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Resource Use of Native Bees: Understanding the Roles of Preference, Nutrition and Competition

By

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A dissertation submitted in partial satisfaction

of the requirements for the degree of

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in

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in the

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of the

University of California, Berkeley

Committee in charge:

Professor Claire Kremen, Chair

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Professor Stephen Welter

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I'd like to dedicate this manuscript to my family who has been there every step of the way.

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## Abstract

Resource Use of Native Bees: Understanding Roles of Preference, Nutrition and Competition

by

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Global declines of pollinators have highlighted the lack of information on pollinator species and communities necessary to improve conservation efforts. Specifically, significant information is missing on resource use of native pollinators and factors at both the species and community level that influence resource selection. My dissertation work uses a variety of methods to examine resource use and patterns at the species level and community level for native bumble bees (*Bombus*) and how preference, nutrition and competition affect foraging and community assembly.

Identifying resource and habitat preference is considered a key component to conserving declining species and is commonly used by conservation biologists for birds and mammals. Although identifying resource preference is also considered critical to conserving pollinators, to date no comparable work has been done on pollinator species. In chapter one, pollen use of the yellow faced bumble bee (*Bombus vosnesenskii*) was conducted using a Dirichlet multinomial model (DM) by comparing three different models of collection: 1) Use=Availability, 2) Use=Preference\* Availability and 3) Use. Although *B. vosnesenskii* was thought to be a generalist forager, it does not collect pollen with respect to its availability and demonstrated significant preferences for some resources when accounting for availability. Additionally, models that excluded availability were typically superior to those that included availability. This suggests that estimates of preference and resource reliance can be adequately estimated without measuring availability which may be critical for cryptic species and those difficult to identify available resources such as pollinators.

To better understand the patterns of resource preference found in chapter one, analysis of features that may influence resource use was conducted. Morphological, nutritional (protein and amino acid composition) and availability (pollen per inflorescence) features of plants were measured for 12 species available to *B. vosnesenskii*. Plants were divided first by origin (native or non-native) and then by collection (collected or not-collected) and compared across groups. Although it is assumed native species should be superior for native pollinators no significant differences were found when compared based on origin. However, when grouped based on collection, collected species were significantly different for amino acid content, protein, and availability. This suggests that not all native plants are adequate resources to support pollinator

communities and that not all invasive species are inadequate and that care should be taken when restoring plant communities for pollinators.

Lastly, phylogenetic methods were used to understand patterns of community assembly and competition in bumble bee communities. Community assembly theory would suggest that communities should attempt to limit similarity, either morphological or phylogenetic similarity, to maintain coexistence. Bumble bees have long been considered to limit overlap in a community by dividing niche space by tongue length and thus communities were thought to be comprised of species with different tongue lengths. To test this, the Nearest Taxon Index (NTI) and Net Relatedness Index (NRI) were calculated for 118 co-occurring communities in Nearctic Areas of North America using a phylogenetic distance matrix and a trait distance matrix. Although, long believed to limit similarity by having different tongue lengths this method found bumble bee communities were both phylogenetically closely related and had more similar tongue length when compared to randomly generated null communities. This could suggest that patterns of community assembly and resource use may be driven by nutritional requirements of bumble bees and require them to share resources rather than divide the niche space as expected.

Although further work is needed to support these findings, the patterns found here have implications for conservation of native pollinators. The observed non-random patterns of resource use, both for preference and floral traits, suggest that significant effort should be made to determine plant species that support native pollinators particularly species that are declining. It is commonly assumed that by restoring native plant communities native bee communities will return but this work highlights that not all native plants can provide necessary protein or amino acids to support bee communities. Lastly, the high level of relatedness of bumble bee communities may suggest that whole communities may respond similarly to threats such as disease or habitat fragmentation and thus may be more susceptible to whole community losses. Efforts should be made to monitor populations and limit damage to communities. While all species have intrinsic value, pollinators are also invaluable to maintaining wild flower and crop plants that support higher trophic level diversity and food security and thus particular efforts should be made to conserve them.

## Chapter 1

### Does local availability drive pollen collection by bees?

#### Introduction

Resource use and availability dictate some of the most important aspects of individual survival and population persistence. Ecological interactions that structure communities, such as competition, predation and mutualism, are all influenced by how organisms use available resources. Resource use, however, is not solely determined by the availability of the resource but also by resource preference at both the species and individual level. Preference, as defined by Beyer et al. (2010), is a statistical description of resource use relative to a sample of resource availability. Preference statistics are commonly used to identify habitat, and more rarely nesting and foraging resources, critical to the survival of endangered and threatened species.

Understanding resource use and preference is especially important for species that providing support services and are also declining globally such as pollinators (Potts et al. 2010; Winfree 2010). However, limited information on floral preference for most pollinator species restricts conservation efforts (Winfree 2010) and few studies have yet. To date, only a few studies have calculated resource preferences for pollinator species (see Kells, Holland, & Goulson 2001; Williams et al. 2010) and these used floral visitation records, which do not distinguish between pollen and nectar resources, and may be a poor proxy for resource availability (Vilà et al. 2009).

By identifying specific resources critical to pollinator survival, preference analysis can improve conservation. The utility of preference analysis has encouraged the growth of methodological and analytical techniques, but concerns about how to calculate and interpret preference estimates persist (Beyer et al. 2010; Johnson 1980; Manly et al. 2002). Identifying preference typically requires comparing use of a resource against its availability to assess whether non-random patterns of resource use occur (Manly et al. 2002). Therefore, inaccuracies in either measures of use or availability can greatly distort preference estimates a problem that is magnified for highly mobile organisms such as bees and birds.

In particular, how available resources are identified and measured can significantly alter preference estimates. As demonstrated by Johnson (1980), excluding a single resource from a preference analysis dramatically shifted the availability of all remaining resources and thus the preference estimates resulting in a resource switching from preferred to avoided. The choice to include or exclude resources from the analysis, however, is often made by the researcher and can differ from study to study. For example, identifying available resources for pollinators requires understanding both what resources are accessible and accurately quantifying the resource. Resources for pollinator are commonly quantified at the flower level but that is not representative of the resource actually being used, nectar or pollen. However, to date, no studies have examined whether different measures of availability produce different or better estimates of preference for any species.

Methods have been developed to standardize availability data for preference analysis (Johnson 1980; Manly et al. 2002). However, because calculation of preference requires a measure of availability, despite standardization, any variability in the measure of availability will influence the estimation of resource preference. Additionally, pollinators may actively seek

certain resources regardless of their availability (Williams & Kremen 2007) which would dramatically influence the preference estimate. Thus, although availability is commonly considered necessary to estimating preference, removing availability from preference estimation could more accurately represent resource preference across multiple sites and individuals. Removing availability data could thus improve the generalizability of preference estimates, but, to date, no calculations of preference have ever been made without reference to availability, nor compared to calculations using availability.

In this paper, we investigate three alternative models of resource preference using empirical data on bee foraging. First, we measure use and availability at the pollen level to determine if bees demonstrate non-random use of pollen resources. Bees are known to be more selective of pollen, the primary larval food and more important to fecundity, than nectar resources (Cane & Sipes 2006; Wcislo & Cane 1996). Second, we estimate pollen preference using availability measured at the pollen, flower and inflorescence level, to identify the most predictive scale for determining resource preference. Lastly, we estimate pollen preferences without using any availability data to determine if this produces better estimates than availability estimates. We hypothesize that bees will collect pollens non-randomly, preferences estimated using pollen-level availability measures will be superior to preferences estimated with coarser scales of availability and exclusion availability data will improve preference estimates.

## Methods

### *Site description*

During 2009, five one-hectare grassland sites were chosen in Briones East Bay Regional Park and Mount Diablo State Park in Contra Costa County, CA. All sites were >1km apart to limit overlap in bees foraging in multiple sites. One to two sites were monitored each day during five bi-weekly sampling periods from mid-May to late July for the presence of the bumble bee, *Bombus vosnesenskii* Radoszkowski. Wind and temperature data were recorded at the beginning and end of each sample day, and sampling was only conducted when temperatures were between 15 and 32 degrees Celsius with wind < 8 m/s. When *B. vosnesenskii* was present, sites were sampled to obtain pollen use and pollen availability data (see below).

### *Organism*

*Bombus vosnesenskii* Radoszkowski 1862 was chosen as a model organism in this system. This species is known to be polylectic (Thorp, Horning, & Dunning 1983) and thus will make foraging choices based on preference instead of specialization. Specialized species would seek particular resources regardless of availability and would not permit accurate testing of hypotheses of the effect of availability measures nor of random collection. *B. vosnesenski* has a wide distribution, occurring along most of the western coast of North America (Stephen 1957), and is known to be an effective pollinator of crop species (Greenleaf & Kremen 2006; Kremen et al. 2004). Additionally, because *Bombus* species collect pollen into loads on their corbicula, the pollen can be sampled non-destructively, providing conclusive records of the pollen species that individual *Bombus* are actively collecting and in what proportions.



### ***Pollen Load Collection***

Bees were netted and then immobilized using a bee-squeezer to remove a single pollen load from their leg. A single load was removed as a marker to prevent recapturing the same bee. The pollen load was placed in a microcentrifuge tube, labeled and filled with 70% ethanol. A minimum of five bees were collected at a site with a mean of 15.4 bees captured per site.

In the lab, pollen loads were vortexed until homogenous and then sub-sampled using a micropipettor. The sample was mixed with glycerin and fuschin dye on a microscope slide. Pollen grains were then identified to plant species by comparing them to reference slides created from pollen collected directly from identified plant species. Three hundred pollen grains were identified to plant species at each site-date  $c_{ij}$ ; only records for which > 95% of grains could be identified were retained in the sample. We assume that presence of pollen in the corbicula load reflects active selection of those pollens unless behavioral observations indicated otherwise.

### ***Floral availability***

Fifty 1m<sup>2</sup> quadrats were evenly spaced along a grid throughout the site and sampled for flowering vegetation. Vegetation was sampled systematically to ensure equal sampling of the entire site. All flowers or inflorescences within the quadrats appearing to have receptive stigmas or productive anthers were counted. When inflorescence was the unit rather than flower, we also counted the average number of flowers per inflorescence for ten individuals and used that information to estimate the total floral availability at each site. This provides estimates of floral availability at both the inflorescence level and flower level. A list of all species blooming within a site was also recorded to account for any species not found within quadrats, so that they were not falsely recorded as having zero availability. If pollen of a plant species not included in quadrat sampling was collected, availability was adjusted to reflect a single inflorescence and scaled accordingly for pollen and flower.

### ***Estimating site level pollen availability***

To estimate pollen availability for all plant species blooming, average pollen production was multiplied by the floral availability found at a site, to provide an estimate of the amount of pollen available at each site.

### ***Average Pollen Production, $u_i$***

In order to estimate pollen production, 5 mature but unopened buds were collected opportunistically for each species. Buds were placed in water and allowed to open in the lab. After maturation, stamens were removed and placed in 100 ul of 70% ethanol. Forty-five  $\mu$ l of fuschin stain were added to each tube to stain the pollen. When necessary more ethanol was used to cover stamens or plant parts and measurements were later scaled to reflect this difference. Samples were vortexed to homogenize the sample and 10ul were prepared on slides. Each sample (one flower head or bud) was subsampled five times. Two photographs were taken under 80x magnification (some samples required higher magnification and were scaled accordingly) of each slide prepared.

Using a digital particle counter called ImageJ (NIH) we counted the number of pollen grains in each photograph (Costa & Yang 2009). A total of fifty photos (5 flower heads or buds x 5 subsamples x 2 photos/subsample) were analyzed per species and an average pollen production

per flower was calculated for each species,  $u_i$ . For some plant species, due to pollen density or size, magnification or concentration adjustments were made and then scaled accordingly prior to calculating average pollen production.

## Model

Assume that the pollen counts  $c_{ij}$  for each bee follow a Dirichlet multinomial (DM) distribution. The DM has  $D-1$  parameters to describe expected proportions and one parameter to describe variation between bees. The parameters of the DM allow determination of the mean and variance of the preferences for each plant species during a sample period.

The parameters of the DM can either be estimated directly or could arise from a model with availability data and preference parameters by setting the mean or changing one of the values used to determine the mean through the below relationship:

$$\mu_i = \frac{p_i a_{ij}}{\sum_{i=1}^D p_i a_{ij}} \quad (1)$$

Where  $\mu_i$  is the mean use of the plant species,  $p_i$  is the preference parameter for each plant species, and  $a_{ij}$  is the availability of plant species  $i$  at site  $j$ . Because preferences in this situation are only meaningful when compared to one another, then we can assume that  $\sum_{i=1}^D p_i = 1$ . Then using Maximum Likelihood Estimation (MLE) we can determine the estimates of  $p_i$  and  $\mu_i$  that produce the fit to the data, as it is constrained or altered through the relationship identified by equation 1.

