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Permalink

Journal
Oikos, 126(5)

ISSN
0030-1299

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Publication Date
2017-05-01

DOI
10.1111/oik.03550

Peer reviewed

Oikos 000: 001–009, 2016
doi: 10.1111/oik.03550

Subject Editor: Stefano Allesina. Editor-in-Chief: Dries Bonte. Accepted 23 August 2016

Estimating resource preferences of a native bumblebee: the effects of availability and use–availability models on preference estimates

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Identifying resource preference is considered essential for developing targeted conservation plans but, for many species, questions remain about the best way to estimate preference. Resource preferences for bees are particularly difficult to determine as the resources they collect, nectar and pollen, are challenging to estimate availability and collection. Resources are traditionally measured at the flower or inflorescence level, but these measures of availability do not correspond to the resources actually used by bees. Additionally, it is unclear as to whether common models including availability are appropriate for bees which may target resources regardless of available quantities. Here we first compare two common hypotheses of resource use – the 'random use hypothesis' and the 'linear preferences hypothesis' – using three different measures of availability (pollen, flower and inflorescence) – to determine if one measure of availability was better for understanding bee pollen use. Next, the superior model using availability was compared to a novel model of bee pollen use the 'target use hypothesis'. This model assumes that bees target some resources regardless of how much of each resource is available (but assuming resources are present at a site), and thus models preference without availability data. Of the models including availability, the linear preference model using inflorescence availability best explained the pollen use data. This suggests that bumblebee pollen use is non-random and that cues to identify and locate resources (i.e. display size and quantity) may be more important than the quantity of the resource available (i.e. pollen availability). Additionally, in most cases the target use model explained the data equal to or better than the other models suggesting bee resource use may be better modeled without measured availability data compared to linear models. These results could be important for expanding resource use analysis of bees that are difficult to quantify availability.

Understanding resource preferences for bees can provide vital information for conservation of declining bee species and restoration of habitat (Kleijn and Raemakers 2008). Despite this, one of the primary methods used to understand preferences for bees are behavioral assays (Forrest and Thomson 2009, Muth et al. 2016) that limit possible choices, and floral abundance and thus do not mimic natural foraging conditions. While these behavioral assays can provide valuable information, they largely cannot be used to inform conservation decisions or estimate preferences of free foraging individuals which may be affected by associational effects between plant species (Underwood et al. 2015). Estimating preferences for wild bees, however, has significant challenges that must be examined before we can appropriately estimate bee floral preferences for bee conservation.

Traditional preference in field studies is a statistical estimation based on the use of a resource relative to the availability of that resource and other available resources (Aarts et al. 2008). Thus, resource use models and preference estimates are dependent upon quantifying both the intentional use of a resource and the availability of that resource. Quantifying both use and availability of resources for freely-foraging, wild bees is extremely difficult as their movements are difficult to track and the resources they are collecting, pollen and nectar, are difficult to measure. In fact, analysis of preference has primarily been conducted using floral visitation records (Kells et al. 2001, Williams et al. 2011, Morandin and Kremen 2013), which pools pollen and nectar collection together (but see Müller et al. 2006, Davis et al. 2012). While this can provide some insight into floral preferences, bees are known to be more selective of pollen resources (Cane and Wcislo 1996, Cane and Sipes 2006) and determinations of diet specialization are typically based on pollen collection (Cane and Sipes 2006). Thus, using floral visitation records to estimate preference may obfuscate the importance of plant species used for pollen versus those used primarily for nectar. For rare or declining species this could be particularly problematic as the more demographically important pollen resource may be overlooked. However, few studies have assessed pollen availability specifically which may limit our understanding of which resources are preferred. Consequently, it is important to assess whether resource use models for bees provide superior estimates of preference if based on different measures of availability.

