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# Colony-Level Variation in Pollen Collection and Foraging Preferences Among Wild-Caught Bumble Bees (Hymenoptera: Apidae)

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**ABSTRACT** Given that many pollinators have exhibited dramatic declines related to habitat destruction, an improved understanding of pollinator resource collection across human-altered landscapes is essential to conservation efforts. Despite the importance of bumble bees (*Bombus* spp.) as global pollinators, little is known regarding how pollen collection patterns vary between individuals, colonies, and landscapes. In this study, Vosnesensky bumble bees (*Bombus vosnesenskii* Radoszkowski) were collected from a range of human-altered and natural landscapes in northern California. Extensive vegetation surveys and Geographic Information System (GIS)-based habitat classifications were conducted at each site, bees were genotyped to identify colony mates, and pollen loads were examined to identify visited plants. In contrast to predictions based on strong competitive interactions, pollen load composition was significantly more similar for bees captured in a shared study region compared with bees throughout the research area but was not significantly more similar for colony mates. Preference analyses revealed that pollen loads were not composed of the most abundant plant species per study region. The majority of ranked pollen preference lists were significantly correlated for pairwise comparisons of colony mates and individuals within a study region, whereas the majority of pairwise comparisons of ranked pollen preference lists between individuals located at separate study regions were uncorrelated. Results suggest that pollen load composition and foraging preferences are similar for bees throughout a shared landscape regardless of colony membership. The importance of native plant species in pollen collection is illustrated through preference analyses, and we suggest prioritization of specific rare native plant species for enhanced bumble bee pollen collection.

**KEY WORDS** foraging behavior, pollinator, *Bombus*, bumble bee, pollen

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In recent years, honeybees and several native bumble bee species have shown sharp declines in population sizes in North America and Europe (Goulson et al. 2008, vanEngelsdorp and Meixner 2010, Cameron et al. 2011, Colla et al. 2012). Given that insect-mediated pollination facilitates increases in fruit, vegetable, and seed production for the majority of global crops, pollinator limitation resulting from population declines could substantially reduce global crop productivity (Klein et al. 2007, Potts et al. 2010). More than US\$224 billion in human food crops depends directly on pollinator activity (Gallai et al. 2009); thus, pollinator conservation represents an important effort toward stabilizing global food security (Steffan-Dewenter et al. 2005).

Bumble bees are a particularly important group of native pollinators for both wild and cultivated plants (reviewed in Kremen et al. 2002, Goulson et al. 2008) and have been found to be essential in maintaining the stability of many plant–pollinator networks (Memmott et al. 2004). Like other bees, bumble bees rely on pollen and nectar as food sources (Plowright et al.

1993), with pollen serving as a protein source and nectar acting as an energy source (Rasheed and Harder 1997). Owing to substantial variation in the nutritional value of various pollen sources (Roulston and Cane 2000, Tasei and Aupinel 2008), bees may show greater selectivity for pollen than nectar (Wcislo and Cane 1996, Cane and Sipes 2006), making research on pollen consumption an understudied priority for landscape biology (Harmon-Threatt 2011, Davis et al. 2012, Jha et al. 2013).

Pollen collection is especially important for bumble bee conservation, given that bumble bee larvae are sensitive to pollen diet composition, with low-diversity pollen diets leading to reduced larval growth compared with higher-diversity pollen diets (Tasei and Aupinel 2008). Experimental studies have also found that bumble bee nests grow more rapidly and achieve greater final weights when supplied with greater pollen diet diversity (Goulson et al. 2002a). In addition, comparisons of historical pollen load composition have found lower species richness in pollen loads of declining species compared with stable species before the onset of decline (Kleijn and Raemakers 2008), suggesting that over long time periods, a narrow range

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of host plant species may reduce bumble bee survivorship and eventually lead to species decline (Kleijn and Raemakers 2008). Contemporary pollinator conservation efforts require an examination of pollen collection patterns at shorter timescales reflective of current land-use practices, individual forager behavior on a daily scale, and colony diet over weeks.