We fit three different models using these methods to address our three questions.

### 1. *Use=Availability*

The first model determines if bees show no preference (i.e. mean pollen use,  $\mu_i$ , is equivalent to pollen availability,  $a_{ij}$ ). In this model, equation one simplifies to  $\mu_i = \frac{a_{ij}}{\sum_{i=1}^D a_{ij}}$  and only the variation parameter needs to be estimated. Then using these parameters we can calculate the likelihood of this model.

### 2. *Use=Preference\*Availability*

In the second model, availability is a measured variable and preference is unknown. To determine the preference parameters ( $p_i$ ) that produce  $\mu_i$  estimates with the maximum likelihood, all possible values of  $p_i$  were input into equation 1,  $\mu_i$  values were calculated and likelihood estimates were determined. Because bees may respond strongly to visual and olfactory cues of plants (Burger, Dötterl, & Ayasse 2010; Campbell et al. 2010) it is possible that preference is better determined by plant or inflorescence availability as opposed to pollen availability. Thus, we create three sub-models to identify preferences ( $p_i$ ) and mean ( $\mu_i$ ) with availability measures of pollen, flower and inflorescence.

### 3. *Use*

For the third model, we calculated  $\mu_i$  and the variance parameter directly without availability data. In this model, the distribution is determined by the pollen used and is not constrained by availability as in model one or by preference as in model 2.

The goodness-of-fit for each model, was determined by parametric bootstrapping observed data, the likelihood was recalculated for each bootstrapped dataset to create distribution

of likelihoods and determine where the observed likelihood value falls within the distribution. To compare the models, parametric bootstrapping of the likelihood-ratios was used. Models were compared in order presented here. Only models using the same type of availability data can be compared using this method. Due to the size of the data set and the number of parameters being estimated, parametric bootstrapping was more appropriate for estimating fit and p-values than traditional goodness-of-fit methods which assume a large sample size.

In total 232 bees were captured during the study. Bees for which more than 5% of the pollen counted was from plants not occurring at the site were removed, leaving 227 bees for analysis. Sites were grouped by their sampling period for analysis, 3-4 sites per sampling period. Sampling periods were found to have significantly more floral species similarity within periods than between sampling periods when compared using multiple response permutation procedure ( $A=0.122$ ,  $p=0.05$ , see Figure 1) (McCune & Grace 2002) which suggests this was an appropriate grouping of sites.

Individual bees typically collected pollen primarily from one plant species with small amounts from other plant species and variability among pollens collected by bees within the same site was high. The Dirichlet multinomial distribution, a generalization of the beta-binomial distribution, is commonly used for compositional data with high variability like the data collected here. This method is similar to the popular method for compositional data used by Aebischer, Robertson, & Kenward (1993) to determine habitat preference for radio tracking data. The DM, however, is more appropriate for our data because it is better able to handle missing or zero use data (Aitchison 1986) and has previously been used for compositional paleopollen analysis (Mosimann 1963; Paciorek & McLachlan 2009).

## Results

Fifteen of the available twenty plant species were collected by *Bombus vosnesenskii* supporting previous findings that *B.vosnesenskii* is a generalist forager. Eighty-three percent of bees captured carried more than one pollen type and thus compositional analysis was most appropriate for this data.

The first model of no preferences (i.e. use = availability) was a poor fit to the data in 3 of 4 measurable sample periods ( $GOF \leq 0.005$ , Table 1). The second model (i.e. use=preference\*availability) was superior to the first model in all sample periods ( $p < 0.005$ ) and for all measures of availability data (pollen, flower and inflorescence). The model based on inflorescence availability had equivalent (4 of 5 cases) or better likelihood values during all sampling periods than the models using either pollen or flower availability data (see Table 2). The third model (Use), however, had equivalent or better likelihoods in 4 of 5 sample periods than all models with availability (Table 2).

Pollen preferences calculated by Model 3, the model with equivalent or better likelihood values in most cases, found that *Vicia villosa* and *Eschscholzia californica* were the most highly preferred plant species during the study (Table 3). Preferences determined using Model 2 based on inflorescence availability were notably different than preferences determined using Model 3.

## Discussion

Identifying resource preference is important for targeting key resources needed to conserve and restore declining species (Cook, Morgan, & Marshall 2010; Winfree 2010) and for understanding their behavior and movement ecology in response to resource distributions that

vary over space and time (Manly et al. 2002). To date, only a few studies have examined whether pollinators have preferences for specific floral resources (Kells, Holland, & Goulson 2001; Williams et al. 2010). We found that *B.vosnesenskii*, a generalist forager, did display strong preferences for particular pollen species, since our first model, which tested whether pollen use by bumble bees was equivalent to pollen availability, was a poor fit to the data and significantly worse than all preference models. This finding supports previous work that bees are highly selective of pollen resources, which they collect for larval provisions (Cane & Sipes 2006; Robertson 1929).

Further, while the terms preference and selection of floral resources are often used with reference to pollinators, they have not been quantified using methods analogous to those used to identify resource preferences for other animal species. We measured the availability of pollen directly, expecting that it would prove superior for preference analysis. However we found that availability measured at the plant and inflorescence level produced better likelihood estimates than the direct measurements of pollen availability. Our results suggest that fine scale measurements of pollen may not be necessary and that inflorescence level analysis may be adequate to estimate preferences for bee species. Inflorescence level availability may better reflect visual and olfactory cues that have been previously shown to be important to bee visitation (Burger et al. 2010; Campbell et al. 2010)(Burger, Dötterl, & Ayasse 2010; Campbell et al. 2010). It is also possible that our estimate of availability, which was based on newly opened buds, did not adequately capture the pollen availability or variability in ambient conditions. Some previous work has shown that bumble bees can discriminate between flowers of the same species based on pollen quantity (Robertson et al. 1999), so although preference models using pollen availability were a worse fit than those based on floral or inflorescence availability, pollen availability may still be a significant factor in determining which flowers within a plant population are visited.

While commonly used for identifying habitats and resources for conservation, preference estimates could be skewed by measures of availability and limited in their generalizability across sites or habitats, because they are calculated in a context-specific fashion (Beyer et al. 2010; Johnson 1980; Manly et al. 2002). Therefore, it may be unsurprising that our third model which excludes availability data was as good as or better than models including availability (in 4 out of 5 measurable cases). This result suggests that innate preferences are better estimated by use data alone. We caution that this finding needs further testing as it may not hold in all study systems; in addition, we found substantial variability in the goodness-of-fit (ranging from .09-.84) which suggests this method is not consistently a good estimator of use. We note that it would be comparatively easy to test whether the estimation of preferences in the resource selection literature are improved by omitting availability data, since it requires no new information, but simply an appropriate statistical comparison of models with and without availability data. If this finding proves robust in other systems, it implies that preference analysis could be more readily applied to species for which resource availability is unknown or difficult to quantify such as cryptic or highly mobile species.

The preferences estimated in Model 3 suggest that, while it is often assumed native plants are a superior resource for native bees (Kearns, Inouye, & Waser 1998; Potts et al. 2010), invasive plants such as *Vicia villosa* (vetch) and *Centaurea solistitalis* (yellow star thistle) can be highly preferred. It is interesting to note that *V. villosa*, the most preferred species during two sampling periods, is the subject of biological control efforts in some ecosystems (Baraibar et al. 2011). Our preference analysis suggests that efforts to remove invasive plant species could

impact preferred foraging resources for *B. vosnesenskii* and might adversely affect bee presence and persistence in some areas if suitable resources are not available. Therefore, when removing invasive species, effort should be made to replace them with highly preferred species such as *Mimulus guttatus* and *Eschscholzia californica*. While early season resource preferences were dominated by invasive species, late season resources included more native plant species despite high availability of invasive species. This may suggest that when producing reproductive individuals the colony is more selective of resources. Further analysis is needed to understand factors influencing preference such as nutrition and morphology (Rasheed & Harder 1997; Roulston, Cane, & Buchmann 2000) and the inclusion of invasive plant species into native bee diets (Stout & Morales 2009).

Many conservation efforts for pollinators focus on providing a suite of floral resources, such as the installation of hedgerows into agricultural land (Winfree 2010), but selection of these plants are often based on visitation records (Frankie et al. 2005; Menz et al. 2010) which may misrepresent the importance of some plant species to pollinator fecundity and survival. Separate records of pollen and nectar collection would help distinguish between preferred pollen resources and incidental nectar foraging. Identifying pollen preferences is especially important for *Bombus* species which are declining globally due to their low effective population size, high resource demands and sensitivity to habitat degradation (Cameron et al. 2011; Goulson, Lye, & Darvill 2008). Some species declines have been directly correlated to narrow pollen use (Kleijn & Raemakers 2008) and diet breadth (Goulson et al. 2005). Additionally, because bumble bees have greater floral demands, due to their large colony size and long flight period, than many other bee species it is suggested that conservation efforts targeted for them will also benefit other bee species (Goulson et al. 2008). We suggest expanding the use of preference estimation to help target conservation efforts for pollinators. Future studies should compare preference estimates based on visitation records versus pollen use to determine if preference estimates are similar using both methods for assessing use.

## Tables

Table 1. The goodness-of-fit values for each model based on pollen availability data. Asterisks indicate significance of GOF compared to the model to the left ( $p < 0.01$ ). Due to limited floral similarities between the four sites in sampling period three, bootstrapping was not possible to produce GOF and p-values.

	$\alpha$	<b>Model 1. Use=Availability</b>	<b>Model 2.Use=Preference and Pollen Availability</b>	<b>Model 3. Use=Preference</b>
<b>Sampling period 1</b>	8	GOF<0.005	GOF<0.005*	GOF=0.55*
<b>Sampling period 2</b>	9	GOF=0.18	GOF=0.375*	GOF=0.42
<b>Sampling period 3</b>	8	-	-	-
<b>Sampling Period 4</b>	7	GOF<0.005	GOF<0.005*	GOF=0.51*
<b>Sampling Period 5</b>	8	GOF<0.005	GOF=0.305*	GOF=0.33*

Table 2. Negative Log-Likelihood values for Model 2 with different types of availability and for Model 3.  $\alpha$  is the number of parameters estimated. Smallest values are bolded and suggest better fit. Italicized values were not significantly different from bolded value for goodness-of-fit.

	$\alpha$	Model 2.Use= Preference* Pollen Availability	Model 2.Use= Preference* Flower Availability	Model 2.Use=Preference * Inflorescence Availability	Model 3.Use=Preference
<b>Sampling Period 1</b>	8	232.8	211.3	211.6	<b>207.1</b>
<b>Sampling Period 2</b>	9	<b>127.6</b>	129.3	128.2	<i>127.7</i>
<b>Sampling Period 3</b>	8	247.2	247.2	<b>234.4</b>	263.8
<b>Sampling Period 4</b>	7	212.3	212.8	212.7	<b>195.7</b>
<b>Sampling Period 5</b>	8	102.9	104.6	102.0	<b>97.6</b>

