Intertegular Distance and not Corbicular Size of *Apis mellifera* Differs in Prairies of Varied Quality

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Pollinators, specifically bees, have an essential mutualistic relationship with plants, but as both bee and plant populations decline, these relationships are now endangered (Papanikolaou *et al.* 2017, Aguirre-Gutierrez *et al.* 2015). Both bees and their prairie habitats are significantly less abundant than before (Papanikolaou *et al.* 2017). Of the 162 million ha of prairie that existed in the United States prior to European settlement, approximately 99.9% has been destroyed (Samson& Knopf 1994). In Illinois, 99.7% of original prairie has been destroyed, and the remnants that remain consist of mostly 5 ha fragments or smaller (Bowles *et al.* 2003), with 75% of the remaining high-quality prairies less than 2 ha (Schwartz 1999). Further, the remnants that are larger are usually low-quality (Schwartz 1999), with previous research documenting a positive correlation between prairie size and prairie degradation (Schwartz 1999). Habitat loss negatively affects bee abundance, which has also declined significantly in recent decades (Winfree *et al.* 2009). In the United States, the population of *Apis mellifera* hives has declined at an average rate of -1.7% annually (Aizen & Harder 2009). Managed *Apis mellifera* colonies have experienced a 100% increase in overwintering mortality (15% to 30%), and overall abundance has steadied since 2007 solely because hives are being replaced when they die (Staveley *et al.* 2013, Winfree *et al.* 2009). The decline of *Apis mellifera* populations is projected to continue to across the world (Winfree *et al.* 2009). The loss of *Apis mellifera* pollination services would not only negatively affect plant communities (Hopwood 2008), but also global food production.

Approximately 35% of global food production depends on pollination services (Hopwood 2008). Bees are considered the most important and effective taxon of pollinators because bees rely on pollen and nectar for their diet (Hopwood 2008, Ricketts *et al.* 2008, Goulson 2003). Specifically, *Apis mellifera* are one of the most important pollinators due to their production of honey, pollination of crops essential to the economy, and their general ability to adapt to new habitats (Staveley *et al.* 2013, Goulson 2003, Winfree *et al* 2009). *Apis mellifera* is also the pollinator most commonly managed for commercialization worldwide (Zhang *et al.* 2015), producing 147,638 thousand pounds of honey in the United States last year (NASS 2018). European *Apis mellifera* was introduced to the United States by European settlers in the 1600s (Zayed & Whitfield 2008) specifically for honey production and is now considered the primary managed crop pollinator (Winfree *et al* 2009). In the past decade, *Apis mellifera* suffered an epidemic known as Colony Collapse Disorder, reducing the species abundance and affecting 20-30% of beekeeper's colonies (Watanabe 2008) compared to the annual average of expected overwintering losses before 2006 (15%) (Staveley *et al.* 2014). While most research has focused on preventing the spread of pathogens infecting *Apis mellifera*, habitat loss is being overlooked as a threat to *Apis mellifera* (Sumpter & Martin 2004), although habitat loss has been documented as one of the major factors associated with recent native bee decline (Potts *et al.* 2010, Naug 2009, Winfree *et al* 2009).

Apis mellifera foraging habitats vary in quality, effecting resource availability (Tonietto *et al.* 2017, Seeley 1994). Land composition and diversity of flowering plants can be used to assess *Apis mellifera* habitat quality (Goulson 2003, Sumpter & Martin 2004, Sponsler & Johnson 2015). Comparatively, prairies provide better pollination resources than agricultural fields, as they are typically more florally diverse and have lower pesticide exposure risk (Migdal *et al.*

2018). Larger bees make up a greater proportion of bees present in foraging habitats with larger grassland areas than in foraging areas with smaller grassland areas (Hinners *et al.* 2012). Habitat quality is so critical that *Apis mellifera* communication has evolved to indicate effectiveness of recent foraging (number of zig zag patterns) (Seeley 1995). If evolution selected so strongly for habitat quality, it might be key to *Apis mellifera* survival. Therefore, the quality of the habitat may be important to *Apis mellifera* health.

Apis mellifera with a more diverse and plentiful diet become healthier, positively influencing the success of *Apis mellifera* (Di Pasquale *et al.* 2016). For example, diverse diets have been found to lead to hives that are more resistant to mite infestation and infection (Sumpter & Martin 2004). Further, recent work suggests that multi-floral pollen is more nutritionally beneficial than mono-floral pollen (Sumpter & Martin 2004). Colonies of *Apis mellifera* near foraging sites with better landscape compositions additionally accumulate more food and collect more wax (Sponsler & Johnson 2015). Finally, *Apis mellifera* colonies with a greater variety of pollen resources for their diet have greater population sizes (Requier *et al.* 2015).

