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Seasonal Variation in Abundance and Thermal Tolerance of Bumble Bees (Bombus) in Grand Teton National Park

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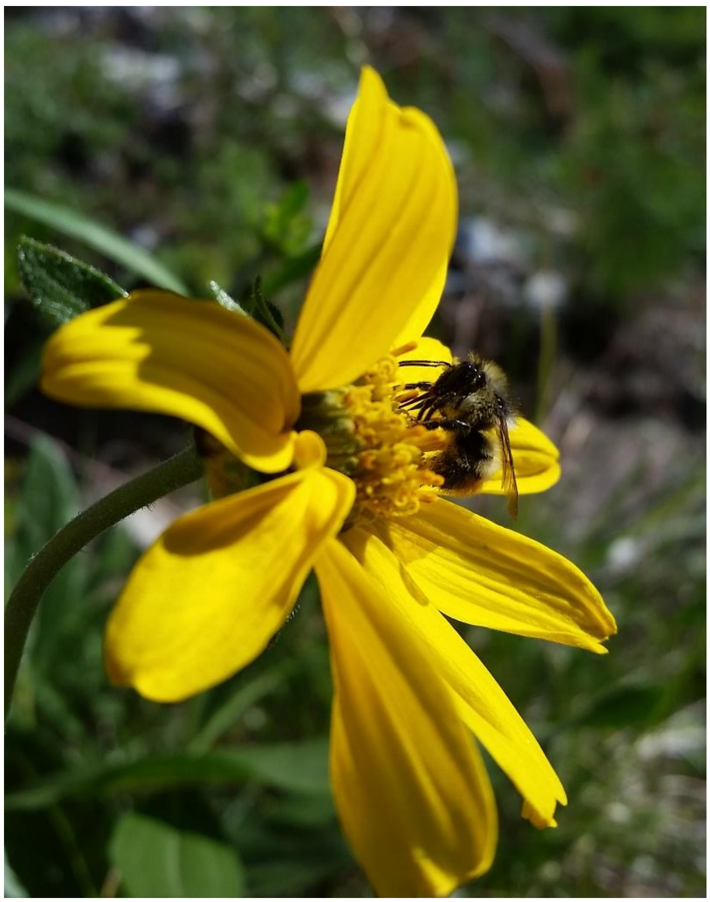

Abstract:

Bumble bee (genus *Bombus*) emergence time is affected by temperature, elevation, and is correlated with flowering time of diverse plants. Critical thermal limits of bumble bees is effected by elevation, latitude, and genetics. However, little research has been done examining variation in thermal tolerance of *Bombus* at high elevation throughout their active season. I sampled for abundance of several species of *Bombus* in Grand Teton National Park throughout the summer and tested for critical thermal limits. I found that bumble bees begin emerging in late May but didn't appear in large numbers until late July. The most abundant species were *B. mixtus*, *B. flavifrons*, and *B. bifarius* with *B. mixtus* emerging the earliest. The critical minimum temperature did not vary between these three species while *B. mixtus* had a lower critical maximum temperature than *B. flavifrons*, and *B. bifarius.* This suggests a possible connection between emergence time and thermal tolerance that could be explored in further studies.

Introduction:

In temperature regions, most bees, and other insects, are only active during parts of the year. In order for bees to survive a lack of resources and cold temperatures they must hibernate during the winter. Bumble bees (genus *Bombus*) are one of the few bees native to the United States that are social; unlike honeybees, bumble bee colonies are seasonal. At the end of the summer a fertilized queen hibernates alone before emerging the following spring to found a colony of her own. Temperature is crucial to emergence time and survival because in order for a queen to be able to fly her flight muscles must be above 30°C (Heinrich, 1975). After the queen emerges she finds a place to build her nest. The first offspring of the new colony are female workers. Males don't emerge until later in the season where they mate with a new queen before she overwinters (Goulson, 2003).