Table 3. Preference estimates for Model 2 and Model 3

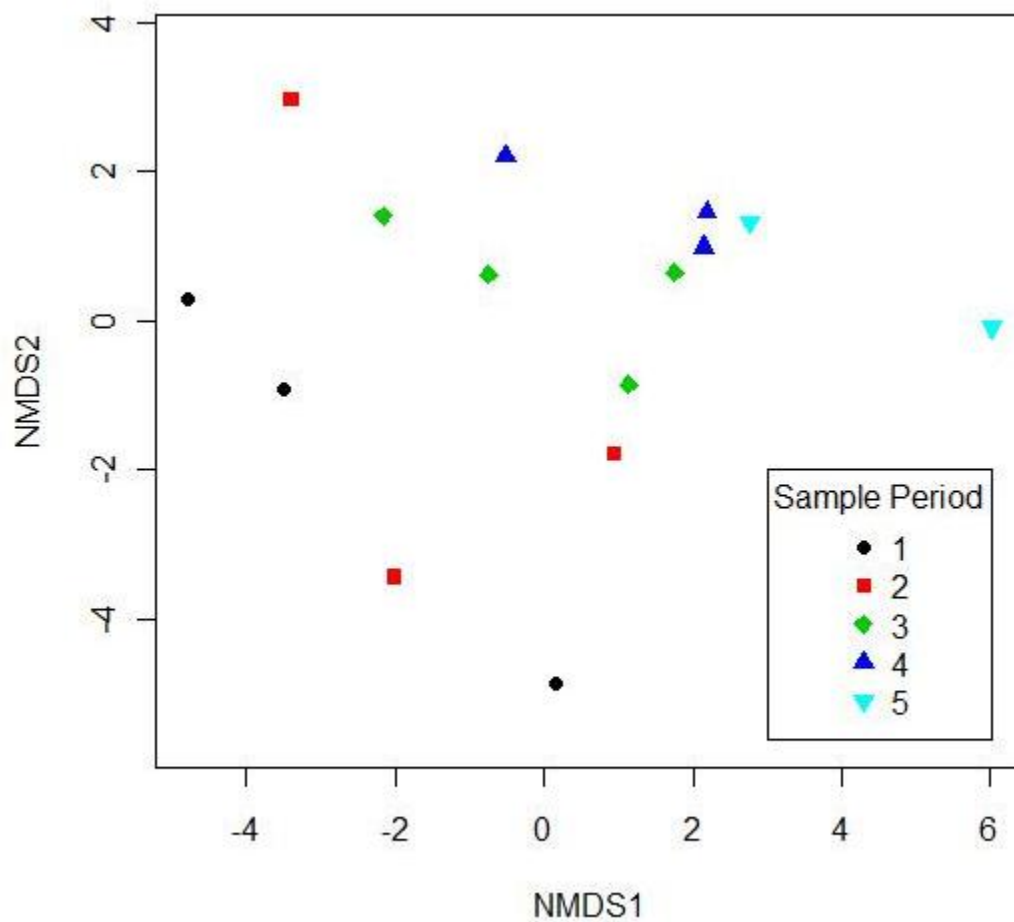
	<b>Plant Species</b>	<b>Model 2. Inflorescence</b>	<b>Model 3. Use</b>
Sample Period 1	<i>Vicia villosa</i>	0.8802	0.7423
	<i>Mimulus guttatus</i>	0.0703	0.1047
	<i>Eschscholzia californica</i>	0.0086	0.0596
	<i>Lupinus microcarpus</i>	0.0316	0.0473
	<i>Trifolium oliganthum</i>	0.0084	0.0452
	<i>Brassica nigra</i>	0.0010	0.0008
Sample Period 2	<i>Vicia villosa</i>	0.0624	0.7801
	<i>Eschscholzia californica</i>	0.1905	0.1605
	<i>Lupinus microcarpus</i>	0.0493	0.0181
	<i>Mimulus guttatus</i>	0.0884	0.0179
	<i>Silybum marubium</i>	0.2603	0.0167
	<i>Trifolium oliganthum</i>	0.3489	0.0059
	<i>Brassica nigra</i>	0.0003	0.0008
Sample Period 3	<i>Eschscholzia californica</i>	0.5321	0.4488
	<i>Centaurea solistitalis</i>	0.0926	0.3281
	<i>Vicia villosa</i>	0.3355	0.1948
	<i>Brassica nigra</i>	0.0020	0.0192
	<i>Lupinus microcarpus</i>	0.0379	0.0091
Sample Period 4	<i>Eschscholzia californica</i>	0.7850	0.7558
	<i>Centaurea solistitalis</i>	0.0127	0.1734
	<i>Lupinus microcarpus</i>	0.0763	0.0676
	<i>Vicia villosa</i>	0.1259	0.0031
	<i>Brassica nigra</i>	0.0001	0.0002
Sample Period 5	<i>Eschscholzia californica</i>	0.6728	0.4548
	<i>Eriogonum spp.</i>	0.2495	0.3256
	<i>Centaurea solistitalis</i>	0.0014	0.1096
	<i>Holocarpha hermanii</i>	0.0053	0.1024



<i>Vicia villosa</i>	0.0705	0.0066
<i>Brassica nigra</i>	0.0003	0.0005
<i>Mimulus guttatus</i>	0.0003	0.0004

## Figures

Figure 1. Non-metric dimensional-scaling plot of floral species similarity of sites grouped by sampling period. Each point is an independent site-date, color-shape combinations indicate sampling period. NMDS(dims=2, stress=8.753)



## Chapter 2

### Untangling the paradox: the use of non-native plants by native bees

#### Introduction

The spread of non-native plant species can homogenize plant communities and threatens the biodiversity of ecosystems globally (Pimentel et al. 2001). By reducing native plant diversity and altering plant communities, non-native plant species can lead to declines of species, such as pollinators, that provide valuable ecosystem services (Aizen & Morales 2006; Morón et al. 2009). Declines of pollinators could threaten the sexual reproduction for the estimated 90% of flowering plant species that require or benefit from animal mediated pollination (Allen-Wardell et al. 1998; Ashman et al. 2004; Potts et al. 2010). Despite the presumed link between pollinator declines and the spread of non-native plant species, few studies have examined the direct effects of non-native plants on native pollinators and a recent review on the impact of non-native plants on native bees found both positive and negative effects (Stout & Morales 2009).

There are two major assumptions about native pollinators' use of non-native plants: 1) non-native plants share a suite of characteristics that affect their use by native pollinators and 2) the origin of a plant species is a significant factor in determining whether a plant species is attractive to pollinators. Non-native plants, however, are extremely taxonomically diverse and thus may not be morphologically similar. While non-native plant species do differ as a group from native species in functional traits related to growth (van Kleunen, Weber, & Fischer 2010), these traits may have little bearing on floral or resource traits that influence pollinator visitation and use. When pairing native and non-native species by family or genus, non-native species were not consistently different from native species in pollinator visitation, autogamy or pollen limitation (Harmon-Threatt et al. 2009) suggesting that origin is not a significant factor in determining attractiveness to pollinators.

Features of plants and pollinators other than origin, however, influence pollinator visitation and use. Plant features such as floral morphology, protein content, amino acid content and availability are known to influence pollinator use (Hanley et al. 2008; Harder 1990; 1986; 1985; Wcislo & Cane 1996); therefore it would be useful to determine whether such traits differ between native and non-native plants. One limitation in distinguishing between the effect of origin on pollinator visitation and use is that researchers rarely record which native plant species are available but not collected by pollinators. Additional information on native plants that are available but not collected could provide valuable insight into the similarities between species that are collected and whether origin is a significant factor in determining collection.

In this paper we examine traits that could affect pollen collection by a native bumble bee *Bombus vosnesenskii*. We focus on pollen collection because bees are known to be more highly selective of pollen than nectar resources (Cane & Sipes 2006) and thus non-native plants may be less likely to be included in pollen diets compared to nectar diets. We examine four traits that could influence pollen selection by bees: floral morphology, essential amino acid content, protein content and per inflorescence pollen reward. We asked: 1) Are non-native plant species significantly different than native plant species for traits that may affect pollen collection? and 2) Are plants whose pollens are collected significantly different from plants whose pollens are not collected? We hypothesize that plants grouped by origin will not be significantly different for

traits that affect pollen collection but that plant species that are collected by *B.vosnesenskii* will be significantly different than non-collected species.

## Methods

### *Site description*

During 2009, five one-hectare grassland sites in Briones East Bay Regional Park and Mount Diablo State Park in Contra Costa County, CA were sampled to identify preference of the bumble bee *B. vosnesenskii* for different floral resources (see Harmon-Threatt et al. chapter 1). During 2009 and 2010 these sites were revisited to measure plant characteristics that may influence visitation to all blooming species. Plant species were classified as collected or not collected based on pollen load records from *Bombus vosnesenskii* recorded during the 2009 sampling year (See Harmon-Threatt et al. Chapter 1). Species were classified as native or non-native using USDA categorization (USDA 2010).

### *Morphological measurements*

All plant species that were present in sites when bees were present were identified and measured. Ten flowers from different individuals were chosen randomly and measured for each species. To maintain consistency, corolla length was measured from the top of the corolla to the top of the pedicel and corolla width was measured across the widest part of the flower. Flower height was measured from the topsoil to the top of the flower being measured. Color, number of flowers per inflorescence, and symmetry were also recorded. Color and symmetry were excluded from analysis because as ordinal variables they could not be analyzed using the same method.

### *Pollen quality*

Pollen contains both protein and free amino acids necessary for bee larval maturation (Roulston & Cane 2000) and pollen of poor quality can affect size, development of hypopharyngeal glands, and ability to reach the adult stage, which can have significant demographic consequences (Herbert, Bickley, & Shimanuk 1970; Peng & Jay 1976; Roulston & Cane 2002). To determine protein and amino acid content of pollen, flowers of all blooming species were collected from field sites and placed in water in the lab. After at least 24 hours, each flower was vibrated with a 512Hz tuning fork (Kearns & Inouye 1993) to collect pollen into Petri dishes. After collection, Petri dishes were placed in a drying oven for at least 48hrs at 40 degrees Celsius. Samples were stored in a -20 degree Celsius freezer for subsequent processing.

Dried samples were cleaned of all plant and insect debris. Pollen samples were then placed in small tin foil packets and divided into individual samples weighing between 1 and 5mg. Percent carbon and nitrogen of pollen was determined by combusting five samples of each plant species. Measurements of percent nitrogen from combustion are highly correlated with measurements of protein using other analysis techniques and are therefore considered an appropriate method to estimate the amount of nitrogen and thus protein available in pollens (Roulston, Cane, & Buchmann 2000). Previous analyses of pollen nutrition have used percent protein or nitrogen as the statistic to determine nutrition (Roulston & Cane 2000). We elected to use carbon:nitrogen ratio for analysis because carbon can contribute significantly to the weight and size of pollen and reduce the amount of nitrogen received per volume of pollen consumed by larvae (see Table 1).

Pollen samples weighing >25mg for each plant species were also analyzed for amino acid composition (at the UC Davis Molecular Structure Facility). The nine essential amino acids, threonine, lysine, methionine, valine, isoleucine, phenylalanine, histine, arginine and tryptophan, identified by DeGroot (1954) for honeybees and previously used for bumble bee pollen analysis (Hanley et al. 2008), were analyzed. Samples were processed using a Na citrate-based hydrolysis analyzer. A second sample analysis was used to determine cysteine and methionine using performic acid because these amino acids are destroyed in the hydrolysis process (see Table 2). Tryptophan could not be reliably quantified and was removed from the analysis.

### ***Floral reward***

Previous studies have shown that bumble bees can discriminate between flowers with different amounts of available pollen (Robertson et al. 1999). Therefore, the amount of pollen available in an inflorescence was estimated by counting the amount of pollen available in a fresh flower (see methods Harmon-Threatt, Chapter 2) and scaling that to the number of flowers in an inflorescence.

### **Analysis**

To compare the difference between plant species grouped based on origin or whether they were collected, we used t-tests whenever single continuous factors were compared (C:N ratio and per inflorescence reward), and a Multiple Response Permutation Procedure (MRPP) for factors that contain multiple non-independent measures (such as amino acid composition and morphological traits) (McCune & Grace 2002). MRPP is a non-parametric method to test for differences among two or more predetermined groups. Using a pairwise Euclidean distance matrix of the entire dataset, MRPP calculates mean within-group distance ( $\delta$ ) for the observed groups and then permutes group membership and pairwise distance and the recalculates  $\delta$  for each permutation. Significance of the observed  $\delta$  is determined by the percentage of permuted  $\delta$ 's less than the observed. MRPP is considered a superior test to MANOVA for community ecological data (McCune & Grace 2002). The effect size of MRPP is described by the chance-corrected within group agreement (A). All MRPP analyses were conducted in R 2.10.1 with the vegan package.

### **Results**

Non-native species and native species were not significantly different for morphology nor essential amino acid composition ( $A = 0.009$ ,  $p = 0.335$  and  $A = -0.016$ ,  $p = 0.593$  respectively). Native and non-native plants also did not exhibit significant differences for per inflorescence reward ( $t = 0.384$ ,  $df = 9$ ,  $p = 0.867$ , Fig 1A) nor C:N ratio ( $t = -0.077$ ,  $df = 9$ ,  $p = 0.709$ , Fig 2 A)

When plant species were grouped by whether their pollens are collected or not collected, the groups differed significantly for essential amino acid composition ( $A = 0.068$ ,  $p = 0.047$ ), per inflorescence reward ( $t = 4.625$ ,  $df = 9$ ,  $p = 0.001$ , Fig 1B) and carbon nitrogen ratio ( $t = -2.187$ ,  $df = 9$ ,  $p = 0.056$ , Fig 2B), but not morphological characteristics ( $A = -0.063$ ,  $p = 0.901$ ).