Characterizing colony-level resource collection is of particular interest to bumble bee conservation, given that successful bee colonies exhibit coordinated foraging efforts to acquire appropriate balances of required resources (Camazine 1993, Goulson et al. 2002b) and that pollen diet directly influences larval survivorship and colony growth (e.g., Goulson et al. 2002a, Tasei and Aupinel 2008). Coexisting colonies within a landscape presumably have access to shared resources based on the capacity of individual bees to forage over great distances (Osborne et al. 1999, Hagen et al. 2011, Rao and Strange 2012, Jha and Kremen 2013a), but it is unknown whether colony membership influences the pollen collection patterns of wild bumble bees. Past research on bumble bee colonies has found that overall colony pollen diet is determined in part by vegetation surrounding colony location (Takeuchi et al. 2005, Munidasa and Toquenaga 2010) and that surrounding landscape influences individual pollen load richness (Goulson et al. 2002a). However, colony-level variables such as colony size and colony identity have also been found to play a role in the use of available plants and novel rewarding flowers (Raine et al. 2006, Munidasa and Toquenaga 2010, respectively), suggesting that colony mates may have similar pollen collection patterns.

In contrast, interactions between conspecifics in a shared landscape can also alter foraging behavior by repelling bees from previously visited flowers (Goulson et al. 2001), suggesting that competitive interactions may drive individuals within a colony or within a landscape to forage on different resources. At high densities of conspecifics, individual bumble bees exhibit reduced floral constancy (Baude et al. 2011) and diet breadth expansion (Fontaine et al. 2008), and studies suggest that the removal of competing conspecifics or congeners allows other wild bees to forage more efficiently (Thomson et al. 1987). If such competitive forces are strong between colony mates or individuals in the same landscape, these forces could drive divergent foraging patterns at the colony or landscape level.

Although previous studies make great contributions to understanding some factors contributing to variability in pollen diet, much still remains unknown about the exhibited "preferences," defined as pollen collection relative to availability (Beyer et al. 2010), of wild bumble bees. Pollen collection preferences have been found to be driven in part by nutritional rewards inherent to pollen of particular plant species (Harmon-Threatt 2011), suggesting that foraging preferences may be influenced by uniform external rewards for bees of a species. In addition, one study of wild bumble bees shows that they can exhibit strong preferences for a small number of plant species (Jha et al.

2013). However, prior studies have not examined how individual wild bees or colonies compare in their collection or preference patterns across study regions. By genetically sorting wild-caught bees into colonies, the current study represents the first effort, to our knowledge, to characterize pollen load composition and pollen collection preferences from wild-caught bees with identified colony membership.

In this study, pollen loads were collected from captured individuals of the Vosnesensky bumble bee, *Bombus vosnesenskii* Radoszkowski. This polylectic species is among the most important crop pollinators on the West coast of the United States (Kremen et al. 2004) and primarily nests underground in colonies of 200–300 workers (Shelly et al. 1991). Although *B. vosnesenskii* is not exhibiting range-wide declines (Cameron et al. 2011), the species exhibits reduced nesting densities and reduced dispersal in highly urbanized landscapes (Jha and Kremen 2013a,b), making it an important conservation target. We analyzed pollen loads collected by foraging bees on the day of capture and incorporated molecular data to subsequently identify bee colony membership. Specifically, we characterized pollen load composition, richness, and diversity at the individual and colony level and examined pollen collection relative to availability.

Based on bumble bee access to similar resources, the potential for colony-level influences, and shared floral rewards, we have developed three major hypotheses: 1) pollen load composition will be more similar for colony mates than individuals within a study region and will be more similar for individuals within a study region than individuals distributed across large biogeographic areas, 2) pollen collection preferences will be uniform for bees across all study regions, and 3) pollen load richness and diversity will correlate with floral cover, floral richness, and natural landscape cover.

## Materials and Methods

**Study Sites and Bee Collection.** Research was conducted from 1st June to 14th July of 2009 within eight independent study regions extending from Sonoma to Sacramento County, in the Bay and Delta bioregions of northern California (e.g., Kremen et al. 2002, 2004). Study regions were separated by 3.89 to 118.25 km (mean  $49.76 \pm 28.09$  km) and were each composed of a 1.2-km transect, subdivided into five sites spaced 300 m apart. A mean of  $104.3 (\pm 2.8)$  *B. vosnesenskii* worker bees were net-collected in a single day from each study region, corresponding to a mean of  $20.8 (\pm 2.18)$  bees from each site. In total, >800 bees were collected from 40 sites (described in Jha and Kremen 2013a,b), a subset of which were systematically selected for pollen load analysis (described in Colony Membership section). On capture, individual bees were temporarily immobilized in a bee squeezer, a small mesh-walled chamber, to allow for the pollen load to be removed from the right corbicula by sterilized forceps and transferred to 1 ml of 95% ethanol.