While population-level effects of foraging quality have been widely investigated, a smaller amount of research has been done on individual-level health effects of habitat quality, particularly for *Apis mellifera*. Environmental differences have been shown to influence individual characters in bees. It is expected that greater *Apis mellifera* health would manifest itself in more physiological growth (Veiga *et al.* 2013). Bee body size provides limited information about bee health. For example, *Melipona flavolineata* body size is positively related to foraging range of their colony (Veiga *et al.* 2013), however body size alone cannot be used to estimate production costs of a solitary bee *Osmia cornuta* (Bosch & Vicens 2002). Alternatively, intertegular distance in bees is an accepted means of estimating body size, wing strength and foraging distance (Kuhn-Neto 2009), which can vary between 10km to 20km (Goulson 2003). Wing measurements are also used to discriminate between subspecies of *Apis mellifera* (Barour & Baylac 2016). When *Melipona flavolineata* have more food, their intertegular distance increases and corbicular area decreases (Veiga *et al.* 2013). *Melipona flavolineata* with a larger corbicula collect proportionally more pollen at one time have a smaller body size, and are produced when colony food reserves are low (Veiga *et al.* 2013). However, bigger bees have a larger intertegular distance have greater foraging distance than smaller bees (Ribbands 1951, Veiga *et al.* 2013). Therefore, both intertegular distance and corbiculae size each have the potential to positively affect *Apis mellifera* success (Papanikolaou 2017, Veiga *et al.* 2013).

To evaluate whether prairie quality can predict *Apis mellifera* corbicula size and intertegular distance, we sampled *Apis mellifera* individuals within two restoration prairies of varied quality in southern Illinois, based on size and floral diversity. Specifically, we predicted that *Apis mellifera* found at a higher quality restoration prairie would exhibit greater intertegular distance and have smaller corbicula than *Apis mellifera* found at a lower quality restoration prairie.

Materials and Methods

Apis mellifera were collected in Heartland Prairie in Madison County, Illinois and Paul Wightman Subterranean Nature Preserve, hereafter referred to as Wightman Prairie, in Monroe County, Illinois in 2017. Heartland Prairie was used as the lower quality site, being a 60-acre tallgrass prairie with approximately 150 native prairie plant species (The Nature Institute 2018). Wightman Prairie was used as the high-quality site, being a 535-acre clifftop prairie (Illinois DNR n.d.). Honeybees were targeted for collection using aerial nets, and then transferred to jars filled with ethyl acetate. All bees were pinned on site. A minimum of 30 specimens from each site were measured. In October 2018, the intertegular distance (mm) and corbiculae size were measured (mm^2) using digital calipers under a light microscope. All caliper measurements were taken three times and averaged. Corbiculae were measured for both distal-end width (mm) and midline length (mm) to determine overall corbiculae size (mm^2). Intertegular distances and corbiculae size were each separately evaluated for normality using Shapiro-Wilk analysis. A Mann Whitney U test (Wilcoxon rank sum test) assuming abnormal distribution was then used for each character (intertegular distance and corbiculae size).

Results

Corbicula size and intertegular distance from the restoration sites were not normally distributed, as shown in table 1, therefore the nonparametric tests Mann-Whitney U, Wilcoxon Ranked Sum test, were used.

Table 1. Normality Test of corbicula size and intertegular distance from the high-quality site, Wightman prairie and the lower-quality site, Heartland Prairie.

Tests of Normality

		Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Site	Statistic	df	Sig.	Statistic	df	Sig.
Corbicula	Wightman	.150	26	.136	.952	26	.254
	Heartland	.098	30	.200*	.977	30	.730
Intertegular Distance	Wightman	.106	30	.200*	.982	30	.873
	Heartland	.130	29	.200*	.947	30	.143

*. This is a lower bound of the true significance.

a. Lilliefors Significance Correction

Apis mellifera from the low-quality restored prairie had an insignificantly less corbicula area than *Apis mellifera* from the high-quality prairie, as shown in table 2.

Table 2. Mean ranks of corbicula size and intertegular distance from the low-quality restoration, Heartland Prairie, and the high-quality restoration site Wightman Prairie.

