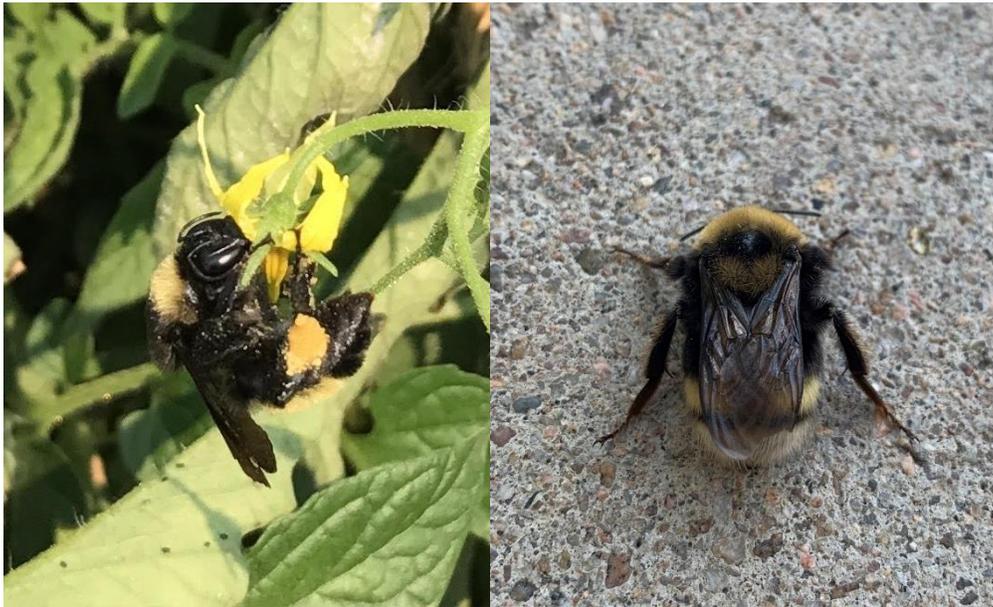


Distribution of declining bumble bees in central and eastern Wyoming

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Bombus pensylvanicus (left) and *B. occidentalis* (right).

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Abstract

Bees are prolific and vital pollinators in both agricultural and natural settings, but some populations are declining, including the Western bumble bee (*Bombus occidentalis*), Suckley's cuckoo bumble bee (*B. suckleyi*), and the American bumble bee (*B. pensylvanicus*). Monitoring declining species is crucial to understand their status and management needs; however, a lack of standardized sampling methods can make range-wide monitoring difficult. Monitoring bees is usually done by two common sampling methods: passive traps and target netting. Here we examine the difference in abundance and richness of bees sampled using these methods in Wyoming, USA, with a particular focus on bumble bees (genus *Bombus*). We sampled in eastern and central Wyoming in 2019. At each site we captured bees using vane traps and target netting. We compared abundance and richness (total number of taxa represented) for species of *Bombus*. We collected two bee genera and one *Bombus* species not observed in our previous sampling. We found no difference in the abundance and richness of *Bombus* species collected in vane traps and target netting, but we encourage using both methods to more comprehensively survey the community, because the methods can collect different species. We collected *B. occidentalis* at 3 new locations and *B. pensylvanicus* at 1 new location; however, we did not collect *B. suckleyi*. Sampling bumble bees will provide up-to-date information about the current distribution and abundance of bumble bees of management concern.