**Vegetation Surveys and Land-Use Classification.** Vegetation surveys recording flowering species richness, the number of flowering inflorescences, and petal area per inflorescence in 1- by 1-m quadrats were conducted on the same day as bee collection. Quadrats were randomly placed at 12 locations within a 250-m radius of each of five sampling sites in each of the eight study regions, as well as at 12 locations between each sampling site 300 m perpendicular to the transect. In total, 108 1- by 1-m quadrats were surveyed per study region, with 60 quadrats at sampling sites along the transect and 48 quadrats perpendicular to the transect between sampling sites. Floral cover was then calculated based on inflorescence count multiplied by the petal area per inflorescence. Inflorescence counts have been found to be reliable predictors of site pollen availability, as documented for similar plant species within the same study region (Harmon-Threatt 2011).

Land cover throughout a 2-km radius surrounding each site was manually assigned to one of seven categories (oak chaparral, riparian forest, weed or grass, annual crop, planted crops, bare, or impervious surface) using aerial imagery at a resolution of 5 m in the computer program ArcGIS (Environmental Systems Research Institute [ESRI] 2006). In addition, land-use classifications were confirmed with ground-truthing surveys at every site. The proportion of human-altered land cover (cultivated crops, bare, and impervious areas mean =  $0.23 \pm 0.24$ ), nonwoody natural land cover (weed or grass mean =  $0.13 \pm 0.11$ ), and woody natural land cover (oak chaparral and riparian forest mean  $0.61 \pm 0.37$ ) varied substantially within a 2-km radius of each study region.

**Colony Membership.** Multiplex polymerase chain reactions were performed on DNA extracted using the HotShot protocol (Truett et al. 2000) from the tarsal segment of the right hindmost leg of individual bees. Thirteen microsatellite loci (Stolle et al. 2009) distributed across 10 different chromosomes (Stolle et al. 2011) based on the *Bombus terrestris* genome were screened. Molecular techniques are detailed in Jha and Kremen 2013b. Colony membership was determined based on genetic similarity using COLONY 2.0 (Wang 2004, described in Jha and Kremen 2013a,b). Colonies with three or more captured individuals carrying pollen loads were selected for pollen load analyses. Forty-one individuals belonging to a total of 12 colonies representing five of the eight study regions were included in the analysis based on the selection criteria of a minimum of three captured individuals carrying pollen loads per colony.

**Pollen Identification.** While conducting vegetation surveys, anthers were collected from each flowering plant species and stored in 95% ethanol. Pollen was then extracted by pipette from solution surrounding the stored anthers, acetolysed following standard protocols with slight modifications, stained with fuschin dye, and mounted (as per Kearns and Inouye 1993). Pollen was photographed at 630 $\times$  magnification to create a reference library for the 63 flowering plant species encountered in the vegetation surveys.

Pollen loads gathered from the corbucula of selected bees were also stored in ethanol. Following the same protocols used for pollen collected throughout the vegetation surveys, bee pollen loads were acetolysed, dyed, and mounted. Each slide was scanned systematically, and the first 300 grains to be encountered were identified based on the reference collection. It was not possible to make species-level identifications of pollen grains belonging to some species in the *Asteraceae* family or to distinguish between three particular species of the *Convolvulus* genera. If a single slide was insufficient for reaching 300 grains, multiple slides were mounted or a new sample of pollen from the original load was acetolysed and mounted. In total, a small fraction of pollen grains were unidentifiable (<1.7%). Owing to dehydration, one pollen load included in subsequent analyses repeatedly resulted in low yield following acetolysis and mounting. Only 50 grains were identified for this load as opposed to the target of 300 grains.

**Data Analysis. Pollen Load Composition and Similarity.** Individual pollen load richness was calculated by counting the number of observed taxa in each individual pollen load. Values were averaged for individuals of a shared colony to calculate colony-level means and standard deviation. Diversity was calculated at the individual level using Simpson's diversity index (Simpson 1949), reported as 1-D, and individual values were averaged to calculate colony-level mean diversity and standard deviation. Pooled pollen loads for all individuals of a shared colony were also used to calculate colony-level diversity. Rarefaction curves, which plot the increase in species richness per added sample, were generated for observed pollen load richness in the computer program EstimateS Version 9 (Colwell 2013) to overcome differences in sample size and to estimate overall richness. The Chao1 estimate of richness was calculated for each individual and for each colony to allow for comparisons of richness across different sample sizes (Colwell et al. 2012).