### **Discussion**

The spread of non-native species can disrupt native plant-pollinator webs and may contribute to pollinator declines (Aizen & Morales 2006; Moroń et al. 2009). However, non-

native plants can be incorporated into diets of native pollinators and in extreme cases are the sole food source for pollinator species (Nienhuis, Dietzsch, & Stout 2009; Stout & Morales 2009). Although it is assumed that non-native plants are different from native plant species in traits that influence use and visitation, we did not find non-native species consistently different than native species for any of the features measured including those regarding nutrition.

Instead, as hypothesized, species that were collected by *Bombus vosnesenskii* provided significantly different per inflorescence reward, essential amino acid content and C:N ratio than plants not collected. This suggests that bees discriminate among resources by nutritional characteristics and availability but not by origin. Previous studies have examined the protein content of plant species collected by numerous different bee species (Roulston et al. 2000) but this assumes all bees have the same protein, species and morphological preferences and thus ignores preferences of each individual species, which can strongly influence pollen collection (Williams 2003). By focusing on a single bee species we can better understand the role of protein as well as other features in pollen collection.

In contrast, the lack of difference between collected and non-collected plants for morphological characters may be because of the focal pollinator species' ability to manipulate flowers. As a large bodied species, *B. vosnesenskii* is capable of opening flowers and removing pollen from flowers that might otherwise be unavailable. For example, *Vicia villosa* is a small narrow corolla flower but is highly collected by *B. vosnesenskii* which pulls open the flowers to access the pollen. Additionally, while foraging efficiency of bumble bees has been linked to the matching of corolla length and tongue length (Harder 1985; Harder 1983; Peat, Tucker, & Goulson 2005), this relates more closely to probing for nectar collection than pollen collection and thus it is unlikely that corolla length influences pollen collection. Thus, the non-significant effect of morphology on collection is not surprising for this species but may not be the case for smaller pollinators that cannot manipulate flowers as easily.

The collection of non-native pollens suggests that some non-native plant species can provide adequate resources for some native bees. While there are numerous other consequences of the spread of non-native plant species, such as the homogenization of plant communities, the use of non-native species by some pollinators should be considered when efforts to restore native plants require widespread eradication of non-native plants that may be sustaining pollinators (Nienhuis, Dietzsch, & Stout 2009).

Although it is often believed that restoring native plants in general is adequate to prevent pollinator declines, this study suggests that not all native plants provide necessary resources to native pollinators and that care should be taken to ensure that plants that are chosen for pollinator restoration are not only native but also collected by native pollinators. This could be especially important for pollinator species that are known to be declining (Kleijn & Raemakers 2008). We suggest that future studies continue to explore the relationship between pollinator species and plant nutrition and origin.

## Tables

Table 1. Plant species and measured values compared using a t-test grouped either by origin or collection. \* indicates value was determined to be a group outlier and removed from analysis

Plant species	Plant Family	Origin	Collected	C:N ratio	log (per inflorescence pollen reward)
<i>Brodiaea elegans</i>	Lilliaceae	Native	No	8.103	2.738
<i>Clarkia purpurea</i>	Onagraceae	Native	No	14.669	1.998
<i>Lupins bicolor</i>	Fabaceae	Native	No	NA	1.824
<i>Eschscholzia californica</i>	Papveraceae	Native	Yes	7.729	4.171
<i>Lupinus microcarpus</i>	Fabaceae	Native	Yes	6.043	3.688
<i>Mimulus guttatus</i>	Scrophulariaceae	Native	Yes	8.929	3.003
<i>Brassica nigra</i>	Brassicaceae	Non-native	No	8.044	2.182
<i>Carduus pyncocephalus</i>	Asteraceae	Non-native	No	11.956	1.784
<i>Silybum marubium</i>	Asteraceae	Non-native	No	10.424	4.175*
<i>Centaurea solistitalis</i>	Asteraceae	Non-native	Yes	9.245	3.665
<i>Trifolium oliganthum</i>	Fabaceae	Non-native	Yes	8.148	2.827
<i>Vicia villosa</i>	Fabaceae	Non-native	Yes	7.460	3.076

Table 2. Essential amino acid composition of plants in study

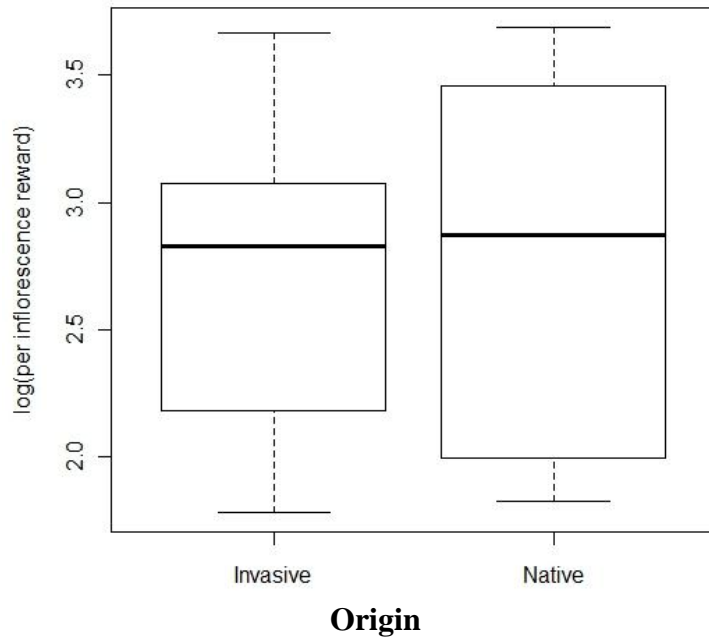
Species	Threonine	Valine	Methionine	Isoleucine	Phenalanine	Histine	Lysine	Arginine
<i>Brodiaea elegans</i>	1.65	2.00	0.27	1.46	1.15	0.62	2.20	1.28
<i>Clarkia purpurea</i>	0.65	0.79	0.11	0.53	0.23	0.31	0.81	0.44
<i>Lupinus bicolor</i>	0.64	0.75	0.09	0.58	0.51	0.26	0.86	0.49
<i>Eschscholzia californica</i>	0.98	1.15	0.12	0.84	0.70	0.43	1.28	0.68
<i>Lupinus microcarpus</i>	0.77	0.56	0.12	0.70	0.30	0.36	1.10	0.60
<i>Mimulus guttatus</i>	0.66	0.88	0.10	0.56	0.49	0.31	0.84	0.54
<i>Brassica nigra</i>	0.66	0.77	0.08	0.49	0.41	0.24	0.92	0.45
<i>Carduus pyncocephalus</i>	0.44	0.52	0.15	0.42	0.17	0.31	0.75	0.27
<i>Silybum marubium</i>	0.51	0.32	0.11	0.44	0.15	0.44	0.81	0.31
<i>Centaurea solistitalis</i>	2.09	2.54	0.41	1.78	0.86	1.45	3.19	1.39
<i>Trifolium oliganthum</i>	0.61	0.84	0.10	0.55	0.45	0.23	0.78	0.42



## Figures

Figure 1. Boxplots of per inflorescence reward when plant species were grouped by origin or whether pollen was collected by *Bombus vosnesenskii*.  
*Per inflorescence pollen reward*

A)



B)

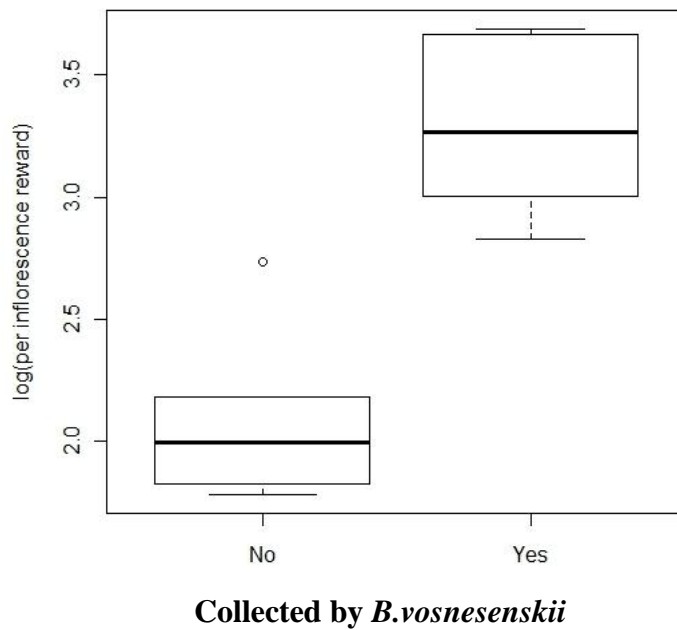
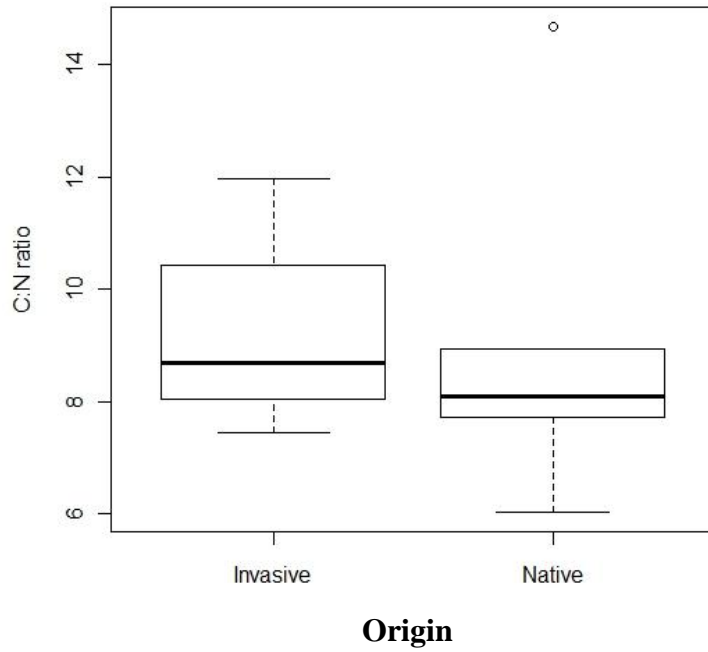


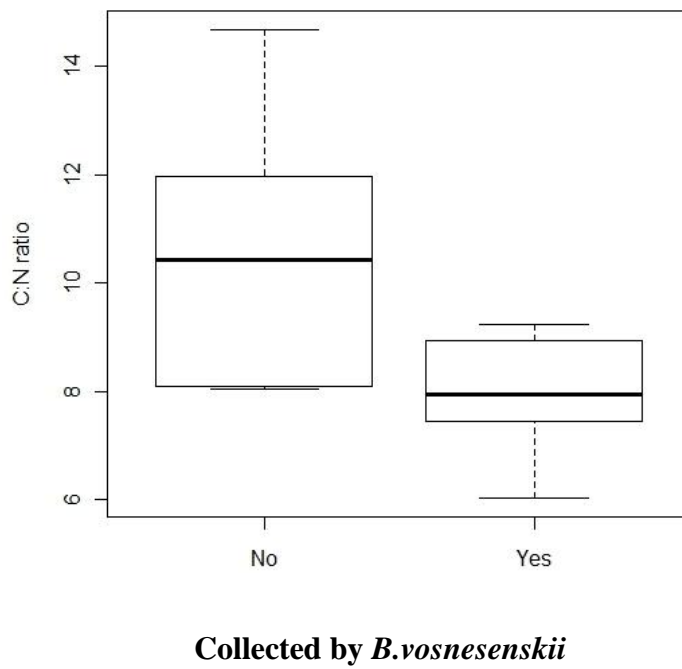
Figure 2. Boxplot of C:N ratio when plant species were grouped by origin or whether pollen was collected by *Bombus vosnesenskii*.

*Carbon Nitrogen Ratio*

A)



B)



## Chapter 3

# Community structure of *Bombus*: The influence of relatedness and tongue length

### Introduction

Competition, predation, abiotic conditions and resource availability are some of the many factors that determine a species distribution and occurrence in ecological communities. How multiple species respond to these same factors can determine what species and morphological traits are included or excluded from a community. Community ecologists and evolutionary biologists have long tried to understand and explain community patterns sparking numerous theories of coexistence.