Highly mobile organisms, like bees, can easily move long distances (Osborne et al. 2008, Jha and Kremen 2012) and may selectively collect from some patches making it difficult to accurately quantify availability. Some of this movement could be to avoid predators (Abbott 2006, Bray and Nieh 2014), reduce competition (Goulson et al. 1998) or to seek better resources that exist in other patches (Lefebvre et al. 2007, Llandres et al. 2012). Thus, resource availability within a patch may poorly reflect resource availability to bees and could significantly affect estimates of resource preference. For example, if the availability of a flower in patch A is low but large amounts of the pollen are found on the bee the estimate for preference would be high but the bee may have come from adjacent patch B where the availability of the plant species is much higher. Consequently, the resource may only be collected proportionally to its availability but over a larger area than was measured. Additionally, resources that are highly preferred will likely be collected regardless of availability (Williams and Kremen 2007) and thus including availability could downplay the importance of a resource if it is highly available. Problems with estimating availability have been noted previously for many organisms (Beyer et al. 2010) and multiple methods have been developed to estimate preference without availability for assessing habitat context on site selection by caribou (Polfus et al. 2011), and ecological niche modelling, among others, but rarely for bees (but see Kleijn and Raemakers 2008). Estimating preference without availability may provide a more robust tool for estimating preference for bees, but it is necessary to compare these models to more standard resource use models to determine how well they perform.

To improve the usefulness of preference analysis for bees, it is necessary to assess the suitability of different preference models and measures of availability for these organisms. While it is often difficult to distinguish between active selection of resources and incidental usage (e.g. individuals passing through habitats to reach nesting locations, Beyer et al. 2010), pollen loads on bees offer quantifiable records of selected resources and typically include only small proportions of incidental use (i.e. pollens not actively collected by the individual but obtained while nectaring on a different flower species). Here, we use pollen loads to more definitively quantify resource use and focus on two issues related to preference and availability for bees: appropriately measuring resource availability and suitability of resource use models for mobile organisms. We assess two models of bumblebee pollen resource use using different measures of availability which can vary greatly in relative abundance and could affect model performance and preference predictions (Fig. 1). Using multiple measures of availability allows comparison of model performance if resources are recorded at the pollen, flower or inflorescence level and will help determine superior methods of measuring availability for modelling bee pollen preference. Resource use models are based on two alternative hypotheses of the relationship between preference, use and availability. First we evaluate a very common null hypothesis of resource use, the "random use hypothesis" (Manly et al. 2002, Beyer et al. 2010). This model tests whether resources are selected randomly and thus are used in direct proportion to availability. The random use model is commonly used to differentiate preferences from random selection in resource use modelling. We then assess one of the most common

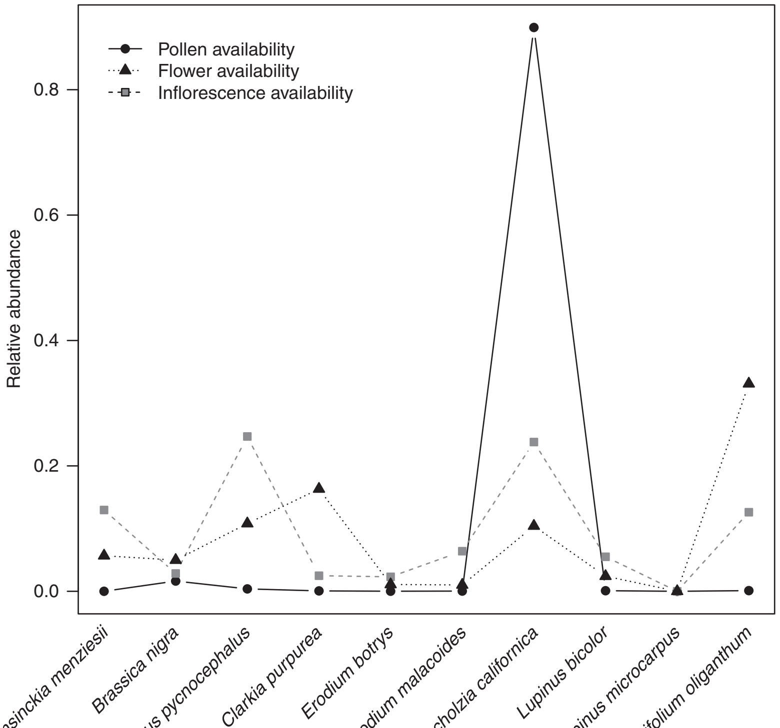

Figure 1. Relative resource abundance for a single site during sample period 1 measured at the pollen, flower and inflorescence level of all available plant species.