Four generalized linear models (GLMs) were constructed in the computer program R (R Core Team 2013) to examine the relationship between three predictor variables: 1) regional average floral richness, 2) regional average floral cover, and 3) landscape-level proportion of riparian forest and oak chaparral, and four response variables: 1) individual estimated pollen load richness, 2) individual pollen load diversity, 3) colony-level estimated pollen load richness, and 4) colony-level pollen load diversity. Colony-level richness and diversity were estimated from all bees assigned to a given colony. The use of GLMs is preferred for nonnormally distributed ecological count data over linear models with log-transformed data (O'Hara and Kotze 2010). Estimated pollen load richness values were reported as nearest-integer values of Chao1. Models with richness as response variables used the Poisson distribution with a log link function, as supported by previous studies (e.g., Vincent and Haworth 1983, Pellissier et al. 2013). Models with diversity as response variables used the binomial distribution with a logit link function, as shown in various models with

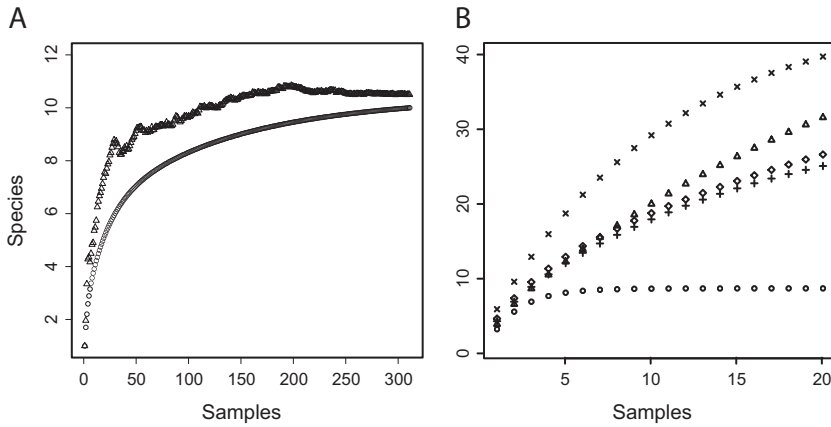


Fig. 1. (A) Smoothed accumulation curve and Chao1 estimate of richness based on >300 pollen grains analyzed from pollen loads belonging to 1 of the 41 individuals analyzed, ( $S(\text{est}) = 10$ ,  $\text{Chao1} = 10.5$ ). Legend: smoothed richness (O), Chao1 estimate of richness ( $\Delta$ ). (B) Extrapolated accumulation curves for 20 samples based on richness for all individuals sampled per study region, with symbols corresponding to study region. Legend: region A (+), region B ( $\diamond$ ), region C (O), region D ( $\Delta$ ), and region E ( $\times$ ).

proportional data restricted to a range between zero and one as response variables (e.g., Haas et al. 2011, Anderson et al. 2013). Each model was compared with a corresponding GLM in the absence of predictor variables in an ANOVA using a chi-squared test to evaluate the overall model.

A permutational MANOVA test with the four predictors, 1) regional average floral richness, 2) regional average floral cover, 3) colony, and 4) region, was conducted on a matrix of plant taxa and individual bee pollen loads in R using the “vegan” package (Oksanen et al. 2013). To parse the effect of colony membership and study-region membership on pollen load composition, the permutational MANOVA was repeated with permutations constrained to individuals with shared study-region membership. In addition, the “vegan” package (Oksanen et al. 2013) was used to perform nonmetric multidimensional scaling (nMDS) on individual pollen collection per plant taxa to generate an ordination plot visualizing the similarity of pollen loads coded by colony membership and study region.