Recent work by Webb et al. (2002) encouraged bridging community ecology and evolutionary biology to understand patterns of species occurrence based on the interrelatedness of phylogeny, morphology and species assemblages. Incorporating phylogenies into community analysis allows more accurate interpretation of how communities assemble and the processes that govern community membership. One of the leading theories of community assembly is Diamond's (1975) assembly rules, which was later expanded by Keddy (1989; 1992) and others. The theory of assembly rules asserts that observed community composition is determined by two primary filters that affect community composition: habitat filters and biological filters. Habitat or abiotic filters can limit community composition by requiring certain traits for survival in a community. Species that pass through the habitat filters then interact with each other to compete for resources and thus are filtered again based on biological interactions. This concept has recently been paired with phylogenetic analysis to determine the traits and relatedness of communities. Various methods have developed to analyze the relationships between phylogeny, traits and species assemblages (Blomberg, Garland, & Ives 2003; Ives & Helmus 2010; Kraft & Ackerly 2010; Pagel 1994; Webb 2000) and are frequently used to understand the genetic and ecological constraints on traits. These methods can also help explain how communities respond as a result of the interaction between traits and relatedness. However, the use of phylogenies to understand animal communities is still constrained by a lack of complete phylogenies (especially for insects), and the difficulty of identifying communities for mobile organisms (but see Blomberg, Garland, & Ives 2003; Ives & Helmus 2010). Using phylogenetic methods can provide new insight into patterns of community assembly which may be particularly useful for species or communities that are in decline.

In 2007, a comprehensive phylogeny of bumble bees (*Bombus*) including 219 of the approximately 250 described species globally was published (Cameron, Hines, & Williams 2007) offering a unique opportunity to look at the community assembly of a group that is both important for pollination of many plant species and known to be in decline (Goulson, Lye, & Darvill 2008a). Additionally, recent global declines of bumble bees have been linked to narrow diet breadth (which may be related to restrictions in foraging caused by tongue length) and range size, among other factors, and are observed disproportionately in some subgenera, suggesting a link between relatedness and susceptibility to decline (Goulson, Lye, & Darvill 2008a; Goulson, Lye, & Darvill 2008b; Kleijn & Raemakers 2008; Williams & Osborne 2009; Williams, Colla, & Xie 2009). Further, as the only native eusocial bees in North America and often among the largest species in a community, bumble bees are assumed to exhibit stronger intra-generic

competition, due to the high resource demand to support colonies, thus permitting analysis of intragenus community assembly. The phylogeny can also be used to test existing theories regarding how bumble bees partition resources and assemble communities. Thus, we can use the *Bombus* phylogeny to test existing theories regarding resource partitioning and community assembly.

Resource partitioning for bumble bee communities has long been linked to tongue length (Hanski 1982; Inouye 1977; Pyke 1982) which has been shown to affect handling efficiency of flowers and extracting nectar (Harder 1983; Ranta 1983; Ranta & Lundberg 1980). Tongue length in bumble bees is also strongly correlated with wing length and other morphological characteristics that can affect foraging and competition (Medler 1962; Morse 1977a). In some communities, tongue lengths of bumble bee species were significantly different and believed to help limit competition for resources (Inouye 1977; Pyke 1982). Resource partitioning and community assembly based on tongue length, however, has also been called into question by various researchers and in European communities tongue length was found to be more similar than expected when compared to randomly created communities (Pekkarinen 1984; Ranta 1982; 1984) which suggests that tongue length alone may not explain community composition for bumble bees.

Temperature tolerance is also a significant factor for distinguishing subgenera or bumble bees and could also influence community composition (Williams et al. 2008). Thus, two factors that may influence *Bombus* community assembly are how tongue length correlates with the phylogeny and how abiotic filters influence the relatedness of community members. To date, neither tongue length nor relatedness of *Bombus* has been tested for their role in community assembly using phylogenetic community methods. Additionally, the relationship between local community relatedness and the relatedness of the regional community has also not been tested. If regional communities are closely related, this limits the possible diversity at the local community and thus can also influence trait patterns as well.

Assembly theory would suggest that co-occurring species would limit similarity to maintain coexistence. Limiting similarity, however, could be achieved by limiting phylogenetic or trait similarity (or some combination of the two) depending on trait conservatism and community relatedness caused by habitat filtering. Using the *Bombus* phylogeny and information on communities, distributions, and tongue lengths, we were interested in 3 questions related to community assembly in *Bombus* communities: 1) Does tongue length show significant patterns of phylogenetic conservatism? 2) Are there non-random patterns of tongue length or relatedness among co-occurring species in *Bombus* communities? 3) Do patterns of similarity of tongue length in regional communities inform patterns in local communities?

## Methods

### *Data collection*

*Bombus* tongue length data were collected through literature searches in ISI Web of Science during the spring of 2009 using search terms: (Bombus or bumble\*) and (proboscis or tongue). Additional sources were acquired by searching literature cited by articles collected through the literature search. Data were quality controlled to ensure that tongue length was for the worker caste and measured directly as the sum of prementum and glossa (Harder 1985). If multiple records existed for a bee species, the weighted average of all records based on sample size of the original study was used. Species from subgenus *Psythirus* were removed from

community and trait level analysis because the group is primarily parasitic and does not have a worker caste, thus it does not partition resources or compete with other species in a similar manner to other *Bombus* species.

Bumble bee communities here are defined as species that co-occur spatially and are active during the same periods of the year in Nearctic areas of North America to ensure that species in a community are potentially competing for and partitioning resources or other niche axes. Communities were identified by contacting authors and researchers with survey data on pollinators or *Bombus* to acquire original databases on bumble bee species presence in Nearctic Areas. Sites were greater than one km apart to be considered distinct and bees were collected across the entire plant community to ensure bee species were not excluded by sampling a single plant species that may not be utilized by all bee species (Inouye 1978; Morse 1977b). If sites were sampled repeatedly, only the sampling date with the highest diversity, a proxy for highest potential competition, was chosen. Abundance data were excluded from the analysis because they were not available for all sites. Original data were required because data were typically pooled spatially or temporally when reported in publications.

To test for non-random patterns in observed communities, we identified regional species pools to compare against observed community phylogenetic distance and trait organization. Regional species pools were created based on equal area grid cells defined by Williams (1996). Each grid cell covers approximately 611,000 km of the earth's surface. Using DiscoverLife.com, a freely available database of global species occurrence, we determined the species that occurred in each grid cell within Nearctic areas of North America (hereafter Nearctic) and compared these to published records of species occurrence when possible. Only data points that had been verified by a taxonomist and had georeferenced location data were used from the Discover Life database.

## Analysis

All analyses were conducted in **R** 2.10.1 using the *picante* package (Kembel et al. 2010) with scripts written by the first author.

### *Trait Conservatism*

Using the ultrametric no gap phylogenetic tree published by Hines (2008), a time calibrated version of the Cameron et al. (2007) tree, we calculated Blomberg's K value, a metric for describing the distribution of phenotypic variation across the tips of a given phylogeny (Blomberg et al. 2003). Species for which we did not have tongue length data were removed from the phylogeny. A value of  $K=1$  is expected for a trait whose distribution matches the expectations for simple random-walk Brownian motion evolution. A value of  $K>1$  suggests high trait conservatism (i.e. related species are more similar than expected based on Brownian motion) while  $K<1$  shows low trait conservatism. A tip-swap null model can be used to test for the presence of phylogenetic signal by comparing the observed K-value to 999 trees created by randomly shuffled taxon labels; the K value under this null model is very low, on the order of  $\sim 0.145$ .

We analyzed trait conservatism for all species with published trait data ( $n=76$ ) and those that occur in Nearctic regions ( $n=33$ ) separately to determine if there were differing rates of trait conservatism in the younger Nearctic areas used for community analysis.

### ***Phylogenetic Community Analysis***

Using the phylogenetic tree, a pairwise phylogenetic distance matrix can be created to calculate the branch lengths separating each species. Using the phylogenetic distance matrix we calculated two metrics for each community and null gridcell: 1) Nearest Taxon Index (NTI) and 2) Net Relatedness Index (NRI) as defined by (Webb 2000) and implemented in *picante* (Kembel et al. 2010). NTI calculates the phylogenetic distance between a species and the nearest related taxon in a community and provides analysis of phylogenetic clustering of closest relatives. NRI, in contrast, calculates the mean total phylogenetic distance separating all community members from each other and allows us to analyze the overall relatedness of the community members. NTI and NRI are both compared to 999 randomly generated communities of equal species richness selected from each observed communities regional species pool. Observed values are expressed as standard deviations from the null distribution, with positive values indicating phylogenetic clustering. Using a Wilcoxon signed-rank test to compare the observed values to the expected values generated by the null communities, we can look for trends across communities in NRI and NTI. The generation of null models for each observed community also allows for significance testing of each individual community.

### ***Trait-based Community Analysis***

Similarly, because NTI and NRI use a distance matrix to determine phylogenetic distance between species we can use a distance matrix based on trait distance to calculate NTI and NRI for the traits of species in a community. Using the tongue length data for Nearctic species we created a distance matrix of all species and calculated NRI and NTI using the same method above. This method results in mean trait distance ( $NRI_{\text{trait}}$ ) and mean nearest trait distance ( $NTI_{\text{trait}}$ ) for each community.

Secondly, we can look at how evenly the trait is spaced within the community by calculating the standard deviation of the nearest neighbor (SDNN) (Cornwell & Ackerly 2009; Kraft, Valencia, & Ackerly 2008; Stubbs & Wilson 2004). This method will allow us to determine if tongue length is consistently spaced along a trait axis in a way to limit competition within a site. For each observed community we sorted the tongue lengths of community members from lowest to highest, found the difference between the tongue lengths and the calculated the standard deviation of these differences. Communities with less than 3 species were removed because it is impossible to calculate a standard deviation with less than 2 values. We then tested whether the traits found in a community had more even trait spacing (a low standard deviation of the trait differences) compared to 999 null communities of the same size, to determine whether tongue lengths in a community are more evenly spaced than by chance. All observed communities were compared to the null means using the Wilcoxon signed-rank test.

### ***Regional Community Analysis***

To assess the underlying patterns of trait and relatedness in the Nearctic areas, we calculated the NRI and NTI using trait and phylogenetic distance matrices ( $NRI_{\text{trait\_regional}}$ ,  $NTI_{\text{trait\_regional}}$ ,  $NRI_{\text{phylo\_regional}}$ ,  $NTI_{\text{phylo\_regional}}$ ) for regional species pools compared to the entire Nearctic species pool.

## Results

### *Trait Conservatism*

We found a total of 17 articles with measured tongue length for 76 species globally and 33 species in the Nearctic (see Table 1). For the global sample, Blomberg's K for tongue length was 0.7245 while for Nearctic species  $K = 0.9151$  (see Figure 1). While both the global and Nearctic areas analysis revealed  $K < 1$ , indicating less trait conservatism than Brownian motion, they also both showed highly significant phylogenetic signal of the trait on the phylogeny, relative to the tip swap null ( $p < 0.001$ ).

### *Phylogenetic Community Analysis*

We identified 118 communities in 8 of the 46 grid cells in Nearctic Areas to analyze tongue length and relatedness across co-occurring species. Overall, observed communities were comprised of species that had lower nearest neighbor distance than nulls (Wilcoxon signed-rank test of  $NTI_{\text{phylo}}$ ,  $p = 0.050$ , Table 2). The net relatedness of observed communities exhibited a non-significant trend towards clustering (Wilcoxon signed-rank test  $NRI_{\text{phylo}}$   $p = 0.117$ , Table 2).

### *Trait Community Analysis*

For the same 118 observed communities above, the trait analysis revealed that tongue length had significantly lower nearest neighbor trait distance ( $NTI_{\text{trait}}$ ) and significantly more similar overall tongue lengths ( $NRI_{\text{trait}}$ ) in observed communities compared to nulls (Wilcoxon signed-rank test,  $NTI_{\text{trait}}$ ,  $p = 3.07 \times 10^{-5}$  and  $NRI_{\text{trait}}$   $p = 1.12 \times 10^{-5}$ , respectively, Table 2).

Only three communities had more evenly spaced tongue length (SDNN) than the generated null communities and when all communities compared together showed no significant trend ( $p = 0.2154$ ).

### *Regional Community Analysis*

Regional communities were comprised of species with lower nearest neighbor phylogenetic distances than the null communities using the (Wilcoxon signed-rank test of  $NTI_{\text{phylo\_regional}}$   $p = 0.0011$ , Table 2). Regional communities were not significantly different from nulls for  $NRI_{\text{phylo\_regional}}$ ,  $NTI_{\text{trait\_regional}}$  or  $NRI_{\text{trait\_regional}}$ .