hypotheses of preference, the "linear preference hypothesis", which is based on the common assumption that use is proportional to availability weighted by preference for each resource (Aebischer et al. 1993, Johnson et al. 2004, Beyer et al. 2010). Numerous methods exist for testing linear preferences but they all similarly calculate preference in a linear fashion. We compare between the random and linear preferences model for each measure of availability to identify the best model of pollen use. By using two models and three levels of availability we can better assess both the method of choice for resources, random or linear preferences, and determine the most informative measure of availability. We expect linear preference models to perform better than random models and models including pollen availability to more closely reflect pollen use since pollen rather than flowers or inflorescences are the units being collected.

The best models of resource use that include availability are then compared to a newly developed model using only the use of pollen and ignoring availability (de Valpine and Harmon-Threatt 2013). We call this the 'target use hypothesis' because it implies that resources essential to breeding, nesting or feeding will be targeted and obtained, as long as they are present at a site. Highly mobile organisms like bumblebees can forage up to 4 km from their nests (Osborne et al. 2008, Jha and Kremen 2012) and are known to target certain resources regardless of availability (Williams 2003, Williams and Kremen 2007); thus, their pollen use may not be adequately described by local floral availability. This 'targeting' of particular resources by bees could cause models including availability, regardless of how it is measured, to generate less robust estimates of resource use and may support use of the target use model over more traditional resource use models.

If resource use is better explained by the target use model than by the linear preference model, it could suggest that resource use is independent of local abundance, or that resource use depends on abundance in a manner more complicated than the linear preferences model. In the former case, resource use could be understood without consideration of availability. In the latter case, the target use model may serve to highlight the limitations of the linear preferences model, but we would need more extensive data and more extensive models to explain resource use.

Material and methods

Site description

During 2009, five 1-ha grassland sites were chosen in Briones East Bay Regional Park and Mount Diablo State Park in Contra Costa County, CA. All sites were >1 km apart to limit overlap in bees foraging between multiple sites in a single foraging bout. 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°C with wind between 0 and 2.2 m s^{-1} when bees are most active. One to two sites were monitored each day within a three to four day sampling period for the presence of the bumblebee *Bombus vosnesenskii*. Five bi-weekly sampling periods occurred from mid-May to late July. Sites sampled during the same bi-weekly sampling period were grouped for analysis. Sites were found to have significantly more floral species similarity within than between sampling periods when compared using multiple response permutation procedure ($A = 0.122$, $p = 0.05$, Supplementary material Appendix 1 Fig. A1) (McCune and Grace 2002) which suggests this was an appropriate grouping of sites across time and that the sampling periods were temporally different enough to be treated independently.

When *B. vosnesenskii* was present at a site, the site was sampled to obtain pollen use and pollen availability data. Availability herein refers to the measured abundance of inflorescences and the estimated pollen and floral availability. Multiple bees sampled at each site and multiple sites sampled represents a replicated type II design as described by de Valpine and Harmon-Threatt (2013). 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 217 pollen loads for analysis. Such pollens may have occurred in the use dataset because they were collected from outside of the one hectare site sampled or transferred between bees within the nest.

Study organism

Bombus vosnesenskii is a widely-distributed species, occurring along most of the western coast of North America (Stephen 1957). This species is known to be polylectic (Thorp et al. 1983) which suggests that observed pollen selection is based on preference rather than specialization. Additionally, because *Bombus* species collect pollen into their corbiculae (a specialized structure on the rear leg), the entire pollen load can readily be removed without collecting the individual bee, providing conclusive records of the pollen species that individual *Bombus* are actively collecting and in what proportions.

Pollen use

A single pollen load was removed from each bee netted in the field. Removing a single load provided a marker to prevent recapturing the same bee. The pollen load was removed with forceps, placed in a microcentrifuge tube, labeled and filled with 70% ethanol. A minimum of five bees were collected from each site during a sampling round with a mean of 15.4 bees captured per site.

