Bumble Bee (Hymenoptera: Apidae) Foraging Distance and Colony Density Associated With a Late-Season Mass Flowering Crop

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ABSTRACT Foraging behaviors of bumble bee workers have been examined in natural habitats, whereas agricultural landscapes, which can provide insights on flight distances to fragmented patches of bloom, have received limited attention. In particular, information on worker flight distances to crops blooming several months after nests have been established is invaluable. Here, we examined foraging patterns of *Bombus vosnesenskii* Radoszkowski in late-season blooming clover in the agricultural-dominated Willamette Valley in Oregon. Workers from 10 fields collected over 2 yr were assigned to full sibling families (colonies) by using eight microsatellite loci. With estimation of numbers of unseen species, we inferred the presence of 189 colonies from 433 bees genotyped in year 1, and 144 from 296 genotyped the next year. Worker foraging distance was estimated to be at least 11.6 km, half the distance between the most remote fields visited by the same colonies. Numbers of nests contributing workers to each field ranged from 15 to 163. Overall, 165 (50%) colonies foraged in two or more fields, and thus used common resources within the landscape. Estimates of average nest densities in the landscape each year ranged from 0.76/km² to 22.16/km², and highlighted the influences of various study parameters incorporated into the calculation including sample size, distances between sites, and analytical tools used to estimate unsampled individuals. Based on the results, bumble bees can fly long distances, and this could facilitate their survival in fragmented agricultural landscapes. This has important implications for the scale of habitat management in bumble bee conservation programs.

KEY WORDS *Bombus*, microsatellites, pollinators, clover

Bumble bees, *Bombus* spp. (Hymenoptera: Apidae), provide valuable pollination services in natural and agricultural landscapes (Corbet et al. 1991). They are social insects that require continuous access to pollen and nectar resources for colony survival and growth (Alford 1975). Typically, bumble bee colonies develop over several months, whereas bloom in most plants lasts for just a few weeks. Hence, each colony is dependent on multiple plant or crop species that bloom in succession for successful completion of the life cycle.

Loss of foraging resources resulting from habitat fragmentation and subsequent agricultural intensification is speculated to be, in part, responsible for the recent declines in bumble bees reported from Europe and North America (Darvill et al. 2006, Goulson et al. 2008, Grixti et al. 2009). In general, landscapes dominated by large commercial agricultural fields of cereals or grasses are not expected to promote bumble bee colony development as foraging resources are limited (Saville et al. 1997, Svensson et al. 2000). The abundance of bloom in a single agricultural crop that is visited by bees can benefit bumble bee colony de-

velopment if the landscape includes seminatural habitats that provide forage during periods when the crop is not in bloom (Herrmann et al. 2007, Knight et al. 2009, Westphal et al. 2009). Alternatively, agricultural landscapes with multiple cropping systems that bloom in sequence can facilitate bumble bee development (Rao and Stephen 2010). However, as the bumble bee nest is fixed in location, access to the multiple foraging resources at different periods during the life cycle is dependent on the flight distances of workers.

Flight distances of bumble bee species based on estimates made with diverse techniques are variable (See Table in Zurbuchen et al. 2010a). It is not possible to follow individual bees and hence the estimates could well-reflect distances covered during foraging trips and not the distance covered between take off and landing. In mark recapture studies *B. terrestris* (L.) and *B. lapidarius* (L.) navigated back to their nests after being released >9.5 km away (Hedtke 1996 from Walther-Hellwig and Frankl 2000, Goulson and Stout 2001). Mark recapture studies by Dramstad (1996) indicated that *B. lucorum* and *terrestris* workers foraged ≈175–300 m away, although one marked *B. lapidarius* was discovered >300 m from the nest. Numbered tags used by Walther-Hellwig and Frankl (2000) highlighted variation in foraging distances of bumble bee species within the same habitat. In their study, *B. muscorum* (L.) foraged within 100 m and is

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thus a 'door step forager', whereas *B. terrestris* is a 'long distance forager' with foraging distances of 500–1,750 m. A third species, *B. lapidarius,* is an intermediate forager with foraging distances ranging from 500 to 1,500 m. Osborne et al. (1999) used harmonic radar and tracked individual *B. terrestris* up to 631 m from the nest. However, only distances up to 700 m could be tracked with this method.