## Discussion

Tongue length has long been assumed to be the primary method by which bumble bees partition resources (Hanski 1982; Inouye 1977; Pyke 1982), though this theory has previously been called into question (Ranta 1984; Ranta & Lundberg 1980). However, prior to this study no analysis had been conducted to test this using phylogenetic analysis nor to consider the role of relatedness in determining species assembly. If tongue length is a significant trait for partitioning resources we would expect communities to be comprised of species that had significantly different or evenly spaced tongue lengths within a community.

We found that tongue length is highly conserved both globally and in Nearctic areas, compared to other morphological traits in animals (Blomberg et al. 2003). This finding is despite high levels of variability in worker size in *Bombus* nests (Johnson 1986; Peat, Tucker, & Goulson 2005) and evidence that alternative methods for measuring may introduce additional

error to the analysis (Harder 1982). Given that tongue length is highly conserved, if tongue length is a significant factor for resource partitioning we would expect communities to be comprised of species that are distantly related to limit trait similarity. However, we found that communities had lower nearest neighbor distances for both phylogenetic and trait distance compared to null communities and that the trait also had lower mean trait distance than the null communities. The lack of even trait spacing in this community supported the finding of similarity of species and traits in observed communities. Thus, communities are comprised of species that are both closely related and have similar tongue lengths than expected for randomly associated species.

We also found regional communities to be made up of closely related species when compared to nulls created from the Nearctic species pool. The relatedness of regional communities observed here may be the result of regional diversification and limited dispersal which would cause the clumping of taxa. Habitat filters could contribute to this pattern by limiting community members to species that are well adapted to certain habitat features such as cold tolerance which could encourage diversification. If local communities are already strongly filtered and the trait is highly conserved one would expect the communities to be both closely related and have similar tongue lengths, as found here. The clumping of taxa and the similar tongue length could suggest that other biotic and abiotic factors may play a more significant role in *Bombus* community assembly than competition and cause communities to be more similar than different (Ranta 1984; Sargent & Ackerly 2008).

Previous work using random draws of communities to determine if tongue length was more similar in observed or random communities found that coexisting species had tongue lengths that were more similar than expected (Ranta 1982; 1984). However, the null species pools considered were extremely small, which has previously been acknowledged to influence error rates (Kembel 2009; Kembel & Hubbell 2006). Additionally, the data were pooled across an entire year and not based on species that competed directly with one another which is a necessary condition for resource partitioning.

Many factors other than tongue length contribute to competition among *Bombus* and could be playing a significant role in community assembly. Nesting limitation has been proposed as one of the most limiting factors for bees and bumble bee species could be more limited by ability to find adequate nesting sites than by floral resources (Potts et al. 2005; Steffan-Dewenter & Schiele 2008). Additionally, our analysis focuses on the evolutionary time scale of bumble bees diversifying to coexist, however, there are ecological factors at the site level that could contribute to observed patterns. Further studies should consider bottom up effects that the floral community has on bumble bee communities and tongue length to develop a better understanding of floral characters on tongue length assembly in communities.

Lastly, most observed declines disproportionately affect longer tongued species than co-occurring shorter tongued species in the United Kingdom (Goulson et al. 2005; Goulson, Lye, & Darvill 2008b). The significant conservatism of this trait would suggest that some subgenera of *Bombus* would be more at risk than others if tongue length is related to declines and as a result some groups of bees may need additional consideration for conservation efforts.



## Tables

Table 1. Tongue Length of 81 *Bombus* species.

species	subgenus	tongue length(mm)	ln(tongue length)	Author(s)
<i>soroensis</i>	Kallobombus	6.602	1.887	Goulson et al (2005); Medler (1962); Goulson et al. (2008)
<i>ardens</i>	Pyrobombus	8.600	2.152	Inoue and Yokoyama (2006)
<i>pyrenaeus</i>	Pyrobombus	6.100	1.808	Goulson et al. (2008)
<i>beaticola</i>	Pyrobombus	8.000	2.079	Inoue and Yokoyama (2006)
<i>frigidus</i> *	Pyrobombus	5.730	1.746	Macior (1974)
<i>jonellus</i> *	Pyrobombus	5.847	1.766	Goulson et al (2005), Ranta (1983), Goulson et al. (2008)
<i>cingulatus</i>	Pyrobombus	6.690	1.826	Pekkarinen (1979)
<i>mixtus</i> *	Pyrobombus	5.090	1.627	Macior (1974)
<i>pratorum</i>	Pyrobombus	6.567	1.914	Goulson et al. (2005); Ranta (1983); Goulson et al.(2008)
<i>lemniscatus</i>	Pyrobombus	6.259	1.834	Williams et al.(2009)
<i>hypnorum</i>	Pressibombus	6.600	1.887	Ranta (1983); Pekkarinen (1979); Goulson et al. (2008)
<i>perplexus</i> *	Pressibombus	7.430	2.006	Medler (1962)
<i>bifarius</i> *	Pressibombus	5.703	1.741	Macior (1974); Medler (1962); Bowers (1985)
<i>ternarius</i> *	Pressibombus	5.924	1.779	Medler (1962); Harder (1983)
<i>huntii</i> *	Pressibombus	6.897	1.931	Medler (1962); Bowers (1985)
<i>vosnesenskii</i> *	Pressibombus	7.710	2.043	Medler (1962)
<i>impatiens</i> *	Pressibombus	7.270	1.984	Medler(1962)
<i>melanopygus</i> *	Pressibombus	6.490	1.870	Macior (1974)
<i>lapponicus</i>	Pressibombus	5.890	1.773	Pekkarinen (1979)
<i>sylvicola</i> *	Pressibombus	5.790	1.756	Macior (1974)
<i>bimaculatus</i> *	Pressibombus	8.265	2.112	Medler(1962); Harder (1983)
<i>monticola</i>	Pressibombus	6.635	1.892	Goulson et al (2005); Pekkarinen (1979)

<i>vagens*</i>	Pressibombus	8.010	2.081	Medler(1962)
<i>centralis*</i>	Pressibombus	7.557	2.022	Macior (1974); Bowers (1985)
<i>flavifrons*</i>	Pressibombus	7.835	2.059	Inouye (1980); Macior (1974); Bowers (1985)
<i>sporadicus</i>	Bombus	7.370	1.997	Pekkarinen (1979)
<i>ignitus</i>	Bombus	9.300	2.230	Inoue and Yokoyama (2006)
<i>terrestris</i>	Bombus	7.692	2.040	Goulson et al. (2005); Corbet et al. (1995); Pekkarinen (1979); Goulson et al.(2008)
<i>hypocrita</i>	Bombus	8.000	2.079	Inoue and Yokoyama (2006)
<i>affinis*</i>	Bombus	6.870	1.924	Medler (1962)
<i>lucorum*</i>	Bombus	6.499	1.872	Goulson et al. (2005); Pekkarinen (1979); Medler (1962); Goulson et al.(2008), Ranta (1983)
<i>patagiatus</i>	Bombus	6.753	1.910	Williams et al. (2009)
<i>occidentalis*</i>	Bombus	5.964	1.786	Macior (1974); Medler (1962); Bowers (1985)
<i>terricola*</i>	Bombus	6.400	1.869	Medler (1962)
<i>balteatus*</i>	Alpinobombus	9.220	2.221	Medler (1962); Macior (1974)
<i>rufocinctus*</i>	Sibiricobombus	6.565	1.882	Medler (1962); Bowers (1985)
<i>fraternus*</i>	Cullumanobombus	7.430	2.006	Medler (1962)
<i>griseocollis*</i>	Cullumanobombus	7.610	2.029	Medler (1962)
<i>morrisoni*</i>	Cullumanobombus	8.250	2.110	Medler (1962)
<i>wurflenii</i>	Alpigenobombus	8.647	2.157	Medler (1962); Goulson et al. (2008)
<i>kashmirensis</i>	Alpigenobombus	7.236	1.979	Williams et al.(2009)
<i>sichellis</i>	Melanobombus	6.593	1.886	Williams et al.(2009)
<i>lapidarius</i>	Melanobombus	7.012	1.948	Goulson et al (2005); Ranta (1983); Corbet et al (1995); Goulson et al. (2008)
<i>rufofasciatus</i>	Melanobombus	8.365	2.124	Williams et al.(2009)
<i>friseanus</i>	Melanobombus	7.729	2.045	Williams et al.(2009)
<i>ruderarius</i>	Thoracobombus	8.500	2.140	Goulson et al (2005)

<i>veteranus</i>	Thoracobombus	8.300	2.116	Ranta (1983); Goulson et al. (2008)
<i>sylvarum</i>	Thoracobombus	8.114	2.094	Goulson et al.(2005); Medler (1962); Goulson et al.(2008)
<i>humilis</i>	Thoracobombus	7.952	2.073	Medler (1962); Goulson (2008)
<i>pascuorum</i>	Thoracobombus	7.998	2.079	Goulson et al (2005); Ranta (1983); Corbet et al (1995); Pekkarinen (1979); Goulson et al. (2008)
<i>honshuensis</i>	Thoracobombus	9.549	2.256	Inoue and Yokoyama (2006); Suzuki (2007)
<i>pseudobaicalensis</i>	Thoracobombus	9.700	2.272	Ishii et al (2008)
<i>impetuosus</i>	Thoracobombus	8.037	2.084	Williams et al.(2009)
<i>muscorum</i>	Thoracobombus	7.874	2.064	Goulson et al (2005); Pekkarinen (1979)
<i>filchnerae</i>	Thoracobombus	8.306	2.117	Williams et al.(2009)
<i>laesus</i>	Thoracobombus	6.773	1.913	Williams et al.(2009)
<i>sonorus*</i>	Thoracobombus	8.860	2.182	Medler (1962)
<i>pensylvanicus*</i>	Thoracobombus	9.670	2.269	Medler (1962)
<i>medius*</i>	Thoracobombus	8.030	2.083	Medler (1962)
<i>californicus*</i>	Thoracobombus	10.010	2.304	Macior (1974)
<i>fervidus*</i>	Thoracobombus	9.690	2.271	Medler (1962)
<i>sylvestris</i>	Psythirus	6.600	1.887	Goulson (2008)
<i>bohemicus</i>	Psythirus	7.000	1.946	Goulson (2008)
<i>rupestris</i>	Psythirus	7.000	1.946	Goulson (2008)
<i>campestris</i>	Psythirus	6.900	1.932	Goulson (2008)
<i>insularis*</i>	Psythirus	8.180	2.102	Macior (1974)
<i>consobrinus</i>	Megabombus	15.295	2.728	Pekkarinen (1979); Suzuki et al.(2007)
<i>hortorum</i>	Megabombus	12.400	2.518	Goulson et al (2005); Ranta (1983); Corbet et al (1995); Pekkarinen (1979); Goulson et al. (2008)
<i>ruderatus</i>	Megabombus	11.199	2.416	Goulson et al (2005); Medler (1962); Goulson et al. (2008)
<i>supremus</i>	Megabombus	11.359	2.430	Williams et al.(2009)

<i>diversus</i>	Megabombus	13.250	2.584	Inoue and Yokoyama (2006); Suzuki (2007)
<i>subterraneus</i>	Subterraneobombus	8.960	2.193	Goulson et al (2005); Medler (1962)
<i>distinguendus</i>	Subterraneobombus	9.610	2.263	Goulson et al (2005); Medler (1962); Goulson et al.(2008)
<i>appositus*</i>	Subterraneobombus	10.505	2.352	Macior (1974); Medler (1962)
<i>borealis*</i>	Subterraneobombus	8.610	2.153	Grixti et al (2009)
<i>haemorrhoidalis</i>	Orientalibombus	11.510	2.443	Dayal and Rana(2007)
<i>confusus</i>	Bombias	8.370	2.125	Medler (1962)
<i>auricomus*</i>	Bombias	10.810	2.380	Medler (1962)
<i>nevadensis*</i>	Bombias	9.983	2.301	Macior (1974); Medler (1962)
<i>convexus</i>	Mendacibombus	9.895	2.292	Williams et al.(2009)
<i>waltoni</i>	Mendacibombus	9.728	2.275	Williams et al.(2009)
<i>Note: A weighted average was used for species with multiple published measurements. Subgeneric classification is based on Williams et al. (2008). *indicates those in Nearctic community</i>				

Table 2. Results from the Local and Regional Phylogenetic and Trait Analysis. P-value is reported from the Wilcoxon signed-rank test. All analysis were two-tailed.