In the lab, individual pollen loads were homogenized and then a 10 μl sample was placed on a slide with a drop of mixed glycerin and fuchsin dye to aid in visualizing features of pollen grains. Pollen grains were then identified to plant species by comparing them to reference slides created from pollen collected directly from identified plant species collected simultaneously at the sampling sites. Three hundred pollen grains were identified to plant species (j) for each bee load (i); only pollen loads for which $>95\%$ of grains could be identified were used in the study. We assume that presence of pollen in the corbicula reflects active selection of those pollens unless behavioral observations indicated that a plant species was only used for nectar (which can be easily distinguished from pollen collecting) and thus pollen observed in the corbicula was considered incidentally collected. A single plant species *Carduus pycnocephalus* was removed from analysis because it has large, sticky pollen and prominent anthers which may increase incidental collection but bees were only observed nectaring on this species. Of the 51 bees with some pollen of this species in only 15% of cases did that collection exceed 5% of all grains collected so removal only affected a small number of all bees caught. Removal of these incidental collections helps reduce possible contamination of samples, a source of concern in other studies of resource use (Keating and Cherry 2004).

Floral availability

Vegetation was sampled systematically to ensure equal sampling of the entire one hectare site. Fifty 1-m^2 quadrats were evenly spaced along a grid throughout the site and sampled for flowering vegetation. All flowers, flower heads, or inflorescences within the quadrats appearing to have receptive stigmas or productive anthers were counted. Inflorescences arranged in dense heads (e.g. Asteraceae spp. and *Trifolium oliganthum*) were counted as individual flowers. For plant species with inflorescences in which a single flower could be visited individually by bees, but not quickly counted in the field, both number of inflorescences and number of flowers on a haphazard sample of ten inflorescences were counted in the field to estimate the total floral availability at each site; thus we obtained estimates of floral availability at both the inflorescence level and flower level. A list of all plant species blooming within a site was also recorded to account for any species not found within quadrats, so that they were not falsely recorded with zero availability. If pollen load samples included pollen of a plant species not occurring in quadrat sampling but recorded at the site, availability was adjusted to reflect a single inflorescence.

Estimate of pollen production and pollen availability

In order to estimate pollen production, five 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 µl of 70% ethanol. Forty-five µl of fuschin stain were added to each tube to stain the pollen. Samples were homogenized and 10 µl were prepared on slides. Each sample (one flower head or bud) was subsampled five times. Two photographs were taken under 80 × magnification (some samples required different magnification and were scaled accordingly) of each slide prepared. Multiple samples and photographs were taken to reduce inaccuracy due to clumping in the sample or on the slide.

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

To estimate pollen availability for each plant species blooming, average pollen production was multiplied by the floral availability found at a site.

Models

We formulated statistical models representing three competing hypotheses for bee pollen use. Under the ‘random use’ hypothesis, bees sample pollen completely randomly, and hence use is directly proportional to availability, with no preference. Under the commonly-used ‘linear preference’ hypothesis, bees have simple relative preferences, such that use equals availability times preference for each pollen species, divided by the sum of availability times preference so that use proportions sum to 1 (Aebischer et al. 1993). Under the ‘target use’ hypothesis (de Valpine and Harmon-Threatt 2013), use is neither random nor proportional to availability times preference. Rather, use is considered unrelated to abundance of the plant availability within the site and instead use is equal to a constant divided by the sum of such constants for all plant species present at a site to produce relative preferences for each species within a site.

Each of these models is thoroughly described by deValpine and Harmon-Threatt (2013) using simple equations to predict pollen use, with some parameters estimated to determine preference or resource use within a sampling period. In order to use maximum likelihood estimation, the models also need realistic distributions for the sampled data of pollen use. Individual bees typically had pollen mostly from one plant species with small amounts from other plant species, and variability among pollens collected by bees within the same site was high. Thus, the resource use data were clearly over-dispersed relative to a simple distribution such as the multinomial. We have proposed using a mixture of the Dirichlet distribution for variation in use proportions between individual bees and the multinomial distribution for variation in sampling of pollen within a given load, which together yield the Dirichlet-multinomial distribution, as a parsimonious distribution for over-dispersed compositional count data (de Valpine and Harmon-Threatt 2013). This distribution requires estimation of one variance parameter and had previously been used for compositional paleopollen analysis (Mosimann 1963, Paciorek and McLachlan 2009) but had not been put into a general framework for resource use models. Each model was estimated by maximum likelihood using this distribution for residual variation.