Recent advances in molecular techniques have enabled the use of microsatellite markers for identification of sisters among sampled bumble bees for estimation of flight distances. With this technique, which assumes single mating by queens, Darvill et al. (2004) estimated foraging distances <312 m for *B. pascuorum* (Scopoli) and <612 m for *B. terrestris.* Knight et al. (2005) estimated that maximum foraging distances for four United Kingdom species ranged from 449 m to 758 m. With similar molecular analyses, dispersing queens and foraging workers of *B. lapidarius* and *B. pascuorum* were detected at distances >5 km by Lepais et al. (2010), whereas Kraus et al. (2009) observed male flight distances close to 10 km. The majority of these studies were conducted in natural or seminatural habitats and hence there is no information on flight distances of workers foraging on isolated patches of crop bloom. Knowledge of the capability to cover varying distances between the nest and the foraging resources is crucial to preservation of the each species (Zurbuchen et al. 2010b). For temperate bumble bees, the size of the population at the end of the year impacts the pollination services provided the next year. Hence, information on flight distances to late season crops that bloom several months after nests have been established will be valuable for insights on bumble bee survivorship in agricultural landscapes.

There is also little information on location of nests of bumble bees foraging in agricultural habitats. Bumble bee nests are difficult to locate but with molecular techniques densities on nests in the landscape can be estimated. Using sample sites with a 50-m radius in an agricultural landscape, Darvill et al. (2004) estimate the presence of 13 *B. terrestris* and 193 *B. pascuorum* colonies per square kilometer. In contrast, a similar study in an urban landscape documented the presence of an average of 96 colonies of *B. terrestris* and 66 colonies of *B. pascuorum* visiting urban parks in the United Kingdom in 2001 (Chapman et al. 2003). At the Rothamsted Research Center in the United Kingdom, 26–117 colonies per km^2 were documented to be present in a study conducted in 2002 and 2003 (Knight et al. 2005). Nest density typically is calculated by using the foraging range as the radius for estimation of the area sampled, and hence it is influenced by estimations of flight distances. This was highlighted in a study by Knight et al. (2009) in which nest density was estimated as 255.8 km^{-2} when forage range was assumed to be 449 m, and as 51.6 km^{-2} with a foraging distance of 1,000 m. In addition, estimations of bumble bee colonies are also being refined with inclusion of estimations of the numbers of nonsampled colonies as it is not possible to capture representatives from every colony contributing foragers to the sampling arena.

However, this additional estimation has not been included in earlier estimations of nest densities.

Agricultural intensification has been correlated with declines in diversity, abundance, and services to crops provided by wild pollinators (Kremen et al. 2007). Yet, in the agriculture-dominated Willamette Valley in western Oregon, a complex of seven bumble bee species is thriving and record crop yields in spring and summer crops have been achieved by producers in this region (Rao and Stephen 2009, 2010; Rao et al. 2009). This is speculated to be because of the presence of multiple mass flowering crops that bloom in sequence and in synchrony with periods of foraging by queens and workers. The region can serve as a model for bumble bee conservation in arable landscapes. However, information is lacking on foraging distances and nest abundances of bumble bee species accessing the bloom in the landscape. We used microsatellite DNA analyses of bumble bee workers collected from western Oregon to estimate 1) numbers of colonies contributing workers to single and multiple mass-flowering fields, 2) foraging distance of bumble bee workers, and 3) nest density of bumble bee colonies across the landscape. The study was conducted with *B. vosnesenskii* Radoszkowski, the dominant native bumble bee species in the region (Rao and Stephen 2010). It was focused on clover crops as they are highly attractive to bumble bees (Free 1993) and, in western Oregon where the study was conducted, their bloom coincides with a dearth of other blooming crops and wildflowers.