		Local		Regional	
	Metric	Z	p	Z	p
Relatedness	NTI <sub>phylo</sub>	-1.641	0.050	-3.068	0.001
	NRI <sub>phylo</sub>	-1.192	0.117	-1.310	0.091
Tongue Length	NTI <sub>trait</sub>	-9.027	0.000	0.997	0.841
	NRI <sub>trait</sub>	-4.239	0.000	-1.036	0.150
	SDNN	-0.788	0.215	NA	NA

## Figures

Figure 1. Traitgram, a visual depiction of the phylogenetic tree where the position of the nodes and tips corresponds to the trait value, of tongue length for all available species, a, and Nearctic species, b.

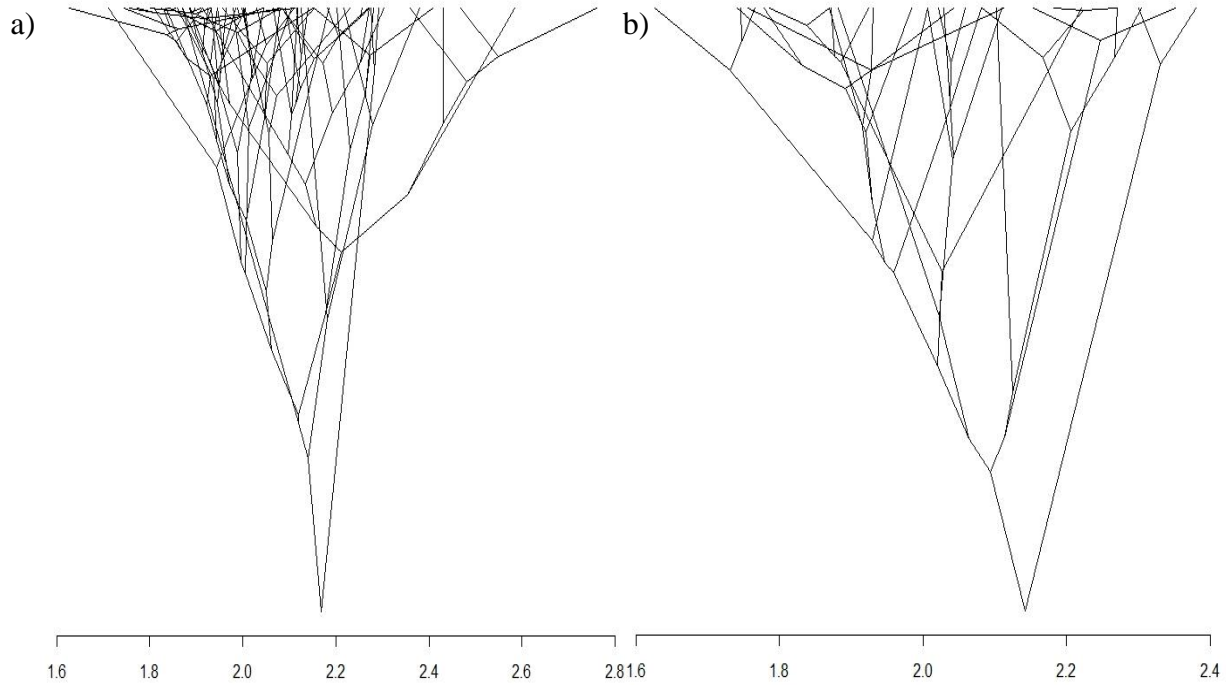
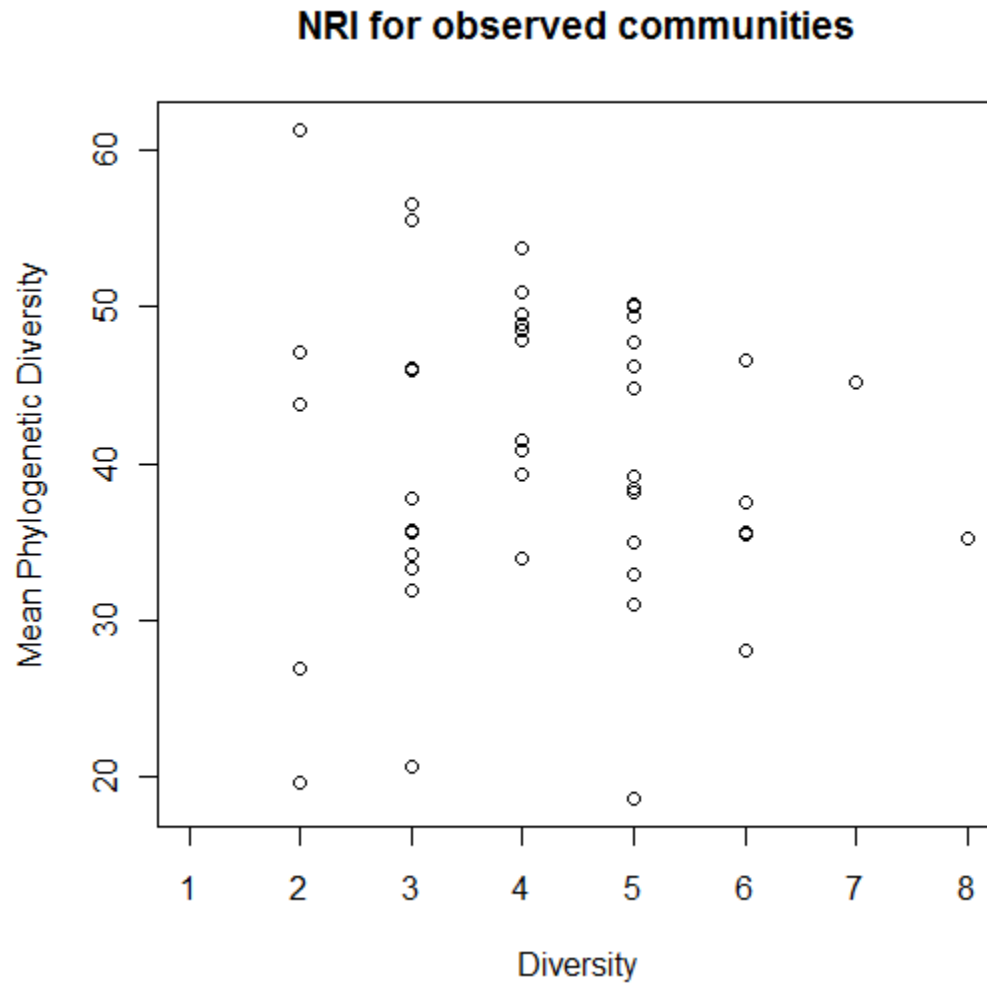


Figure 2. Mean phylogenetic diversity ( $NRI_{\text{phylo}}$ ) and species diversity at all communities.



## Literature Cited

- Aebischer, N., Robertson, P. & Kenward, R. (1993) Compositional analysis of habitat use from animal radio-tracking data. *Ecology*, **74**, 1313-1325.
- Aitchison, J. (1986) *The analysis of compositional data*. Chapman and Hall, London, UL.
- Aizen, M.A. & Morales, C.L. (2006) Invasive mutualisms and the structure of plant-pollinator interactions in the temperate forests of north-west Patagonia, Argentina. *Journal of Ecology*, **94**, 171-180.
- Allen-Wardell, G., Bernhardt, P., Bitner, R., Burquez, A., Buchmann, S., Cane, J., Cox, P., Dalton, V., Feinsinger, P., Ingram, M., Inouye, D.W., Jones, C., Kennedy, K., Kevan, P., Koopowitz, H., Medellin, R., Medellin-Morales, S., Nabhan, G., Pavlik, B., Tepedino, V., Torchio, P. & Walker, S. (1998) The potential consequences of pollinator declines on the conservation of biodiversity and stability of food crop yields. *Conservation Biology*, **12**, 8-17.
- Ashman, T.-L., Knight, T.M., Steets, J.A., Amarasekare, P., Burd, M., Campbell, D.R., Dudash, M.R., Johnston, M.O., Mazer, S.J., Mitchell, R.J., Morgan, M.T. & Wilson, W.G. (2004) Pollen limitation of plant reproduction: Ecological and evolutionary causes and consequences. *Ecology*, **85**, 2408-2421.
- Baraibar, B., Carrión, E., Recasens, J. & Westerman, P.R. (2011) Unravelling the process of weed seed predation: Developing options for better weed control. *Biological Control*, **56**, 85-90.
- Beyer, H.L., Haydon, D.T., Morales, J.M., Frair, J.L., Hebblewhite, M., Mitchell, M. & Matthiopoulos, J. (2010) The interpretation of habitat preference metrics under use-availability designs. *Philosophical Transactions of the Royal Society B: Biological Sciences*, **365**, 2245-2254.
- Blomberg, S.P., Garland, T. & Ives, A.R. (2003) Testing for phylogenetic signal in comparative data: Behavioral traits are more labile. *Evolution*, **57**, 717-745.
- Burger, H., Dötterl, S. & Ayasse, M. (2010) Host-plant finding and recognition by visual and olfactory floral cues in an oligolectic bee. *Functional Ecology*, **24**, 1234-1240.
- Cameron, S.A., Hines, H.M. & Williams, P.H. (2007) A comprehensive phylogeny of the bumble bees (*Bombus*). *Biological Journal of the Linnean Society*, **91**, 161-188.
- Cameron, S.A., Lozier, J.D., Strange, J.P., Koch, J.B., Cordes, N., Solter, L.F. & Griswold, T.L. (2011) Patterns of widespread decline in North American bumble bees. *Proceedings of the National Academy of Sciences of the United States of America*, 1014743108-.



- Campbell, D.R., Bischoff, M., Lord, J.M. & Robertson, A.W. (2010) Flower color influences insect visitation in alpine New Zealand. *Ecology*, **91**, 2638-2649.
- Cane, J.H. & Sipes, S. (2006) Characterizing floral specialization by bees: Analytical methods and a revised lexicon for oligolecty. *Plant-Pollinator Interactions: From Specialization to Generalization*. (eds N.M. Waser & J. Ollerton), pp. 99-122. University of Chicago Press, Chicago.
- Cook, C.N., Morgan, D.G. & Marshall, D.J. (2010) Reevaluating suitable habitat for reintroductions: lessons learnt from the eastern barred bandicoot recovery program. *Animal Conservation*, **13**, 184-195.
- Cornwell, W.K. & Ackerly, D.D. (2009) Community assembly and shifts in plant trait distributions across an environmental gradient in coastal California. *Ecological Monographs*, **79**, 109-126.
- Costa, C.M. & Yang, S. (2009) Counting pollen grains using readily available, free image processing and analysis software. *Annals of Botany*.
- DeGroot, A. (1954) Qualitative amino acid requirements of the honeybee. *Acta Physiologica et Pharmacologica Neerlandica*, **3**, 433-434.
- Frankie, G., Thorp, R.W., Schindler, M., Hernandez, J., Ertter, B. & Rizzardi, M. (2005) Ecological patterns of bees and their host ornamental flowers in two northern California cities. *Journal of the Kansas Entomological Society*, **78**, 227-246.
- Goulson, D., Hanley, M.E., Darvill, B., Ellis, J.S. & Knight, M.E. (2005) Causes of rarity in bumblebees. *Biological Conservation*, **122**, 1-8.
- Goulson, D., Lye, G.C. & Darvill, B. (2008a) Decline and conservation of bumble bees. *Annual review of entomology*, **53**, 191-208.
- Goulson, D., Lye, G.C. & Darvill, B. (2008b) Diet breadth, coexistence and rarity in bumblebees. *Biodiversity and Conservation*, **17**, 3269-3288.
- Greenleaf, S.S. & Kremen, C. (2006) Wild bee species increase tomato production and respond differently to surrounding land use in Northern California. *Biological Conservation*, **133**, 81-87.
- Hanley, M.E., Franco, M., Pinchon, S., Darvill, B. & Goulson, D. (2008) Breeding system, pollinator choice and variation in pollen quality in British herbaceous plants. *Functional Ecology*, **22**, 592-598.
- Hanski, I. (1982) Structure in bumblebee communities. *Annales Zoologici Fennici*, **19**, 319-326.