We compared the explanatory role of each type of availability data (pollen, flower and inflorescence) by comparing negative log-likelihood values, which are equivalent to half of the AIC values since the models have the same number of parameters and AIC involves log-likelihood multiplied by 2. For each type of availability data, we compared the three hypotheses (models) and evaluated goodness-of-fit of each hypothesis using parametric bootstraps. For each bootstrap we simulated 200 data sets according to one of the estimated models, keeping the availability data and the number of bees sampled at each site constant. To compare hypotheses, we used likelihood ratio test statistics. The random use model was compared to the linear preference model, and the linear preference model was compared to the target use model. In each case the bootstrap replicates were refit to both models

Table 1. Negative log-likelihood values for random use models using each scale of availability (pollen, flower and inflorescence). Smallest values in bold and suggest better fit within the random use models.

	Pollen random use	Flower random use	Inflorescence random use
Sampling period 1	311.3	249.9	**246.3**
Sampling period 2	**148.4**	231.8	196.6
Sampling period 3	**271.4**	445.4	362.2
Sampling period 4	**272.3**	433.8	373.1
Sampling period 5	**172.3**	199.4	186.7

to determine the null distribution of the likelihood ratio test statistic. When possible, we also considered the usual χ^2 null distribution from large-sample theory, which gave similar results, but we present the bootstrap results. To assess goodness of fit for each model, we compared the maximum likelihood of the model to the bootstrap distribution of maximum likelihoods for simulations from that model. These tests evaluate whether the data could have reasonably been generated by a model, without comparison to a specific alternative model.

Data deposition

Data available from the Dryad Digital Repository: <http://dx.doi.org/10.5061/dryad.6kn05> (Harmon-Threatt et al. 2016).

Results

In total 230 bees were captured during the study. Removal of bees for which more than 5% of the counted pollen was unknown or from plants not occurring at the site reduced the total sample to 217 pollen loads for analysis. Over the course of the sampling, *Bombus vosnesenskii* collected pollen from fifteen plant species, supporting previous findings that *B.vosnesenskii* is a generalist forager. Eighty-three percent of bees captured carried more than one pollen type and thus the compositional analysis used here was most appropriate for this data. Availability for each species at a site was highly variable when the scale of measurement used changed (e.g. *Trifolium oliganthum* provided 10% of the available pollen, 49.5% of the available inflorescences and 83% of the available flowers in a single site).

Table 3. Hypothesis testing, based on inflorescence availability. Models were compared to determine if they were significantly different in their fit of the data. p-values shown represent the significance of the one- way test of the first compared to the second model. Bootstrapping to compare the linear preferences model to the random use model for sampling period 3 was not conducted because the random use model had a much worse likelihood.

	Inflorescence linear preference to random use	Target use to inflorescence linear preference
Sampling period 1	$p < 0.005$	$p < 0.005$
Sampling period 2	$p < 0.005$	$p = 0.165$
Sampling period 3	–	$p = 0.970$
Sampling period 4	$p < 0.005$	$p < 0.005$
Sampling period 5	$p < 0.005$	$p = 0.010$

The random use model had much worse log-likelihoods for all measures of availability than the corresponding linear preference models (Table 1, 2) clearly suggesting better AIC scores for all linear models when number of parameters is considered. Estimates of preference from the linear preference models using inflorescence availability were equivalent (i.e. differing by less than 2 in AIC or 1 in log-likelihood) or better than models using pollen or flower availability across all sampling periods (Table 2, left side of vertical line). More specifically, the linear preference model using inflorescence availability better fit the data in sample period 3 and was as good or slightly better in sample periods 1, 2, 4 and 5. In sampling period 1, inflorescence and flower availability were much better than pollen availability but were nearly tied with each other. In sampling periods 2 and 5, inflorescence and pollen availability were similar, while flower availability was slightly worse. In sampling period 4 the three types of availability data were virtually tied. Thus, measuring availability at the scale of inflorescence produced as good or better estimates than floral or pollen availability in the linear preference model.