Materials and Methods

Study Sites. The study was conducted in commercial seed production fields of red clover (*Trifolium pratense* L.; family Fabaceae) and arrowleaf clover (*T. vesiculosum* Savi; family Fabaceae). The fields, 6–25 ha in size, were located in Polk County in the Willamette Valley in western Oregon. Both crops are rotated with grass crops raised for seed in this region. In 2007, bumble bees were sampled from four fields including two of red clover (Fields A, B) and two of arrowleaf clover (Fields C, D) (Fig. 1). The next year we sampled six fields, four of red clover (Fields E, F, I, J) and two of arrowleaf clover (Fields G, H) (Fig. 1). In each year, sampling was conducted between early July and mid-August. During this period, the two clovers were the only commercial crops in bloom.

Sample Collection. During visits to each field we collected *B. vosnesenskii* workers foraging on clover bloom. Individuals were collected using hand-held clear plastic vials with a snap cap. Samples were chilled in the field in a portable cooler for transport to the laboratory, and then frozen at −40°C.

In the laboratory, a single leg, usually the mesothoracic leg, was cut and placed in an eppendorf tube. Bumble bee legs from the same locality and date of collection were placed in the same tube and shipped to the USDA-ARS, Bee Biology and Systematics Laboratory at Logan, UT, for microsatellite DNA analysis.
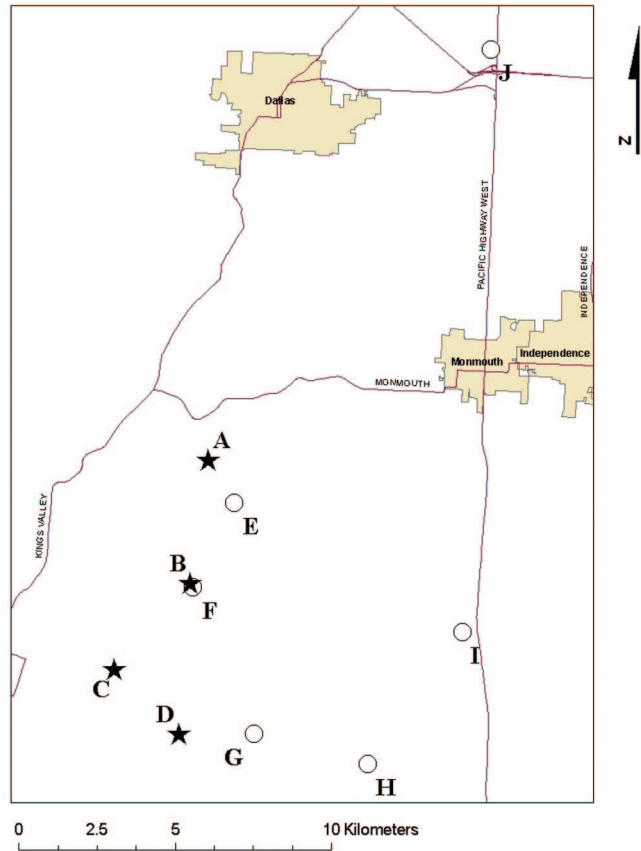
Fig. 1. Aerial photo of the clover fields sampled for collection of *B. vosnesenskii* workers. Stars (A–D) represent 2007 collection fields; Circles (E–J) represent 2008 collections fields. Distances between fields listed in Table 3.

Microsatellite DNA Analyses. The leg from each *B. vosnesenskii* worker bee was cut into four to six pieces, and leg fragments were placed in a 0.2-ml well of a 96 well polymerase chain reaction (PCR) plate. A solution of 150 μl of a 5% Chelex in ddH₂O and 5-μl Proteinase K (10 mg/ml) was added to each well (modified from Walsh et al. 1991). Samples were incubated for 1 h at 55°C, 15 min at 99°C, 1 min at 37°C, and 15 min at 99°C to extract DNA. Extracted DNA was stored at 4°C until PCR amplification.