Ranks

	Site	N	Mean Rank	Sum of Ranks
Corbicular Size	Wightman	26	29.81	775.00
	Heartland	30	27.37	821.00
	Total	56		
IntertegularDistance	Wightman	30	42.40	1272.00
	Heartland	30	18.60	558.00
	Total	60		

Therefore, *Apis mellifera* corbicula area from the high-quality prairie was not significantly different than *Apis mellifera* corbicula area from the low-quality prairie (U = 356, p = 0.576), as shown in table 3.

Table 3. Statistical significance of the Mann-Whitney U test for intertegular distance and corbiculae size of *Apis mellifera* from high compared to low-quality restoration sites.

Test Statistics[a]

	Corbicula	Intertegular Distance
Mann-Whitney U	356.000	93.000
Wilcoxon W	821.000	558.000
Z	-.559	-5.284
Asymp. Sig. (2-tailed)	.576	.000

a. Grouping Variable: Site

Alternatively, *Apis mellifera* from the high-quality prairie can be considered to have greater intertegular distance than *Apis mellifera* from the low-quality restoration prairie as shown in table 2. It can also be concluded that *Apis mellifera* intertegular distance from the high-quality restoration prairie was statistically significantly higher than *Apis mellifera* intertegular distance from the low-quality restored prairie (U = 93.000, $p = 0.000$), as shown in table 3.

Discussion

Originally, the experimental procedure was to collect *Apis mellifera* from May to October 2018, specifically at hill prairie restoration sites of various quality grades around Madison County, Illinois. This collection procedure was carried out and other bee species were found during this collection procedure; however, the abundance of *Apis mellifera* was negligible. Only 3 individual *Apis mellifera* were collected during this time, rendering the collection procedure insufficient for data analysis. Temperature could be a factor in the lack of *Apis mellifera* in southern Illinois hill prairies between May and October 2018. The lack of *Apis mellifera* collected in this experiment may demonstrate the struggle for the survival of the species altogether (Papanikolaou *et al.* 2017). *Apis mellifera* are managed in controlled settings, but even those colonies are subject to significant declines (Suryanarayanan & Klienman 2013). *Apis mellifera* research is important enough for the United States Senate to implement a bill funding conservation research because managed *Apis mellifera* colonies are being lost at rates ranging from 30% to 90% (Suryanarayanan & Klienman 2013), and pollinators add more than 180 billion dollars to crop values for the country every year (Boxer 2007). Habitat quality, but also by temperature affect bee diversity significantly (Papanikolaou *et al* 2017). To preserve the study, *Apis mellifera* collected in 2017 were measured from two different size and quality of restored prairie.

I expected that both greater intertegular distance and lesser corbicula area of *Apis mellifera* would be positively related to increased habitat quality based on the assumptions that higher quality sites would have more successful and healthier *Apis mellifera* and that intertegular distance and corbiculae area are valid indicators of *Apis mellifera* health and success (Papanikolaou 2017); however, corbicula area was not significantly related to the quality of the prairie site. Corbicula area was not significantly different between the restoration prairie sites, it appears that corbicula area may not be an indicator of success in *Apis mellifera* in the same way that it is an indicator in *Melipona flavolineata* (Veiga *et al.* 2013).

Intertegular distance is an established means of determining body size, wing strength and foraging distance all with positive relationships (Kuhn-Neto 2009, Goulson 2003). As expected, the specimens collected at the high-quality restoration prairie had significantly larger intertegular distances. Based on these findings, I suggest that *Apis mellifera* from the higher quality restoration are on average bigger, have greater wing strength and foraging distance, assuming the quality of Wightman prairie is greater than the Heartland prairie, based on established indicators such as relative prairie size and flora diversity (Veiga *et al* 2013). Since the data supports hypothesis that *Apis mellifera* health is increased at the higher quality prairie, I would also hypothesize that the *Apis mellifera* at higher quality restoration prairies are on average bigger and healthier because *Apis mellifera* intertegular distance has been positively correlated with bee size and health in future research (Veiga *et al* 2013). Further testing is necessary to confirm.

Considering that intertegular distance does appear to be an indicator of success in *Apis mellifera* in the same way that it is an indicator in *Melipona flavolineata*, this work also provides support for the overall view that *Apis mellifera* will continue to decline without adequate increase in habitat (Veiga *et al.* 2013). Considering that individual *Apis mellifera* express healthier attributes when foraging in higher quality prairies, efforts to maintain *Apis mellifera* populations should focus on keeping its individual foraging bees healthy by increasing foraging habitat in quantity and quality. *Apis mellifera* preservation efforts should strive to increase size, quantity and floral diversity of prairies.

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