Introduction

Pollinators are vital to most terrestrial ecosystems for plant reproduction in both wild and agricultural settings. As many as 80% of native plant species depend on insect pollination to reproduce (Potts et al., 2010), including 35% of crops grown for human consumption (Klein et al., 2007). However, some native bee populations have been experiencing declines (Cameron et al., 2011; Graves et al., 2020; Jacobson et al., 2018; Meiners et al., 2019, etc.). Native bee declines may be caused by habitat loss and fragmentation, pesticide use, climate change, invasive species, and pathogens (Potts et al., 2010). Declines in abundance of pollinators could be detrimental to agricultural crops and native plants that require insect pollination (Potts et al., 2010), and may cause cascading effects for herbivorous wildlife that feed on insect-pollinated plants like forbs and shrubs. Pollinator declines in the United States have been so precipitous that two species, the rusty-patched bumble bee (*Bombus affinis*) (US Fish and Wildlife Service, 2017) and Franklin's bumble bee (*B. franklini*) (US Fish and Wildlife Service, 2021b), have been listed as Endangered under the Endangered Species Act (ESA), and five other species have been petitioned for ESA listing: the yellow-banded bumble bee (*B. terricola*) (Defenders of Wildlife, 2015a), the western bumble bee (*B. occidentalis*) (Defenders of Wildlife, 2015b), Suckley's cuckoo bumble bee (*B. suckleyi*) (Center for Biological Diversity, 2020), the American bumble bee (*B. pensylvanicus*) (Center for Biological Diversity, 2021a), and the variable cuckoo bumble bee (*B. variabilis*) (Center for Biological Diversity, 2021b). In Wyoming, we have historically observed *B. occidentalis*, *B. suckleyi*, and *B. pensylvanicus*. We have observed *B. occidentalis* and *B. pensylvanicus* in our previous research, but we have yet to collect any *B. suckleyi* (Bell et al., 2019).

Monitoring the decline of pollinator populations is crucial to understand their status and to identify potential causes for their declines (Joshi et al., 2015; Meiners et al., 2019; Rhoades et al., 2017). Several methods are commonly used to sample pollinators including vane traps and aerial netting (Rhoades et al., 2017; Roulston et al., 2007; Wilson et al., 2008). Vane traps, a more novel passive sampling method, are plastic jars fitted with a lid with two intersecting vanes (Stephen & Rao, 2005). Netting involves active sampling by sweep netting vegetation or visually targeting bees and capturing them in an aerial insect nets. These two methods can vary in both the abundance and diversity of bees sampled (Bell et al., 2019; Grundel et al., 2011; Rhoades et al., 2017). Understanding what bees each sampling method collects is useful to better evaluate the status of bees of interest, like bumble bees.

Bumble bees are efficient generalist pollinators that provide invaluable ecosystem services to both native forbs and insect-pollinated crops (Losey & Vaughan, 2006). In the last few decades, notable bumble bee declines have been observed in the United Kingdom (Goulson et al., 2008) and in the U.S. (Cameron et al., 2011; Colla & Packer, 2008; Graves et al., 2020; MacPhail et al., 2019). Different sampling methods – particularly vane traps and target netting – are known to be more effective for collecting bumble bees (Bell et al., 2019; Stephen & Rao, 2005; Strange & Tripodi, 2019); however, we are aware of one study that compared the abundance and richness of bumble bees collected using vane traps and netting (Bell et al. in review).

We sampled central and eastern Wyoming, USA with vane traps and netting in 2019 to assess the bees captured and compare how sampling method altered bumble bee abundance and richness. In particular, we sampled bumble bees to gain more information on the status of declining bumble bee species in the state. We sampled several habitat types including shortgrass prairie, sagebrush steppe, conifer and mixed forests, and alpine meadows. Our specific questions were 1.) How does sampling method affect abundance and richness of bumble bees collected? and 2.) Where did we observe bumble bees of conservation concern? Our results provide baseline information about bees in Wyoming, including three species of bees petitioned for listing under the ESA.

Methods

We sampled pollinators in central and eastern Wyoming at 22 sites in locations with varying climatic and landscape characteristics from mid-July to early September 2019. We set out three blue vane traps (vane traps hereafter; SpringStar©) at 19 of the sites for 24-48 hours. We placed traps at least 15 m apart and considered them to be independent samples (Droege et al., 2010). We actively target-netted bumble bees for 30 minutes at all 22 sites (3 sites were only netted). We visited 3 sites once, 11 sites twice, and 8 sites three times over the summer. Specimens were brought back to the laboratory where they were processed and identified to genus (Michener et al., 1994) and bumble bees were identified to species (Williams et al., 2014). Due to cryptic speciation, all individuals in the *B. fervidus* species complex were recorded as one species (Koch et al., 2018). Additionally, some male *B. centralis* and *B. flavifrons* are morphologically indistinguishable and thus excluded from data analysis (P. Williams, personal communication). We calculated catch rate and taxonomic richness of bumble bee species to compare collection methods using ANOVA in R (R Development Core Team 2013) and the packages *plyr* (Wickham, 2011), *Matrix* (Bates & Maechler, 2013), and *vegan* (Oksanen et al., 2013).