The average activity span for bumble bee species in North America begins as early as late March, with the emerging queens, and lasts until as late as September, when the bulk of the males are seen (worker numbers peak in late summer). The last to emerge are the newly fertilized queens preparing for overwintering. The majority of literature on bee emergence patterns is taken from pinned specimens that may not accurately reflect population fluctuations in the wild. This data is taken from a combination of ranges of various species and doesn't account for differences in collection sites in elevation, longitude and latitude, or climate (Williams et al., 2014).

Insect thermal tolerance—usually measured as the range of temperatures at which the animal can physiologically function--may vary with latitude and elevation. Organisms must deal with temperature extremes that can vary both seasonally and diurnally (Kimura, 2004). For example, limited data suggest that bumble bees from higher elevations are more cold tolerant than those from lower elevations. When *Bombus* species from different elevations within Wyoming were compared *B. bifarius*, which are generally found at higher altitudes, were found to have a lower critical thermal minimum (CT_{min}) than *B. huntii*, which is typically found at lower elevations (Oyen et al., 2016).

Bee emergence time is also correlated with flowering. Because flowering occurs later in areas of higher elevation this shifts the emergence of workers and males later in the season (Pyke et al., 2011). Studies suggest that the emergence of queens is likely due to the increase of soil temperature (Kudo et al., 2013). Snow insulates the ground that it covers such that snow-covered ground will be warmer than bare soil in the same environment. Therefore when snowmelt occurs early in the season it can take longer for the soil to warm. This could potentially lead to a phenological mismatch between pollinator emergence and flowering time (Kudo et al., 2013).

This is of particular importance in alpine areas where bumble bees are the most effective and often most abundant pollinators. In a study conducted in Japan it was found that in a year with typical temperature patterns for the area peak flowering times corresponded with queen emergence and when workers became active. In a subsequent year that was unusually warm this synchrony was disrupted (Kudo et al., 2014). This suggests that as the climate changes and becomes warmer alpine areas may be disproportionately affected.

I chose to explore the seasonal variation in species abundance and thermal tolerance in bumble bee populations in Grand Teton National Park (GTNP). GTNP is located at a high elevation (6834ft where sampled) where the summer season is short and weather can be volatile. By surveying bee species and testing critical thermal limits of *Bombus* species in the park I wanted to see if there was any difference between species and if that difference was correlated with emergence time.

Materials and Methods

Bee abundance:

In order to collect data on the seasonal activity of bees in the park I used vane traps. These traps mimic the appearance of a flower, attracting diverse pollinators; when a bee falls in it cannot escape the funnel-shaped entrance. I put out five traps twice a week for twenty-four hours starting at the end of May and continuing through August as weather allowed (traps were not put out if rain or snow was in the forecast for the day). The sites sampled were lightly wooded areas with wildflowers (Fig. 1). After twenty-four hours I collected the traps and pinned the specimens.

Figure 1: Setting out vane traps for 24 hour sampling.

Thermal Tolerance:

To test thermal tolerance I collected bumblebees (from the same locations as the vane trap sampling) via netting. All specimens were collected within a twenty minute time window before bringing brought back to the lab. All bees were weighed before testing to the nearest mg (Acculab Portable scale). To measure thermal tolerance the bees were placed in vials in wells in an aluminum block mounted on a thermoelectric plate (Fig. 2). Both minimum (CT_{min}) and maximum critical temperatures (CT_{max}) were reached by ramping the temperature either up or down from room temperature at a rate of 0.25°C/min. Temperature of each well was measured individually to account for differences in temperature across the plate.

Figure 2: Bombus being tested for CT_{min} and CT_{max}.