When compared directly using inflorescence availability, the linear preferences model was superior to the random use model in all measurable sample periods ($p < 0.005$, Table 3). Indeed, the random use model failed the goodness-of-fit test for 3 out of 5 sampling periods (data not shown). For sampling period 3, the random use model provided such a poor fit that bootstrapping was hindered for numerical reasons, and no comparison could be made. The target use model, however, was a significantly better model than the linear preference model in 3 of 5 cases (Table 3, Supplementary material Appendix 4). Note that the target use and linear preference models estimate the same number of parameters and thus can be compared directly. The goodness-of-fit test for the linear preference model across different scales was acceptable for all sampling periods except sample period 4 and for all sampling periods for the target use model (Supplementary material Appendix 4), even though these models were sometimes significantly different.

Table 2. Negative log-likelihood values for linear preference and the target use models. Smallest values are bolded and suggest better fit comparing across the linear preference and target use models. α is the number of parameters estimated which includes the variance parameter of the Dirichlet-multinomial. Italicized values were not significantly different than values in bold.

	α	Pollen linear preference	Flower linear preference	Inflorescence linear preference	Target use
Sampling period 1	8	232.3	211.3	211.6	**207.1**
Sampling period 2	9	**127.6**	129.3	128.2	*127.7*
Sampling period 3	8	247.2	247.2	**234.4**	263.8
Sampling period 4	7	212.3	212.8	212.7	**195.7**
Sampling period 5	8	102.9	104.6	102.0	**97.6**