Results

We captured a diverse assemblage of bees using vane traps and target netting. We collected 30 genera of bees from 5 families ($n = 1064$, Table 1) in vane traps. Two of the genera, *Neolarra* and *Pseudopanurgus*, are genera that we have not recently collected, but we have historical observations in Wyoming. We collected 18 species of bumble bees using vane traps ($n = 436$; Table 2). A total of 20 *Bombus* species were collected via target netting ($n = 531$). One of the species of bumble bee, *B. vagans*, has not been collected recently, though the species was previously known in Wyoming. There was no difference in the abundance ($F = 3.217$, $df = 1$, $p = 0.076$) or richness ($F = 0.018$, $df = 1$, $p = 0.894$) between *Bombus* collected in vane traps versus target netting (Fig. 1).

We collected four *B. occidentalis* and one *B. pensylvanicus* in 2019. We did not observe any *B. suckleyi*. Three of the four *B. occidentalis* observations were at new locations, as was the single *B. pensylvanicus* observation (Fig. 2).

Discussion

Wyoming is home to three species of bumble bees that are of management concern. All of three of these bumble bees have received a substantial 90-day Finding and are under review by the US Fish and Wildlife Service currently (US Fish and Wildlife Service, 2016, 2021b, 2021a). Previous surveys revealed that *B. occidentalis* currently lives throughout Wyoming in mountainous and urban areas (Bell et al. in review). Our new observations of *B. occidentalis* at Devils Tower National Monument, the Bear Lodge Mountains and the Laramie Range further confirm our predictions of their habitat. We have not observed any *B. occidentalis* in sagebrush steppe habitats. Although the other bumble bees were not petitioned yet, our surveys collected information about all bees. We discovered that *B. pensylvanicus* lived in southeastern Wyoming (Bell et al. in review, Williams et al. 2014) and our new observation in the Bear Lodge Mountains widens their range to the eastern half of Wyoming. *Bombus pensylvanicus* primarily live in prairie habitats. We did not capture any *B. suckleyi* in 2019 nor in previous surveys (Bell et al. in review). In fact, we are not aware of any bumble bee sampling that captured *B. suckleyi* in the last 10 years (Center for Biological Diversity, 2020). *Bombus suckleyi* are cleptoparasites, which are bees that parasitize other bees by subduing the queen and forcing the workers to raise their young. Interestingly, *B. suckleyi* mainly parasitize *B. occidentalis*. Cleptoparasitic bees are not generally abundant, but their presence is indicative of a healthy bee population. We have captured many other species of cleptoparasitic bumble bees using both methods; therefore, our methods should also capture *B. suckleyi*. These bees appear to have very low numbers or may be extirpated from Wyoming based on the lack of individuals we have observed.

Bees are vastly understudied in the western United States, and particularly in Wyoming. As far as we know, no statewide sampling has been completed since the 1970s. Our research is helping to establish new baseline information for native bees including bumble bee species of management concern. Our sampling in 2019 yielded a similar number of bee genera from previous years (Bell et al., 2019). We observed *Eucera*, *Svastra*, and *Augochloropsis* in previous year and the lack of these genera in 2019 samples was likely due to seasonal disparities. These genera tend to be active earlier in the summer. Additionally, the abundance of bee taxa tends to fluctuate among years likely due to both abiotic and biotic variables.

We were excited to find two bee genera that we have not seen in our previous collections, *Neolarra* and *Pseudopanurgus*. The historical records for these genera in the state are very few (less than 5 total observations for each) and none are recent (WYNDD Data Explorer: https://wyndd.org/data_explorer.php).

Similar to our previous research, we did not see a difference in the abundance or richness of bumble bees collected in vane traps or by target netting. We did not have enough data to statistically examine differences in collection methods for each species of bumble bee; however, the data does show some trends. For example, some species of bumble bees were more readily caught in vane traps than netting (e.g. *B. appositus*, *B. fervidus*, *B. nevadensis*) while others were more likely to be captured by target netting (e.g. *B. mixtus*, *B. kirbiellus*, *B. rufocinctus*). This indicates that using both methods of sampling is beneficial to more comprehensively observe the *Bombus* community present. These disparities are likely due to differences between active sampling (netting) and passive sampling (vane traps). Blooming flowers are needed to target net bumble bees. Thus, bumble bees are nearly impossible to capture in areas with few blooming flower (Pei et al. 2021), likely because they are searching for floral resources. Netting can be challenging and yield few bees in drier habitats or later in the season when fewer flowers are blooming. Target netting also catches larger bees (Pei et al., 2021) and is highly influenced by collector bias (Westphal et al., 2008). On the other hand, vane traps perform best in areas with few blooming flowers (Crawford et al. in prep) and can be used in a variety of habitat types. Vane traps collect small to large bees (Bell et al., in review) and are not influenced by collector bias. These methods perform best in opposite conditions making them ideal to use in tandem.