Eight polymorphic microsatellite loci were used for analysis of *B. vosnesenskii* each year (Table 1). The 2007 samples were analyzed using the following eight loci: B124, B10, B119, B96, (Estoup et al. 1996) BL13, BT10, BTern01, and BT28 (Funk et al. 2006). The same loci were used for the 2008 samples except that BL11 (Funk et al. 2006) was used in place of BT28 because of inconsistent amplification at the locus. DNA was PCR-amplified in two 10-μl multiplex reactions containing 1-μl extracted DNA, 1x reaction buffer (Pro-

Table 1.   Parameters associated with the microsatellite loci used to construct multilocus genotypes of *B. vosnesenskii* workers collected over 2 yr from clover fields in the agriculture-dominated Willamette Valley in western Oregon

Locus[a]	Size range (bp)	No. alleles	Ht 2007	Ht 2008	PW$_{R2007}$	PW$_{R2008}$
B124	215–233	7	0.6565	0.7061	0.153	0.165
BL13	160–178	10	0.7666	0.7844	0.145	0.154
BT10	144–184	14	0.7815	0.7648	0.202	0.220
BTern01	125–150	10	0.7204	0.7035	0.148	0.137
B119	123–125	2	0.4876	0.4955	0.000	0.000
B96	235–251	9	0.8089	0.8206	0.192	0.189
BT28[b]	174–222	8	0.5463	na	0.132	na
BL11[c]	116–122	4	na	0.5965	na	0.118

[a] B10 used initially was subsequently excluded (see text for details).

[b] Used only for 2007 samples.

[c] Used only for 2008 samples.

Table 2. Summary statistics for *B. vosnesenskii* workers collected over 2 yr from clover fields in the agriculture-dominated Willamette Valley in western Oregon

| Fields | No. bees genotyped | No. colonies detected | Estimation including undetected colonies | | Effective population size (N_e) | F*is* |
			Chao	Truncated Poisson		
2007						
A[a]	312	163	187.0	413[d]	282	0.0556
B[a]	33	31	241.3	51	113	−0.2699
C[b]	22	20	101.0	34	87	−0.2748
D[b]	66	54	127.5	98	119	−0.0285
Total	433	184[c]	188.7[c]	558[c,d]	379	
2008						
E[a]	63	54	166.5	94[d]	133	0.0281
F[a]	71	57	137.7	103	178	0.0302
G[b]	19	19	–	–	257	−0.0693
H[b]	15	15	–	–	79	0.2662
I[a]	58	48	181.3	85	108	−0.0191
J[a]	70	55	231.3	100[d]	101	0.1591
Total	296	137[c]	144.2[c]	347[c,d]	284	

[a] Red clover field.
[b] Arrow leaf clover field.
[c] Total number of distinct colonies.
[d] Samples that deviated significantly from the Poisson distribution.

mega, Madison, WI), 0.6-mM dNTP mixture, 0.2–0.4-μM primer, 0.001-mg BSA, 0.4-U *Taq* polymerase (Promega, Madison WI), and the MgCl$_2$ concentration was adjusted to 1.4 mM. The PCR conditions for both multiplex reactions were one 7 min cycle at 95°C, 30 cycles of 95°C for 30 s, annealing temperature 53°C for 30 s, 72°C for 30 s, and a final extension period of 10 min at 72°C. The DNA amplifications were performed with fluorescent 5′ dye-labeled primers and separated on an Applied Biosystems 3730xl automatic sequencer (Applied Biosystems, Foster City, CA). The allele sizes were scored using GeneMapper v4.0 Software (Applied Biosystems).

Statistical Analysis. Tests for departure from Hardy–Weinberg Equilibrium, genotypic disequilibrium, and population statistics (F_{IS} and F_{ST}) were calculated using GENEPOP On The Web (Raymond and Rousset 1995). Sequential Bonferroni corrections were applied to results from multiple comparisons (Rice 1989). Presence of null alleles and allelic dropout were estimated using MICROCHECKER (van Oosterhout et al. 2004).

Colony (family, assuming single father and single queen colonies) assignment of individual bee genotypes was performed using COLONY v.2.0.1.1, which allows for construction of full sibling colonies incorporating allelic drop out and genotyping errors (Wang 2004). Individuals with missing data at more than one locus were removed to avoid artificial assignment of false sibling groups, and thus prevent unrelated individuals at distant fields being assigned erroneously to the same colony because of paucity of genotypic data. This reduced the number of individuals in the analysis, and resulted in a conservative estimate of the number of colonies contributing foragers. Allelic dropout rates were set at 0.001 for all loci, and the typing error rate was set at 0.005 and for all loci.

