0.5123	0.010	Yellow
Diagoniado	2o. oa_op i	5. 10 <del>-1</del> 0	5.5	0.011	. 5115 44

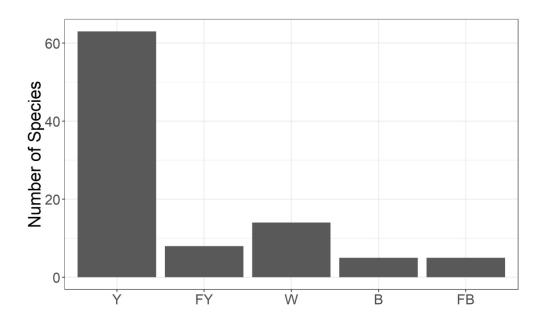
D	D'autaine a	0.0000	0.05	0.007	M. II.
Braconidae	Dinotrema_sp6	0.8333	0.25	0.007	Yellow
Braconidae	Dinotrema_sp7	0.7143	0.1875	0.054	Yellow
Braconidae	Heterospilus_sp6	0.4483	0.5625	0.003	Yellow
Braconidae	Microctonus_sp1_poss_mellinus	0.4694	0.875	0.001	Yellow
Braconidae	Microctonus_sp2_poss_mellinus	0.4783	0.5	0.001	Yellow
Braconidae	Microctonus_sp5	0.8518	0.3125	0.011	Yellow
Braconidae	Opius_sp2	0.6667	0.25	0.028	Yellow
Braconidae	Opius_sp8	0.5454	0.3125	0.020	FY
Braconidae	Orgilus_consuetus	0.75	0.3125	0.005	Yellow
Braconidae	Orgilus_sp1	0.7857	0.5625	0.001	Yellow
Braconidae	Parahormius_sp_poss_new	0.8	0.25	0.010	Yellow
Braconidae	Schizoprymnus_sp_prob_texanus	0.6182	0.625	0.001	Yellow
Ceraphronidae	Aphanogmus_01	0.3881	1	0.001	Yellow
Ceraphronidae	Ceraphron_01	0.383	1	0.001	Yellow
Ceraphronidae	Ceraphron_02	0.3761	0.75	0.002	Yellow
Chalcididae	Haltichella_xanticles	0.3708	0.6875	0.002	Yellow
Cynipidae	Andricus_01	0.7333	0.5625	0.001	Yellow
Cynipidae	Diastrophus_kincaidii	0.6354	0.875	0.001	Yellow
Cynipidae	Dryocosmus_01	0.7203	1	0.001	Yellow
Cynipidae	Dryocosmus_02	0.6471	0.5	0.001	Yellow
Diapriidae	Belyta_sp01	0.5714	0.25	0.061	Yellow
Diapriidae	Diapriid_01	0.3828	1	0.001	Yellow
Diapriidae	Diapriid_04	0.6667	0.1875	0.080	Yellow
Eulophidae	Achrysocharoides_guizoti	0.6667	0.25	0.070	FB
Eulophidae	Aulogymnus_nsp1	1	0.25	0.003	Yellow
Eulophidae	Euderus_spnr_masoni	0.9118	0.5	0.001	FY
Eulophidae	Neochrysocharis_diastatae	0.2527	0.6875	0.084	FY
Eulophidae	Omphale_vulgaris	0.8	0.3125	0.007	Yellow
Eurytomidae	Eurytoma_sp1	0.56	0.5	0.001	FY
Eurytomidae	Eurytoma_sp2	0.3143	0.5	0.050	Yellow
Eurytomidae	Sycophila_sp1	0.6875	0.3125	0.005	Yellow
Eurytomidae	Sycophila_sp2	0.449	0.6875	0.001	Yellow
Eurytomidae	Tetramesa sp1	0.3086	0.8125	0.028	Yellow
Figitidae	Didyctium_01	0.4375	0.375	0.010	Yellow
Figitidae	Ganaspis_mundata	0.6053	0.8125	0.001	Yellow
Figitidae	Leptopilina_boulardi	0.5217	0.375	0.011	Yellow
Figitidae	Neralsia_01	0.4667	0.3125	0.039	Yellow
Figitidae	Trybliographa 01	0.6531	0.75	0.001	Yellow
Ichneumonidae	,	0.8182	0.4375	0.001	Yellow
	Cryptinae_sp13	0.375	0.625	0.004	Yellow
	Cryptinae_sp20	0.6035	0.375	0.046	Yellow
Ichneumonidae	* · · · · · · · · · · · · · · · · · · ·	0.5556	0.25	0.048	Yellow
	Ichneumoninae_sp1	0.5882	0.875	0.001	Yellow
	Ichneumoninae_sp3	0.3529	0.375	0.077	Yellow
Ormyridae	Ormyrus_rosae	0.6268	1	0.001	Yellow
Pamphiliidae	Onycholyda_amplecta	0.7143	0.3125	0.002	Yellow
Platygastridae	Fidiobia_01	0.3478	0.4375	0.100	Yellow
Platygastridae	Innostemma 01	0.5948	1	0.001	Yellow
Platygastridae	Platygaster_02	0.6349	1	0.001	Yellow
Platygastridae	Platygaster_sp01	0.3526	0.9375	0.001	Yellow
Scelionidae	Gryon_bracypt_01	0.2206	0.875	0.085	Red
Scelionidae	Gryon_winged_01	0.2279	1	0.003	Yellow
Scelionidae	Opistacantha_01	0.2279	1	0.019	Yellow
Scelionidae	Scelio_01	0.2776	0.6875	0.001	White
Scelionidae	Trissolcus_01	0.3333	0.875	0.000	Yellow
Cochornad	1110001000_01	5.0000	0.070	0.001	I CHOW

Tenthredinidae Monophadnoides rubi

Torymus fagopirum

Torymidae

0.8 0.25 0.005 Yellow 0.5333 0.3125 0.017 Yellow



635x375mm (96 x 96 DPI)