What bees we captured not only depended on the collection method, but ecosystem characteristics and bee behavior as well. The number of blooming flowers has a large effect on the bees collected for both sampling methods. For example, target netting is very inefficient when few flowers are blooming and usually yield very few bees (Crawford et al., in prep). Conversely, we captured the most bees in vane traps when few flowers were blooming. Vane traps have no scent added, but the brightly colored container acts like a large flower that attracts pollinators. We probably catch more bees and a more diverse assemblage in vane traps in dry years with fewer floral resources. Bee behavior also alters what bees we capture. For example, *B. nevadensis* tend to fly high and fast, and are very difficult to net, which could explain why we collected far most individuals in vane traps. Some species are also more skittish by leaving flowers when a collector comes near, and thus are more frequently missed when netting.

Wyoming contains valuable habitat for declining bumble bee species yet remains a vastly understudied area. Continued surveys will be vital to collect more information about these species including more questions about their status and distribution. Our surveys have helped establish baseline information for bees in the state which have already been useful for the 2 newly petitioned bumble bees. Monitoring is essential to estimate which bees are common and rare, and to track their abundance. Understanding the best methods to monitor with is critical to limit bias and collect the best information available. We recommend using both vane traps and target netting to obtain a broader representation of the bumble bee community present. Using both methods enables us to assess bees in different ecosystems, varying floral abundance, and bees with different behaviors.

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Table 1. Number of bees collected in vane traps in central and eastern Wyoming in 2019. Bolded taxa indicate new observations for our research.

Taxa	<i>Abundance</i>
<u>Andrenidae</u>	9
<i>Andrena</i>	5
<i>Perdita</i>	3
<i>Pseudopanurgus</i>	1
<u>Colletidae</u>	21
<i>Colletes</i>	4
<i>Hylaeus</i>	17
<u>Halictidae</u>	210
<i>Agapostemon</i>	16
<i>Augochlorella</i>	2
<i>Dufourea</i>	7
<i>Halictus</i>	40
<i>Lasioglossum</i>	140
<i>Sphecodes</i>	5
<u>Megachilidae</u>	114
<i>Anthidium</i>	3
<i>Ashmeadiella</i>	3
<i>Coelioxys</i>	1
<i>Dianthidium</i>	4
<i>Heriades</i>	1
<i>Hoplitis</i>	14
<i>Megachile</i>	22

<i>Osmia</i>	65
<i>Stelis</i>	1
<u>Apidae</u>	1241
<i>Anthophora</i>	90
<i>Apis mellifera</i>	2
<i>Bombus</i>	436
<i>Ceratina</i>	79
<i>Diadasia</i>	1
<i>Epeolus</i>	1
<i>Melecta</i>	4
<i>Melissodes</i>	95
<i>Neolarra</i>	1
<i>Nomada</i>	1
TOTAL BEES	1064

Table 2. Number of *Bombus* species collected in vane traps and targeted netting in central and eastern Wyoming in 2019. Male *B. centralis/flavifrons* were not included in analysis due to lack of species determination because there are no morphological differences separating these species. Bolded taxa indicated a new species observation for our research.