**Preference Analysis.** Preference analysis was conducted as per classical methods (Johnson 1980) for the 14 plant taxa that represented over 0.1% of pollen loads and which were observed in vegetation surveys. Ranked preference lists were generated for each individual by ranking plant taxa in order of usage and separately ranking plant taxa in order of study region-level availability. Differences between usage and availability were used to infer preference, with negative values representing preference or usage above availability and positive values representing avoidance or usage below availability (as per Johnson 1980). This method has been used in studies examining resource use relative to availability in previous bee studies (Williams et al. 2011), as well as for resource use by numerous other organisms. Ranked preference lists for all individuals located at study regions with two or more identified colonies were compared with pairwise

Kendall rank correlation tests using the “Kendall” package in R (McLeod 2011). Pairwise comparisons of individual ranked preference lists were categorized as within-colony comparisons for colony mates, within-region comparisons for individuals of separate colonies located at a shared study region, and between-region comparisons for individuals from separate study regions. The proportion of significant Kendall rank correlation values ( $P < 0.05$ ) was recorded for each category.

## Results

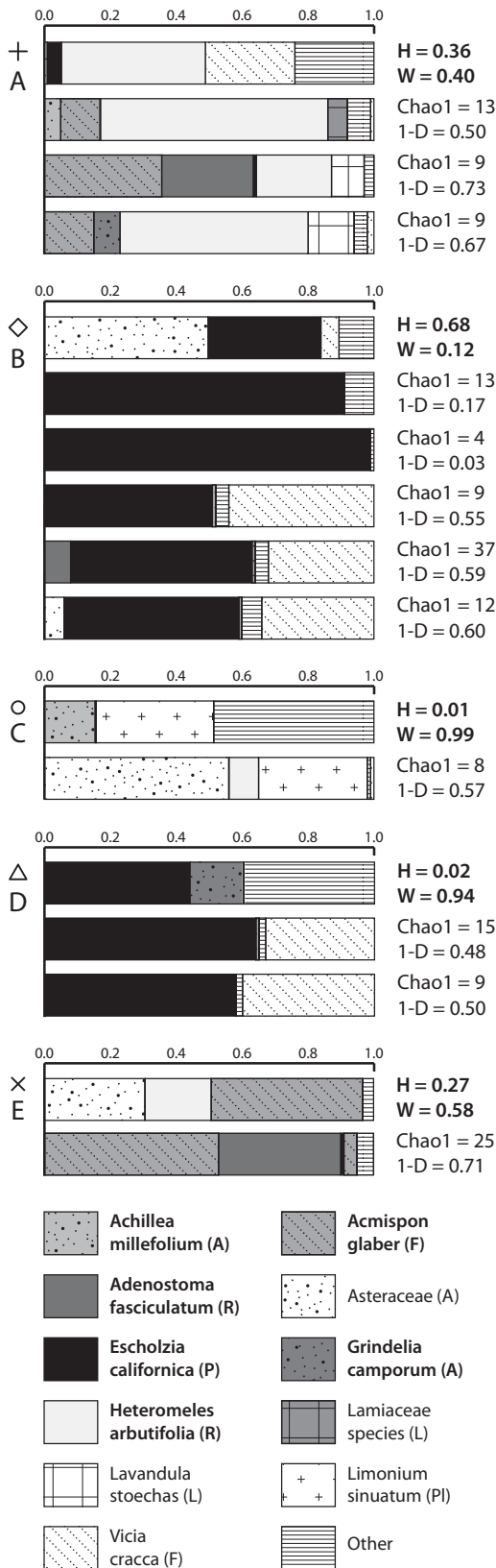
**Individual, Colony, and Overall Pollen Load Composition.** At the individual level, pollen load richness ranged from 1 to 10, with a mean richness of  $4.5 \pm 2.6$  plant taxa. Estimated individual richness using Chao1 ranged from 1 to 13. Individual pollen load diversity calculated as Simpson’s D (1-D) ranged from 0 to 0.75, with a mean diversity of  $0.21 \pm 0.23$ . Accumulation curves for individual pollen loads reached an asymptote at the sample size of 300 grains for 34 of 41 individuals (83%; Fig. 1A).

Pooled colony-level richness ranged from 4 to 14 plant taxa, with a mean of  $9.75 \pm 3.04$ . Estimated richness at the colony level ranged from 4 to 37 plant taxa. Mean colony-level diversity, calculated by finding the mean of Simpson’s D values for all individuals sampled in a colony, ranged from  $0.02 \pm 0.02$  to  $0.39 \pm 0.11$ .

Examining overall pollen loads across all 41 bees distributed across eight study regions and 12 colonies yielded 38 pollen taxa. The Chao1 estimate of richness calculated a total richness  $>46$ . A rarefaction curve for the Chao1 estimate of richness based on all 41 sampled bees reached an asymptote at  $\approx 35$  samples.