CT_{min} was determined by observing bee behavior and looking for entrance into a chill coma (often marked in larger bees by a distinct fluttering and curling). In this state the bee is inactive until it warms again. Once a bee reached its CT_{min} it was removed from the block to recover at room temperature. All bees were allowed to recover for fifteen minutes and had all regained normal movement patterns and coordination before measuring maximum critical temperature. . CT_{max} was also determined by behavior: at CTmax, bees lost motor control and began twitching and making uncoordinated movements

Results

I began sampling May 23rd and found the first *Bombus* (queen) on May 24th. There were few *Bombus* until late July when abundance peaked (Fig. 3; black dotted line). The first male *Bombus* (*B. mixtus*) was trapped on the 28th of July. I found nine different species of *Bombus*: *B. bifarius, B.mixtus, B. nevadensis, B. appositus, B. rufocintus, B. flavifrons, B. fervidus, B.*

suckleyi, and *B. occidentalis*. Out of these, the three most common were *B. mixtus*, *B. bifarius*, and *B. flavifrons*. *B. mixtus* peaked in abundance in late July whereas *B. bifarius* and *B. flavifrons* peaked in mid-August (Fig. 3). A dip in abundance around August 1st (Fig. 3) corresponds to a period of rainy weather where not many bees were flying.

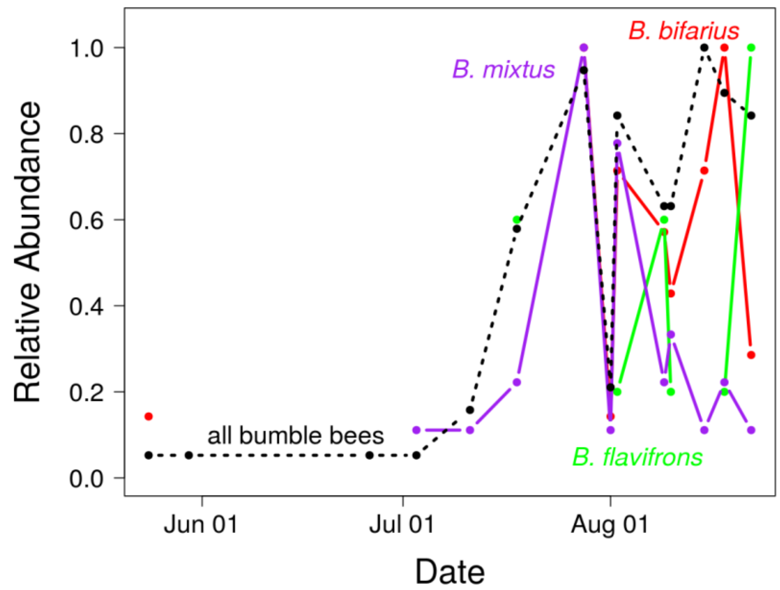

Figure 3: Relative abundance of *Bombus* in Grand Teton National Park from 5/24/2017 to 8/23/2017.

Out of sixty bees tested for thermal tolerance the most common species were *B. bifarius* (16), *B. flavifrons* (7), and *B. mixtus* (19) (Fig. 4). CT_{min} and CT_{max} varied independently of each other and of mass and sex (ANOVA, all $P>0.05$). *B. bifarius* CT_{min} ranged from 0.9 °C to 5.4 °C, with a mean of -2.7 °C, and a standard deviation of 2.2 °C. *B. bifarius* CT_{max} ranged from 41 °C to 50.9 °C, with a mean of 44.7 °C, and a standard deviation of 3.1°C. *B. flavifrons* CT_{min} ranged from -0.3 °C to -5.1 °C, with a mean of -2.6 °C, and a standard deviation of 1.7 °C. *B. flavifrons* CT_{max} ranged from 37.6 °C to 55.4 °C, with a mean of 45.7 °C, and a standard deviation of 6.5 °C. *B. mixtus* CT_{min} ranged from 0 °C to -7.3 °C, with a mean of -3.3 °C, and a standard deviation of 2.3 °C. *B. mixtus* CT_{max} ranged from 27.0 °C to 50.9 °C, with a mean of 40.3 °C, and a standard deviation of 5.7 °C.