Species	Vane Traps	Aerial Netting
<i>B. appositus</i>	103	15
<i>B. bifarius</i>	62	26
<i>B. bimaculatus</i>	2	1
<i>B. centralis</i>	47	34
<i>B. centralis/flavifrons</i> *	13*	-
<i>B. fervidus</i>	87	17
<i>B. flavidus</i>	6	3
<i>B. flavifrons</i>	18	29
<i>B. frigidus</i>	-	2
<i>B. griseocollis</i>	10	14
<i>B. huntii</i>	6	9
<i>B. insularis</i>	35	19
<i>B. kirbiellus</i>	2	21
<i>B. melanopygus</i>	7	1
<i>B. mixtus</i>	9	38
<i>B. nevadensis</i>	44	4
<i>B. occidentalis</i>	3	1
<i>B. pennsylvanicus</i>	-	1
<i>B. rufocinctus</i>	26	88
<i>B. sylvicola</i>	16	5
<i>B. vagans</i>	31	108
TOTAL BOMBUS	436	531

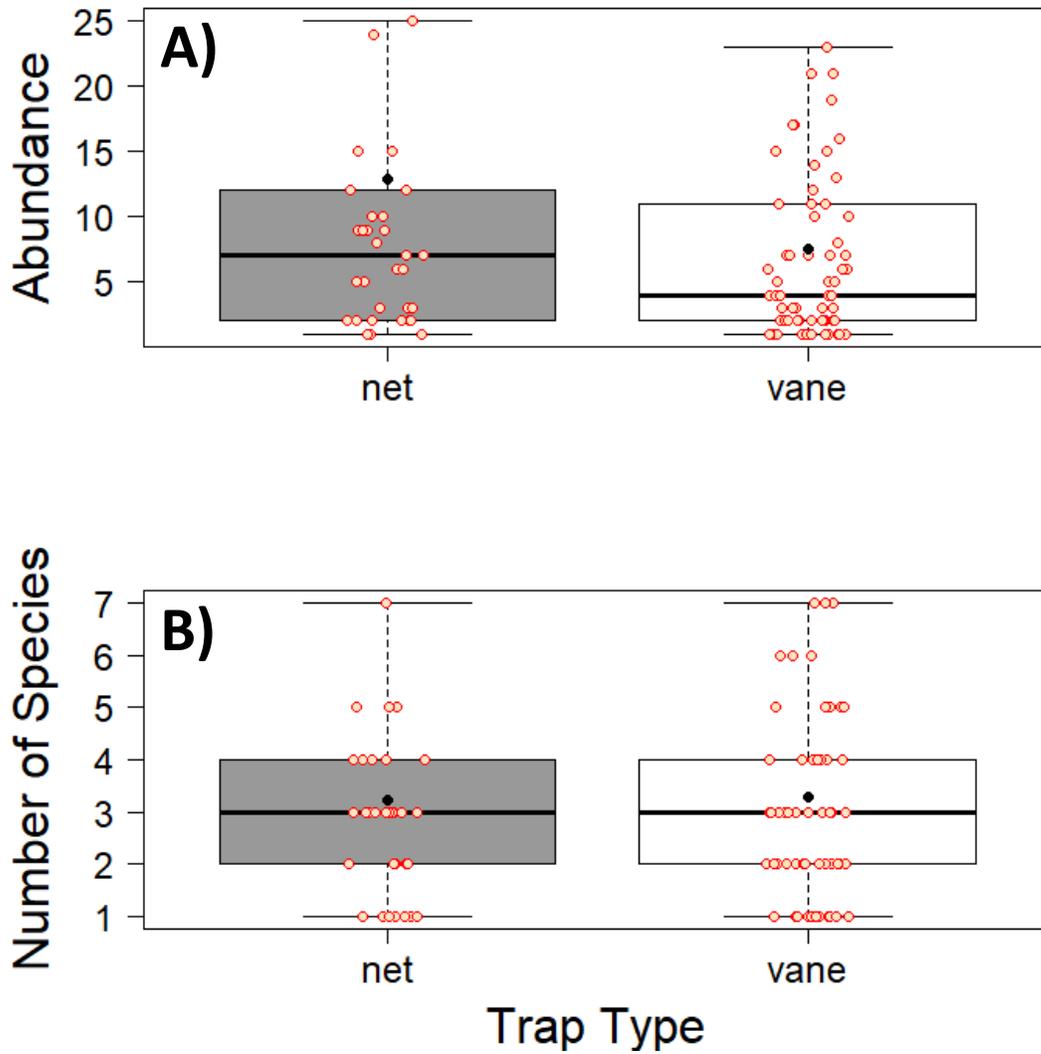


Figure 1. Abundance (A) and richness (B) of *Bombus* species sampled by target netting and in vane traps in central and eastern Wyoming in 2019. Red dots are individual sampling events, black dots are mean values, bold lines are median values, lower and upper limits of the box are the 25th and 75th percentiles and whiskers indicate the lower and upper limits of the data excluding outliers.