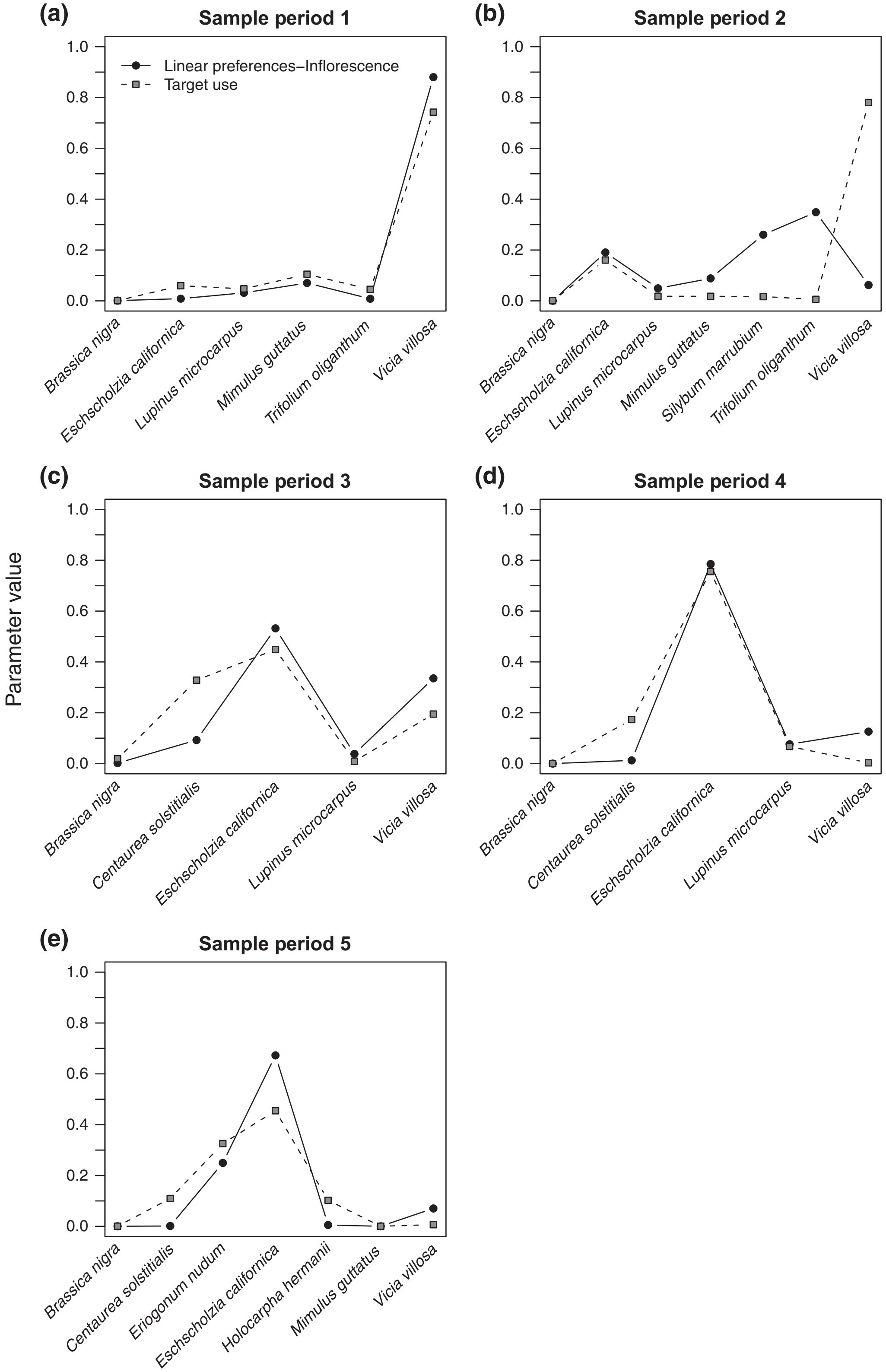

Figure 2. Parameter estimates for the linear preferences model using inflorescence and target use models during each sample period for plants species from which any pollen was collected. Note: confidence intervals were calculated for all cases but in some cases they overlap the estimated preference point and are not drawn.

The difference in interpretation between the linear preferences model and the target use model is illustrated by comparing their parameters for *Trifolium oliganthum* and *Vicia villosa* (Fig. 2b). In the linear preferences model, *T. oliganthum* is estimated to be highly preferred because it is used much more than its low availability, while *V. villosa* is not preferred because it is used less than its high availability. The target use model, however, conveys the opposite message, that *T. oliganthum* is not preferred because it is not collected much, while *V. villosa* is highly preferred because it is the most common pollen collected. Since the target use model does not include availability, 'preference' for this model is just the expected use proportion.

Discussion

Identifying resource preference is important for targeting key resources needed to conserve and restore declining bee species (Cook et al. 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). However, preference for these vulnerable pollinators is difficult to assess for pollen and nectar due to problems quantifying selection and availability as well as identifying proper models for determining resource selection. Bees are known to be highly selective of pollen resources so the poor performance of the random use models was expected. However, although we expected linear preference models including pollen availability, the finest scale of availability measured and the only one that is representative of the actual resource used, we found that use of pollen was equally and occasionally better explained by the coarsest scale of analysis, inflorescence. This could suggest that visual or olfactory cues, which are better represented at coarser scales, are more important for identifying resources than the actual amount of the resource available (Campbell et al. 2010). Alternatively, it is possible that our pollen estimate, which was based on newly opened buds, oversimplifies the variability in pollen availability which can change throughout the day and over the life span on the flower (Barp et al. 2011). However, previous work has shown that bumblebees can discriminate between conspecific flowers based on pollen quantity (Robertson et al. 1999), so although resource selection models using pollen availability were a worse fit than those based on floral or inflorescence availability in this study, pollen availability may still be a significant factor in determining which flowers within a plant population are visited. Further work looking at bee species level preferences between plant species, such as those done here, paired with preferences between bee individuals would help elucidate the relationship between the fine and coarse scale preferences found here and in other studies.

Most interestingly, the target use model, which assessed whether use of plant species was unrelated to availability, was as good or better in four out of five sampling periods than either the linear preferences or random use models. There are many possible reasons behind the superiority of the target use model for identifying preferences of bees. First, many species of bees are known to target higher quality resources and exhibit floral constancy – the tendency to collect from a single species – when foraging (Grüter and Ratnieks 2011, Somme et al. 2015). Consequently, target use models may more accurately reflect resource use of pollinators that are known to exhibit behaviors that cause targeting of certain resources. Additionally, the target use model could perform better when availability is difficult to estimate. All 'measures' of availability in natural systems are really estimates based on subsamples. It is possible that these estimates, regardless of the scale (pollen, flower or inflorescence) do not effectively capture the availability as perceived by bees. Lastly, prior behavioral work found that both visual and olfactory cues play a role in bees identifying host plants even when they specialize on them (Burger et al. 2010, Doetterl et al. 2011). Olfactory cues are often perceived over longer scales than visual ones suggesting that adequately understanding bee's perception of availability may require data at multiple scales that are not traditionally recorded and more complex models for each scale. Each of these factors individually – behavior, measures of availability and scales of perception – could explain why linear preference models were inferior to target use models for modelling bee pollen use.