Three species contributed to  $>70\%$  of overall pollen diet, whereas most other species were observed infrequently. *Eschscholzia californica* Cham, *Vicia*





*cracca*, and *Heteromeles arbutifolia* accounted for 42, 16, and 13% of overall pollen loads, respectively. *Acmispon glaber* (Vogel) and *Adenostoma fasciculatum* each represented 6% of overall pollen collection. All other plant taxa represented <5% of overall pollen load composition. Proportional usage of plant taxa varied between colonies and across study regions (Fig. 2).

**Pollen Load Diversity and Floral Resource Levels.** Estimated individual pollen load richness was not correlated with regional average floral richness ( $\beta = -0.059$ ;  $P = 0.805$ ), regional average floral cover ( $\beta = -0.002$ ;  $P = 0.124$ ), or proportion of riparian forest and oak chaparral cover ( $\beta = -0.54$ ;  $P = 0.368$ ; overall  $P = 0.223$ ;  $df = 40$ ). Estimated colony-level richness was not correlated with regional average floral richness ( $\beta = 0.071$ ;  $P = 0.796$ ), was negatively correlated with regional average floral cover ( $\beta = -0.004$ ;  $P = 0.021$ ), and was not correlated with proportion of riparian forest and oak chaparral cover ( $\beta = -0.534$ ;  $P = 0.425$ ; overall  $P = 0.039$ ;  $df = 11$ ). Individual pollen load diversity was not correlated with regional average floral richness ( $\beta = -1.283$ ;  $P = 0.340$ ), regional average floral cover ( $\beta = 0.002$ ;  $P = 0.792$ ), or proportion of riparian forest and oak chaparral cover ( $\beta = -3.577$ ;  $P = 0.313$ ; overall  $P = 0.778$ ;  $df = 40$ ). Colony-level diversity was also not correlated with regional average floral richness ( $\beta = -1.201$ ;  $P = 0.557$ ), regional average floral cover ( $\beta = -0.001$ ;  $P = 0.976$ ), or proportion of riparian forest and oak chaparral cover ( $\beta = -2.371$ ;  $P = 0.632$ ; overall  $P = 0.886$ ;  $df = 11$ ).

**Pollen Load Composition Similarity Analysis.** Individuals from a shared study region were significantly more similar than individuals in general (MANOVA,  $P = 0.001$ ), whereas after accounting for study region, individuals within a colony were not more similar than at random (MANOVA,  $P = 0.806$ ). Clustering of individual pollen loads belonging to particular study regions is illustrated in a nMDS ordination plot (Fig. 3).

**Pollen Collection Preference Analysis.** Overall means for individual Johnson preference rankings showed highest preferences exhibited for *Lotus corniculatus*, *Pickeringia montana*, and *Lavandula stoechas* (Supp Fig. 1 [online only]). Means of Johnson rankings for all individuals per study region produced non-identical preference lists at all study regions (Supp Fig. 1 [online only]). The Kendall rank correlation

Fig. 2. (A-E) Stacked bar graphs showing pollen load composition by colony, grouped by study region. First bar graph per study region shows proportions of floral resources at corresponding study region along with values for surrounding land cover proportions throughout a 2-km radius (H, human-altered; W, natural woody; remaining proportion is weed or grass). Chao1 estimate of pollen load richness based on all individuals sampled per colony and colony-level pollen load diversity (Simpson's diversity as 1-D) are also indicated. Native plant species in bold. Plant families indicated in parentheses: Asteraceae (A), Fabaceae (F), Lamiaceae (L), Papaveraceae (P), Plumbaginaceae (Pl), Rosaceae (R).