CT_{max} varied significantly among species (ANOVA, $F_{2,38} = 4.66$, P = 0.016), with *B. mixtus* having significantly lower CT_{max} than *B. bifarius* (by 4.6 °C, Tukey HSD, P = 0.035) and *B. flavifrons* (by 5.4 °C, Tukey HSD, P = 0.050), which were indistinguishable in CT_{max} (Tukey HSD, P = 0.903). There were no significant differences in CT_{min} between species. *B. mixtus* had individuals with the most extreme cold tolerance (lowest CT_{min}), but these differences were not statistically significant (ANOVA, P>0.05).

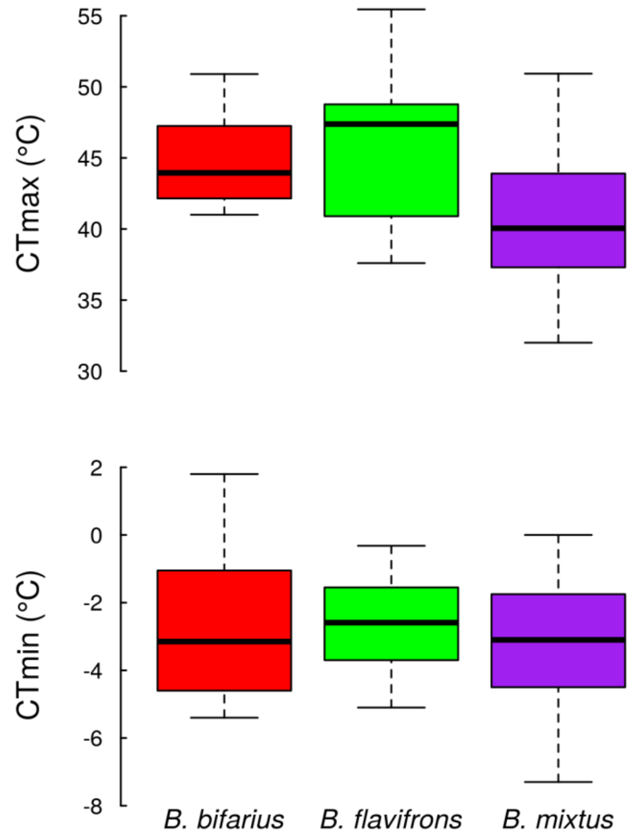

Figure 4: Critical thermal limits of *B. bifarius*, *B. flavifrons*, and *B. mixtus*. *B. mixtus*. *B. mixtus* has a significantly lower CT_{max} than *B. bifarius* and *B. flavifrons*. There is no statistically significant difference between CT_{min}.

Discussion

A possible reason for a difference in emergence time and pattern for these three species from the literature is length of the season. GTNP has a high elevation and a relatively short summer. In May and early June temperature lows are near 0°C and snow is still possible. July is typically the warmest month in the area and that is where the amount of bees found starts to climb. The emergence of *B. mixtus* earlier in the season than other species is interesting. Some possible explanations to be explored would be: if the species had any variation in flower preference and if so, does the flowering time correlate with the difference in emergence times. Differences in thermal tolerance which allow them to be active in colder weather.

The largest issue when looking at these data is the sample size. Ideally, enough samples would be netted to compare males and females across all species present in equal numbers. Other sources of error in this experiment include variation of temperature within the vials. Bees tended to crawl up the sides of the vials during ramping. To counter this bees were poked back down to the bottom of the vial but it is likely there was still variation in temperature among vials on the block.

B. bifarius, *B. flavifrons*, and *B. mixtus* share a similar range in the mountain west which suggests that they would have similar critical thermal limits (Williams et al., 2014). However, *B. mixtus* was the first species to emerge, has the lowest CT_{max}, and had individuals within the population with the lowest CT_{min} (although there wasn't a statistically significant difference between species). This suggests that there may be a correlation between emergence time and critical thermal limits. There wasn't enough data to compare critical thermal limits of species across the season however this would be an interesting question for further research.

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