By removing availability from resource use models, the target use model allows us to produce better estimates of flower preference by avoiding the aforementioned problems with accounting for behavior and estimating availability. Consequently, this could allow the target use model to more easily be extended to other situations when resource availability is difficult to quantify. For example, the target use model could be particularly useful in identifying essential resources for cryptic, declining or rare bee species. Additionally, the target use model could be used to identify changes in resource use by comparing pollen on historic specimens in museum collections to recently-collected specimens (Kleijn and Raemakers 2008). These significant benefits of the target use model could expand the use of resource selection analysis to numerous species and time periods that are difficult to estimate availability but important to understand resource preference.

While estimated preferences cannot be directly compared between models due to differences in computation and plant species availability, it is interesting to note that linear preferences based on inflorescence availability were occasionally quite different than target use preferences (Fig. 2b). This suggests that plants recommended for restoration based on different use models could be notably different and could impact pollinator conservation. Additionally, the resource use estimated by the target use model suggests that, while it is often assumed native plants are a superior resource for native bees (Kearns et al. 1998, Potts et al. 2010), invasive plants such as vetch *Vicia villosa* and yellow star thistle *Centaurea solistitalis* can be highly preferred. It is interesting to note that *V. villosa,* the most preferred species during two sampling periods (based on the target use model), is the subject of biological control efforts in some ecosystems (Baraibar et al. 2011). Additionally, *C. solistitalis* pollen was occasionally collected in greater relative proportion even in sites when its availability was low (Supplementary material Appendix 2 and 3). Our 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 alternative suitable resources are not available (Goulson et al. 2011). Therefore, when removing invasive species, effort should be made to replace them with highly preferred native species (such as *Mimulus guttatus* and *Eschscholzia californica* in our study system). 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 the colony is more selective of resources when producing reproductive individuals later in the season. Further analysis is needed to understand factors influencing preference such as nutrition, development and morphology (Rasheed and Harder 1997, Roulston et al. 2000) and the inclusion of invasive plant species into native bee diets (Stout and Morales 2009, Harmon-Threatt and Kremen 2015).

Many conservation efforts for pollinators focus on providing a suite of floral resources, such as the installation of hedgerows into agricultural land (Winfree 2010, Morandin and Kremen 2013), but selection of these plants are often based on visitation records, which pool pollen and nectar visits (Frankie et al. 2005, Menz et al. 2010), and 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, many of which are declining globally due to their low effective population size, high resource demands and sensitivity to habitat degradation (Goulson et al. 2008, Cameron et al. 2011). Some species declines have been directly correlated to narrow pollen use (Kleijn and Raemakers 2008) and diet breadth (Goulson et al. 2005). Additionally, because bumblebees have greater floral demands than many other bee species due to their large colony size and long foraging period, it is suggested that conservation efforts targeted for them will also benefit other bee species (Goulson et al. 2008, Kleijn and Raemakers 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.

Conclusions

The target use model proposed here, which determines pollen preferences without consideration of availability, was as good or better than more classic models of preference. If this model proves robust in other systems this could significantly expand the ability to determine preferences for bees that are rare, declining or specimen only represented in collections. A better understanding of preferences for bees is necessary for helping improve conservation efforts and methods that expand preference analysis are needed.

Acknowledgements – The authors thank the Harmon-Threatt and Kremen Lab Groups for helpful suggestions to improve the manuscript, Greer Ryan and Wanda Bonneville for assistance with data collection, the California State Parks Department and East Bay Regional Parks Department for access to public lands for research purposes.

Funding – This study was funded by the Western Sustainable Agriculture Research and Education grant (GQ09-018). Additional funding to AHT was received from the NSF- Graduate Research Fellowship and UC Chancellor's Fellowship.

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Supplementary material (available online as Appendix oik-03550 at <www.oikosjournal.org/appendix/oik-03550>). Appendix 1–3.