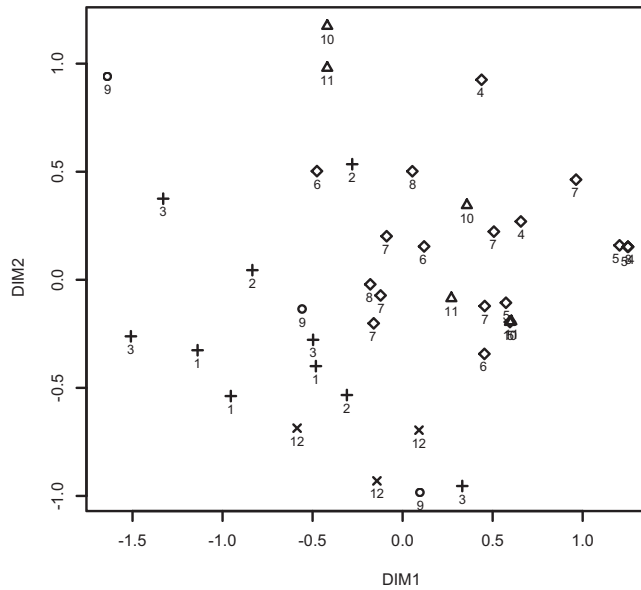


Fig. 3. Ordination plot based on nMDS of individual pollen loads. Colony membership labeled by numbers (1–12) and study region indicated by symbol. Legend: region A (+), region B (◇), region C (O), region D (△), region E (×).

was rarely significant ( $P < 0.05$  for 5.5% of comparisons) when Johnson preference rankings were compared for individuals from separate study regions. Individuals from shared study regions but separate colonies exhibited significantly similar preference rankings ( $P < 0.05$ ) for 69% of such comparisons. Colony mates had significantly similar preference rankings ( $P < 0.05$ ) for 73% of comparisons.

### Discussion

Results from this study describe the first analysis of pollen loads from wild-caught bumble bees with identified colony membership. Overall, 38 discrete plant taxa were observed in pollen loads, with the three species, *E. californica*, *V. cracca*, and *H. arbutifolia* accounting for >70% of total pollen load composition. Consistent with our first hypothesis, pollen load composition was significantly similar for individuals belonging to a shared study region. However, pollen load composition was not more similar for colony mates than other individuals within the same study region. Preferences for pollen taxa were significantly correlated for the majority of individuals located at a shared study region, but contrary to our second hypothesis, we observed disparate pollen preferences between individuals located at separate study regions. Lastly, although neither individual pollen load richness nor diversity correlated with regional floral cover or floral richness, estimated richness at the colony level was negatively correlated with floral cover.

**Pollen Load Composition Similarity.** Our primary finding, that pollen loads are similar between bumble bee individuals within a study region, suggests that competitive forces between colony mates and colo-

nies within a landscape are not strong enough to drive differential foraging patterns. Our results contrast with one past study of pollen analyzed from larval feces, which found that colonies in proximity differed significantly in the proportional usage of different plant species (Munidasa and Toquenaga 2010). The previous study relied on comparisons of pollen diet between three to four coexisting colonies located within a 2.5-km square area, whereas our study compared pollen collection by wild-caught bees belonging to 12 colonies distributed across a much larger spatial scale. In addition to inherent differences in analyzing corbicula pollen compared with fecal pollen, the latter of which may be impacted by digestion, pollen composition differences may be owing to the temporal scale of analysis, given our focus on short-term pollen collection as opposed to long-term colony pollen diet. However, our finding that pollen loads were dissimilar for bees captured from separate study regions is consistent with other past studies that examined pollen collected from colonies across broader time periods (Takeuchi et al. 2005) and across similarly large spatial scales (Goulson et al. 2002a, Takeuchi et al. 2005), which have also revealed that pollen composition varies for colonies located in distinct landscapes. Our findings suggest that location and surrounding landscape, rather than colony membership, play a primary role in driving pollen load composition and similarity between individual foragers.

**Pollen Collection Preferences.** Combining data on floral resources with pollen load composition confirms that foraging is nonrandom, with some plants preferred over others. Despite the predominance of *E. californica*, *V. cracca*, and *H. arbutifolia* in pollen loads, preference analysis reveals that the most abundant taxa in pollen loads are not always the most preferred

taxa. Several species with low overall representation in pollen loads appeared to be highly preferred considering availability (e.g., *L. corniculatus*, *P. montana*, and *La. stoechas*). These highly preferred species represent a mix of native and nonnative plants belonging to the Fabaceae, Lamiaceae, and other plant families. Several plant species belonging to the Fabaceae family were foraged on heavily relative to availability, suggesting potential resource limitation of pollen belonging to a plant family that has previously been shown to dominate pollen loads from multiple bumble bee species (Goulson and Darvill 2004). Other plant species, like certain plants belonging to the *Asteraceae* family, were avoided or used minimally in relation to their availability.