Estimations of Number of Colonies. For estimating the number of full sib families (colonies) that con-

tributed foragers to single or multiple fields, we performed colony assignments thrice for each field to ensure that individual colony designations were stable. One individual from each detected family was used to determine allelic frequencies and F_{IS} values. Effective population (*Ne*) sizes were calculated in COLONY by using the full likelihood method assuming random mating. We analyzed individuals, field-by-field within each year, using the full data set. The power (PW_R) of the multilocus genotypes for each locus was calculated using KinInfor (Wang 2004).

As sampling could not practically capture representatives from every colony contributing foragers to fields, we estimated the number of nonsampled colonies within each field (Table 2) using the following two analyses:

1) Chao's method (Chao 1984), used in diversity studies, which uses a ratio of species detected only once to species detected twice to estimate the frequency of undetected species.

2) The truncated Poisson distribution which estimates the number of null events in a distribution when the distribution is truncated below one (Chapman et al. 2003, Knight et al. 2005).

Correlations of sample size to the number of observed colonies and the number of inferred colonies by using both methods were calculated using SPSS 15.0.1 (SPSS 2006). The truncated Poisson distribution was tested with a goodness-of-fit χ^2 test. Significance for all tests was set at $\alpha = 0.05$.

Estimations of Foraging Distance and Nest Density

Average Foraging Distance. We initially multiplied the number of workers from each field (Table 3, Column 3) with estimated foraging distances (described below) to determine the total distance traveled by all workers that foraged in the field (Table 4, Column 3). The total was then divided by the total number of

Table 3. Estimates of numbers of *B. vosnesenskii* colonies and foragers in single and multiple fields, and distances between fields and foraging distances of workers from combinations of fields that shared workers

Field[a]	No. colonies shared between fields[b]	Number of foragers[c]	Distance between fields (km)	Foraging distance of workers (km)[d]
2007				
A	92	203	–	
B	7	8	–	
C	2	3	–	
D	5	7	–	
Total	106	221		
A-B	17	25–18	3.98	49.75–35.82
A-C	11	16–11	7.33	58.64–40.32
A-D	38	61–46	8.80	268.40–202.40
B-C	1	1–1	3.66	1.83–1.83
B-D	2	2–2	4.83	4.83–4.83
C-D	3	4–3	2.90	5.80–4.35
Total	72	190		
A-B-D	3	4–3–4		17.03–0.90–18.17
A-C-D	2	3–2–3		15.93–4.55–10.86
B-C-D	1	1–1–1		2.69–1.50–2.36
Total	6	22		
2008				
E	11	15	–	
F	13	19	–	
G	3	3	–	
H	0	0	–	
I	10	16	–	
J	13	21	–	
Total	50	74		
E-F	11	12–13	3.00	18.0–19.50
E-G	3	3–3	7.40	11.10–11.10
E-I	5	6–6	8.37	25.11–25.11
E-J	11	12–15	16.63	99.78–124.73
F-G	2	2–2	5.05	5.05–5.05
F-H	2	3–2	7.92	11.88–7.92
F-I	8	10–9	8.70	43.50–39.15
F-J	6	9–7	19.62	88.29–68.67
G-H	1	1–1	3.76	1.88–1.88
G-I	2	2–1	7.41	7.41–7.41
G-J	3	3–3	23.11	34.67–34.67
H-I	3	3–4	5.19	7.79–10.38
H-J	1	1–1	23.14	11.57–11.57
I-J	8	8–8	18.61	74.44–74.44
Total	66	151		
E-F-G	1	1–1–1		3.37–1.26–4.10
E-F-H	2	2–2–2		7.62–4.98–11.38
E-F-I	2	2–2–2		6.04–6.65–11.20
E-F-J	1	1–1–1		4.54–7.54–12.08
E-G-I	1	1–1–1		3.02–3.33–5.60
E-H-I	1	1–1–1		5.66–4.20–3.43
E-H-J	2	2–2–3		9.26–20.76–39.19
E-I-J	2	2–2–2		12.41–15.72–22.85
F-G-J	1	1–1–2		5.65–9.03–28.39
F-H-J	1	1–1–1		6.33–9.49–14.06
F-I-J	4	4–5–4		31.99–35.70–49.65
Total	18	57		
E-F-I-J	1	2–1–1–1		8.05–6.69–7.09–13.39
E-G-H-J	1	2–1–1–1		6.57–7.54–8.09–15.58
F-G-H-I	1	1–1–1–1		5.81–3.42–3.59–4.32
Total	3	14		