- Harder, L.D. (1990) Behavioral responses by bumble bees to variation in pollen availability. *Oecologia*, **85**, 41-47.
- Harder, L.D. (1986) Effects of nectar concentration and flower depth on flower handling efficiency of bumble bees. *Oecologia*, **69**, 309-315.
- Harder, L.D. (1985) Morphology as a predictor of flower choice by bumble bees. *Ecology*, **66**, 198-210.
- Harder, L.D. (1982) Measurement and estimation of functional proboscis length in bumble bees (Hymenoptera, Apidae). *Canadian Journal of Zoology*, **60**, 1073-1079.
- Harder, L.D. (1983) Flower handling efficiency of bumble bees-Morphological aspects of probing time. *Oecologia*, **57**, 274-280.
- Harmon-Threatt, A.N., Burns, J.H., Shemyakina, L.A. & Knight, T.M. (2009) Breeding system and pollination ecology of introduced plants compared to their native relatives. *American Journal of Botany*, **96**, 1544-1550.
- Herbert, E., Bickley, W. & Shimanuk, H. (1970) Brood-rearing capability of caged honeybees fed dandelion and mixed pollen diets. *Journal of Economic Entomology*, **63**, 215-&.
- Hines, H.M. (2008) Historical biogeography, divergence times, and diversification patterns of bumble bees (Hymenoptera: Apidae: Bombus). *Systematic biology*, **57**, 58-75.
- Inouye, D.W. (1977) Resource partitioning in bumble bees. *Journal of the New York Entomological Society*, **85**, 253-254.
- Inouye, D.W. (1978) Resource partitioning in bumble bees- Experimental studies of foraging behavior. *Ecology*, **59**, 672-678.
- Ives, A.R. & Helmus, M.R. (2010) Phylogenetic Metrics of Community Similarity. *The American Naturalist*, **176**, 000-000.
- Johnson, D.H. (1980) The Comparison of Usage and Availability Measurements for Evaluating Resource Preference. *Ecology*, **61**, 65 - 71.
- Johnson, R.A. (1986) Intraspecific resource partitioning in the bumble bees *Bombus ternarius* and *B. pensylvanicus*. *Ecology*, **67**, 133-138.
- Kearns, C.A. & Inouye, D.W. (1993) *Techniques for Pollination Biologists*. University Press of Colorado, Niwot.
- Kearns, C.A., Inouye, D.W. & Waser, N.M. (1998) Endangered Mutualisms: The Conservation of Plant-Pollinator Interactions. *Annual Review of Ecological Systems*, **29**, 83-112.

- Keddy, P.A. (1992) Assembly and response rules: two goals for predictive community ecology. *Journal of Vegetation Science*, **3**, 157-164.
- Keddy, P.A. (1989) *Competition* ( , Eds.). Chapman and Hall, London.
- Kells, A., Holland, J. & Goulson, D. (2001) The Value of Uncropped Field Margins For Foraging Bumblebees. *Journal of Insect Conservation*, **5**, 283-291.
- Kembel, S.W. (2009) Disentangling niche and neutral influences on community assembly: assessing the performance of community phylogenetic structure tests. *Ecology letters*, **12**, 949-60.
- Kembel, S.W., Cowan, P.D., Helmus, M.R., Cornwell, W.K., Morlon, H., Ackerly, D.D., Blomberg, S.P. & Webb, C.O. (2010) Picante: R tools for integrating phylogenies and ecology. *Bioinformatics*, **26**, 1463-4.
- Kembel, S.W. & Hubbell, S. (2006) The phylogenetic structure of a neotropical forest tree. *Ecology*, **87**, S86:S99.
- Kleijn, D. & Raemakers, I. (2008) A retrospective analysis of pollen host plant use by stable and declining bumble bee species. *Ecology*, **89**, 1811-1823.
- Kleunen, M. van, Weber, E. & Fischer, M. (2010) A meta-analysis of trait differences between invasive and non-invasive plant species. *Ecology Letters*, **13**, 235-45.
- Kraft, N.J.B. & Ackerly, D.D. (2010) Functional trait and phylogenetic tests of community assembly across spatial scales in an Amazonian forest. *Ecological Monographs*, **80**, 401-422.
- Kraft, N.J.B., Valencia, R. & Ackerly, D.D. (2008) Functional traits and niche-based tree community assembly in an Amazonian forest. *Science*, **322**, 580-2.
- Kremen, C., Williams, N.M., Bugg, R.L., Fay, J.P. & Thorp, R.W. (2004) The area requirements of an ecosystem service: crop pollination by native bee communities in California. *Ecology Letters*, **7**, 1109-1119.
- Manly, B., McDonald, L., Thomas, D., McDonald, T. & Erickson, W. (2002) *Resource Selection by Animals*. Kluwer Academic Publishers, Dordrecht, The Netherlands.
- McCune, B. & Grace, J.B. (2002) *Analysis of Ecological Communities*. MjM Software Design, Glenden Beach, Oregon, USA.
- Medler, J. (1962) Morphometric Studies on Bumble Bees. *Annals of the Entomological Society of America*, **55**, 212-218.

- Menz, M.H.M., Phillips, R.D., Winfree, R., Kremen, C., Aizen, M.A., Johnson, S.D. & Dixon, K.W. (2010) Reconnecting plants and pollinators: challenges in the restoration of pollination mutualisms. *Trends in plant science*, **16**, 4-12.
- Moroń, D., Lenda, M., Skórka, P., Szentgyörgyi, H., Settele, J. & Woyciechowski, M. (2009) Wild pollinator communities are negatively affected by invasion of alien goldenrods in grassland landscapes. *Biological Conservation*, **142**, 1322-1332.
- Morse, D. (1977a) Proboscis and wing lengths of bumblebees (*Bombus* spp). *Annals of the Entomological Society of America*, **70**, 311-315.
- Morse, D. (1977b) Resource partitioning in bumble bees: The role of behavioral factors. *Science*, **197**, 678-680.
- Mosimann, J.E. (1963) On the compound negative multinomial distribution and correlations among inversely sampled pollen counts. *Biometrika*, **50**, 47-54.
- Nienhuis, C.M., Dietzsch, A.C. & Stout, J.C. (2009) The impacts of an invasive alien plant and its removal on native bees. *Apidologie*, **40**, 450-463.
- Paciorek, C.J. & McLachlan, J.S. (2009) Mapping ancient forests: Bayesian inference for spatio-temporal trends in forest composition using the fossil pollen proxy record. *Journal of the American Statistical Association*, **104**, 608-622.
- Pagel, M. (1994) Detecting correlated evolution on phylogenies-A general method for the comparative analysis of discrete characters. *Proceedings of the Royal Society-Biological Sciences*, **255**, 37-45.
- Peat, J., Tucker, J. & Goulson, D. (2005) Does intraspecific size variation in bumblebees allow colonies to efficiently exploit different flowers? *Ecological Entomology*, **30**, 176-181.
- Pekkarinen, A. (1984) Resource Partitioning and coexistence in bumblebees. *Annales Entomologici Fennici*, **50**, 97-107.
- Peng, Y. & Jay, S. (1976) Effect of diet on queen rearing by caged worker honeybees. *Canadian Journal of Zoology*, **54**, 1156-1160.
- Pimentel, D., McNair, S., Janecka, J., Wightman, J., Simmonds, C., O'Connell, C., Wong, E., Russel, L., Zern, J., Aquino, T. & Tsomondo, T. (2001) Economic and environmental threats of alien plant, animal, and microbe invasions. *Agriculture, Ecosystems & Environment*, **84**, 1-20.
- Potts, S.G., Biesmeijer, J.C., Kremen, C., Neumann, P., Schweiger, O. & Kunin, W.E. (2010) Global pollinator declines: trends, impacts and drivers. *Trends in Ecology & Evolution*, **25**, 8.

- Potts, S.G., Vulliamy, B., Roberts, S., O'Toole, C., Dafni, A., Ne'eman, G. & Willmer, P. (2005) Role of nesting resources in organising diverse bee communities in a Mediterranean landscape. *Ecological Entomology*, **30**, 78-85.
- Pyke, G. (1982) Local geographic distributions of bumblebees near Crested Butte, Colorado-competition and community structure. *Ecology*, **63**, 555-573.
- Ranta, E. (1983) Foraging differences in bumblebees. *Annales Entomologici Fennici*, **49**, 17-22.
- Ranta, E. (1984) Proboscis length and the coexistence of bumblebee species. *Oikos*, **43**, 189-196.
- Ranta, E. (1982) Species structure of North European bumblebee communities. *Oikos*, **38**, 202-209.
- Ranta, E. & Lundberg, H. (1980) Resource partitioning in bumblebees-The significance of differences in proboscis length. *Oikos*, **35**, 298-302.
- Rasheed, S. & Harder, L. (1997) Economic motivation for plant species preferences of pollen-collecting bumble bees. *Ecological Entomology*, **22**, 209-219.
- Robertson, A., Mountjoy, C., Faulkner, B., Roberts, M. & Macnair, M. (1999) Bumble bee selection of *Mimulus guttatus* flowers: The effects of pollen quality and reward depletion. *Ecology*, **80**, 2594-2606.
- Robertson, C. (1929) *Flowers and insects. Lists of visitors to four hundred and fifty three flowers*. Carlinville, IL.
- Roulston, T. & Cane, J. (2000) Pollen nutritional content and digestibility for animals. *Plant Systematics and Evolution*, **222**, 187-209.
- Roulston, T. & Cane, J. (2002) The effect of pollen protein concentration on body size in the sweat bee *Lasioglossum zephyrum* (Hymenoptera : Apiformes). *Evolutionary Ecology*, **16**, 49-65.
- Roulston, T., Cane, J. & Buchmann, S. (2000) What governs protein content of pollen: Pollinator preferences, pollen-pistil interactions, or phylogeny? *Ecological Monographs*, **70**, 617-643.
- Sargent, R.D. & Ackerly, D.D. (2008) Plant-pollinator interactions and the assembly of plant communities. *Trends in Ecology & Evolution*, **23**, 123-30.
- Steffan-Dewenter, I. & Schiele, S. (2008) Do resources or natural enemies drive bee population dynamics in fragmented habitats? *Ecology*, **89**, 1375-1387.
- Stephen, W. (1957) *Bumble bees of Western America (Hymenoptera:Apoidea)*. Oregon Technical Bulletin, Corvallis, OR.

- Stout, J.C. & Morales, C.L. (2009) Ecological impacts of invasive alien species on bees. *Apidologie*, **40**, 388-409.
- Stubbs, W. & Wilson, B. (2004) Evidence for limiting similarity in a sand dune community. *Journal of Ecology*, **92**, 557-567.
- Thorp, R., Horning, D. & Dunning, L. (1983) *Bumble Bees and Cuckoo Bumble Bees of California (Hymenoptera: Apidae)*. University of California Press, Berkeley.
- Vilà, M., Bartomeus, I., Dietzsch, A.C., Petanidou, T., Steffan-Dewenter, I., Stout, J.C. & Tscheulin, T. (2009) Invasive plant integration into native plant-pollinator networks across Europe. *Proceedings of the Royal Society-Biological sciences*, **276**, 3887-93.
- Wcislo, W.T. & Cane, J.H. (1996) Floral Resource Utilization by solitary bees and exploitation of their stored foods by natural enemies. *Annual review of entomology*, **41**, 257-286.
- Webb, C.O. (2000) Exploring the phylogenetic structure of ecological communities: An example for rain forest trees. *American Naturalist*, **156**, 145-155.
- Webb, C.O., Ackerly, D.D., McPeck, M.A. & Donoghue, M.J. (2002) Phylogenies and community ecology. *Annual Review of Ecology and Systematics*, **33**, 475-505.
- Williams, N.M. (2003) Use of novel pollen species by specialist and generalist solitary bees (Hymenoptera: Megachilidae). *Oecologia*, **134**, 228-37.
- Williams, N.M., Cariveau, D., Winfree, R. & Kremen, C. (2010) Bees in disturbed habitats use, but do not prefer, alien plants. *Basic and Applied Ecology*.
- Williams, N.M. & Kremen, C. (2007) Resource distributions among habitats determine solitary bee offspring production in a mosaic landscape. *Ecological Applications*, **17**, 910-921.
- Williams, P.H. (1996) Mapping Variations in the Strength and Breadth of Biogeographic Transition Zones Using Species Turno... more. *Proceedings of the Royal Society-Biological sciences*, **263**, 579 - 588.
- Williams, P.H. & Osborne, J.L. (2009) Bumblebee vulnerability and conservation world-wide. *Apidologie*, **40**, 367-387.
- Williams, P., Colla, S. & Xie, Z. (2009) Bumblebee vulnerability: common correlates of winners and losers across three continents. *Conservation biology : the journal of the Society for Conservation Biology*, **23**, 931-40.
- Winfree, R. (2010) The conservation and restoration of wild bees. *Annals of the New York Academy of Sciences*, **1195**, 169-97.