Based on uniform floral rewards regardless of colony membership or location, we hypothesized pollen collection preferences would be similar throughout the research area. This hypothesis is in contrast to evidence of workload partitioning in bumble bee colonies (e.g., Goulson et al. 2002b), which suggests that individual bees in a colony may be expected to exhibit differing foraging preferences. Distinct foraging patterns would be beneficial at the colony level to obtain diverse and stable nutrient sources. However, we hypothesized that shared colony-level variables such as colony size (Munidasa and Toquenaga 2010) or behavioral attributes (Raine et al. 2006) may lead to similar foraging preferences for colony mates while introducing variation between colonies. Our results support this hypothesis, as we found that a majority of ranked preference lists were significantly similar for colony mates as well as for individuals located at a shared study region. Although experimental studies on nectar foraging suggest that colonies may differ inherently in foraging behavior and learning (Raine et al. 2006), we found no evidence for variation in pollen collection preferences between individuals of different colonies in a shared study region. We posit that variation between colonies in terms of learning and foraging behavior may differ for nectar and pollen and may not translate to significant differences in pollen species collection. At a larger scale, we observed variation in foraging preferences between individuals located in separate study regions, indicating floral rewards alone are not likely to explain observed foraging preferences.

**Pollen Load Richness and Diversity and Floral Resource Levels.** Neither individual pollen load richness nor individual pollen load diversity correlated with regional average floral cover, regional average floral richness, or proportion of woody natural cover. This comports with previous studies from the same bioregion, in which floral resource variables were not correlated with the richness or diversity of pooled pollen loads for multiple individuals per site (Jha et al. 2013). The lack of correlation between vegetation features and individual pollen load composition may be owing to the strong responsiveness of individual bees to landscape features at larger spatial scales (e.g., Osborne et al. 1999, Steffan-Dewenter et al. 2002, Jha and Kremen 2013a) or may be explained by strong preferences for

particular taxonomic plant groups (Jha et al. 2013) potentially driven by floral rewards (Harmon-Threatt 2011) and learning behavior; however, these mechanisms remain to be explored further.

Additionally, colony-level analysis revealed that the overall estimated richness of all pollen collected by individuals of a shared colony correlated negatively with floral cover. Our detection of a correlation between particular floral resource variables and pollen load richness at the colony level but not at the individual level suggests that both scales of analyses may be required for gauging interactions between pollen collection and floral resource variables. Previous studies with colony-level analyses have found habitat composition to be predictive of pollen load and pollen diet composition for comparisons of some bee species across distinct landscapes (Goulson et al. 2002a, Williams and Kremen 2007). In addition, a similar negative correlation between pollen load richness and local floral cover has been observed for the collection of native pollen sources in the same bioregion (Jha et al. 2013). This trend may be explained in part by principles of optimal foraging theory, which predict an increase of diet breadth in low-resource areas and a reduction of diet breadth in high-resource areas (MacArthur and Pianka 1966). This prediction is based on the idea that pollinators may adapt foraging behavior to specialize on plant species with learned rewards when these species are readily available, whereas pollinators faced with low floral resource levels may be forced to generalize visits to include a larger number of plant species to meet resource needs. Overall, although individual short-term pollen collection was not correlated with the floral resource variables we measured, our observation of increased colony-level pollen richness at areas of low floral cover is consistent with optimal foraging predictions.

**Broader Impacts.** Identifying species targeted as pollen sources among foraging bees is critical to pollinator conservation and restoration efforts (Williams et al. 2010, Winfree 2010), which could be improved by considering pollen collection patterns in addition to nectar usage (Frankie et al. 2005, Menz et al. 2011). Similar to past studies within the bioregion (Jha et al. 2013), the current study found that two native plant species *E. californica* and *H. arbutifolia* were heavily used by *B. vosnesenskii* and could be good candidates for inclusion in pollinator restoration efforts in northern California. Based on preference lists, the native plants *P. montana* and *Lotus purshianus* may also be suited for pollinator conservation. Retaining diverse floral resources in agricultural landscapes is especially important for provisioning pollinator communities threatened by habitat degradation and low floral diversity resulting from agricultural systems (Nicholls and Altieri 2012). Conservation of important forage species could facilitate the provisioning of bumble bee colonies and other essential wild pollinators, in turn benefitting agriculture through enhanced pollinator services.



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