[a] Combinations of two or more fields that did not share colonies not listed.
[b] Includes only colonies shared by corresponding set of fields alone. For example, colonies included in the A-B-D combination are not included in the A-B or A-D or B-D combinations.
[c] For two or more fields that shared workers from the same colony, numbers of foragers genotyped from each field separated with hyphens.
[d] For two or more fields that shared workers from the same colony, foraging distances of workers from each field separated by hyphens. Foraging distances estimated by multiplying the number of foragers with 1) half the distance between two fields for two field combinations, and 2) the distance from the centroid for the three and four field combinations.

bumble bees genotyped from the field (Table 4, Column 2) to estimate the average foraging distance of workers in the field (Table 4, Column 4). We also estimated the average foraging distance across all fields sampled in each year.

To estimate the average foraging distance, the total distance flown by foragers in the study needs to be estimated first. For estimation of the total foraging distance traveled by all workers that foraged in a field (Table 4, Column 3), foragers from colonies found at

Table 4.   Estimations of average foraging distance of workers contributing to each field, foraging area, and nest densities based on three estimations of the numbers of colonies detected (with no estimation of unsampled colonies, with Chao's estimator and with the truncated Poisson distribution)

Field	No. foragers	Total distance travelled (km)	Average foraging distance (km)	Foraging area (km^2)	Nest density estimate (colonies/km^2)		
					DNA analysis	Chao	Poisson
2007							
A	312	409.76	1.31	5.42	30.08	34.51	76.21
B	33	46.07	1.40	6.12	5.06	39.40	8.33
C	22	54.00	2.45	18.93	1.06	5.34	1.80
D	66	242.97	3.68	42.58	1.27	2.99	2.30
Average			2.21		9.37	20.56	22.16
2008							
E	63	223.32	3.54	39.48	1.37	4.22	2.38
F	71	242.33	3.41	36.60	1.56	3.76	2.81
G	19	87.52	4.61	66.67	0.28	–	–
H	15	86.67	5.87	104.89	0.14	–	–
I	58	239.56	4.13	53.60	0.90	3.38	1.59
J	70	509.26	7.28	166.30	0.33	1.39	0.60
Average			4.79		0.76	3.19	1.85

only one field were given a forage range of zero, to conservatively estimate average forage range. For colonies that contributed workers to two fields, we assumed that that nest was located exactly halfway between fields, and estimated the foraging distance for each of those foragers as half the distance between those fields. For colonies that contributed workers to more than two fields, the foraging distance for each forager was estimated as the distance of each field from the centroid. Distances between fields (Table 3, Column 4) were estimated using GPS co-ordinates. The estimated foraging distances between combinations of fields were then multiplied by the numbers of foragers from the colonies detected at the shared fields (Table 3, Column 3). This resulted in an estimate of the total foraging distance flown by workers that foraged in a field (Table 4, Column 3).

For example, for estimation of the total foraging distance of workers from Field A, the numbers of foragers from colonies shared with Field B, Field C and Field D, were multiplied by half the distance between Field A and Field B, Field A and Field C, and Field A and Field D, respectively. In addition, the numbers of foragers from colonies shared with Fields B and C, and Fields C and D were multiplied by the distances of Field A from the corresponding centroids. The sum of all these five distances was then estimated as the total foraging distance of workers from Field A.

Maximum Foraging Distance. The maximum foraging distance covered by workers was estimated as half the distance between the most remote fields visited by the same colonies.

Foraging Area. The foraging area for bees from each field (Table 4, Column 5) was calculated as the area around the nests within the average flight range of workers from a field ($A = \pi r^2$), where r is the average foraging distance (Table 4, Column 4). By using the average foraging distance across all fields sampled in the year as the value for r, the foraging area of all bees sampled in a year was estimated.