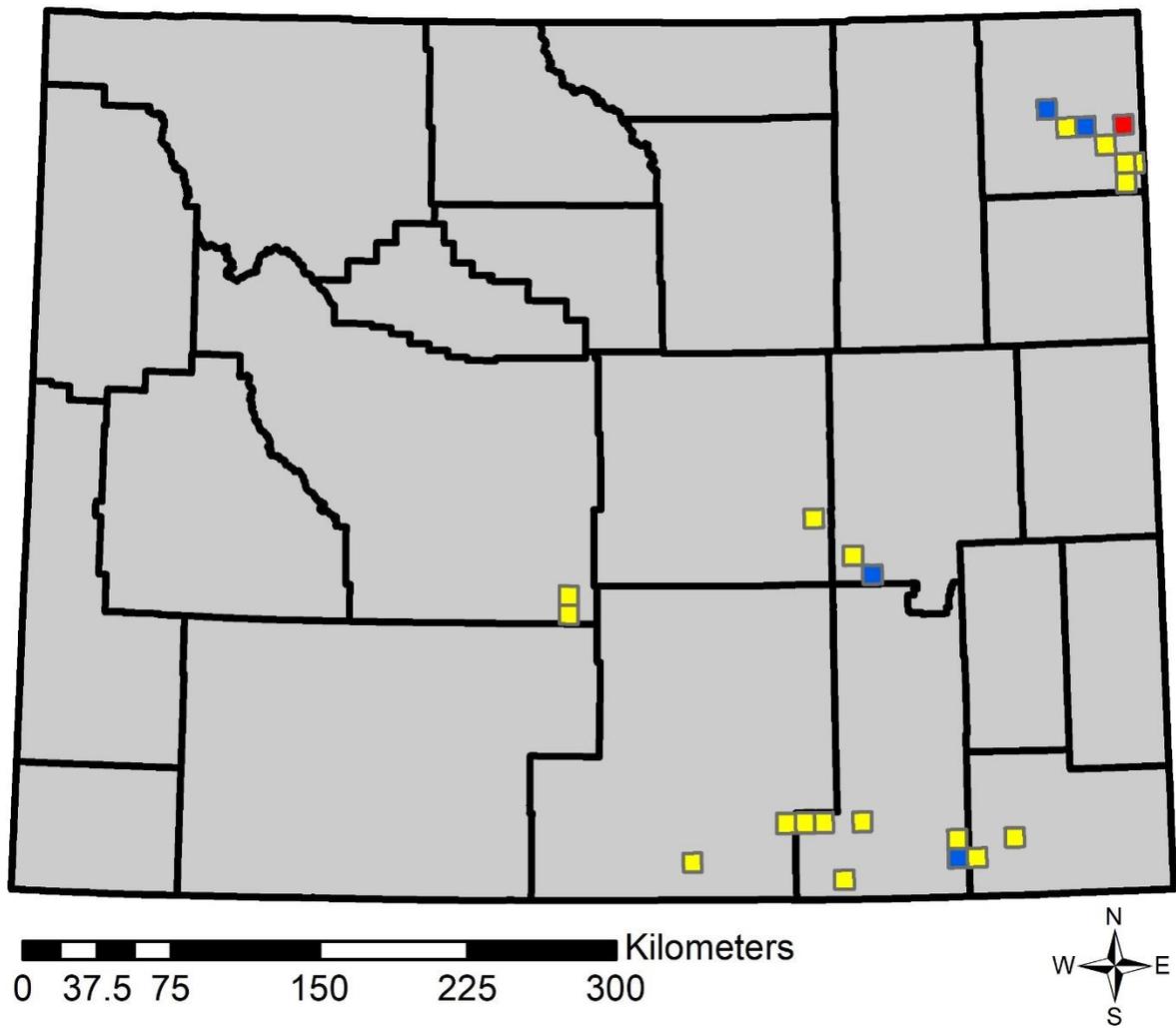


Figure 2. Yellow squares indicate locations we sampled bees in 2019 ($n = 22$) in Wyoming separated by county boundaries (black lines), and locations we collected *Bombus occidentalis* (blue squares) and *B. pennsylvanicus* (red square).