Nest Density. The densities of nests contributing workers to each field were estimated by dividing the number of colonies detected in each field by the for-

aging area (Table 4, Column 5). Nest densities (Table 4, Columns 6–8) were calculated using all three estimations of the numbers of colonies detected: with no estimation of unsampled colonies (Table 2, Column 3), with Chao's estimator (Table 2, Column 4), and with the truncated Poisson distribution (Table 2, Column 5).

Results

Numbers of Colonies Detected and Inferred. From bumble bees collected in the study, we included 433 *B. vosnesenskii* in 2007 and 296 in 2008 (Table 2) in the analysis. Extensive sampling was conducted in Field A for another study (S. R., unpublished data), resulting in a high number of bees (312) included in the current study. In 2008, both arrow leaf clover fields (G, H) were sampled in early August when bloom had almost ended and hence fewer than 20 bees were collected from these fields.

The locus (B10) presented null alleles in multiple field samples in both years, and was excluded from subsequent analyses. After removal of locus B10, populations were in Hardy–Weinberg Equilibrium, and linkage disequilibrium was detected at two locus pairs at two fields after Bonferroni corrections were applied. No large allelic dropout was detected, thus we chose to retain the other markers for further analyses. Pairwise F_{ST} values revealed no significant population differentiation among any fields. F_{IS} values revealed reduced heterozygosity at several fields reflecting the relatedness of individuals in the samples despite the removal of sisters from the data set before the calculation of F statistics (Table 2).

We detected 184 distinct colonies from the 433 *B. vosnesenskii* genotyped in 2007 and 137 colonies from the 296 genotyped in 2008. Inferred class I errors were detected in family reconstruction totaling nine in 2007 and two in 2008. Estimates for the number of colonies including unsampled colonies within each field by using Chao's method and the truncated Poisson are listed in Table 2. These could not be calculated for

fields G and H because no sister pairs were found on those fields as a result of the small sample size.

Comparative estimates of colonies including unsampled colonies using both Chao's method and the truncated Poisson indicated a correlation between total number of families detected in a sample and the number of undetected colonies. The truncated Poisson estimation method was highly correlated to the number of directly detected colonies ($r^2 = 0.998$; $P < 0.001$), whereas the relationship of Chao's method to the directly detected colonies was less linear ($r^2 = 0.762$; $P = 0.011$) but significant nonetheless. Thus, the truncated Poisson tended to give a more variable estimate than Chao's method, which returned more consistent estimates across fields irrespective of actual number of colonies detected. The truncated Poisson estimation method performed well for the smaller sample sizes, but goodness-of-fit was not achieved for the majority of samples with over 60 individuals genotyped (Table 2, Column 5). This appears to be because of the large number of colonies represented by two or more individuals in those samples.

Number of Colonies at Single and Multiple Fields. In 2007, we observed 163 colonies from Field A. This was the largest number of colonies per field resolved in this study because of the large number (312) of bees analyzed from this location (Table 2). In Fields B, C, and D, where sample sizes were much smaller (33, 22, and 66 bees, respectively), the number of detected colonies was accordingly lower (31, 20, and 54 colonies, respectively). With inclusion of estimates of non-detected colonies, the estimated number of colonies foraging on all four fields ranged from 101 to 241 under Chao's method and 34–413 with the truncated Poisson method. In 2008, the number of observed colonies ranged from 15 to 57. With the inclusion of nondetected colonies, the numbers of colonies ranged from 138 to 231 by Chao's method and from 85 to 103 by using the truncated Poisson. Although the fields varied widely in numbers of foragers collected, across both years, the number of colonies observed was highly correlated to the sample size ($r^2 = 0.993$; $P < 0.001$).

Based on the 2007 data, 106 colonies (of 184 detected) were found foraging at only one field, 72 at two fields only, and six colonies at three fields only (Table 3). Colonies were shared between all possible 2-field combinations except between fields E and H. Sharing between one to three colonies was observed in three of the four possible 3-field combinations. No colonies were shared between fields A, B and C, and thus no colonies were shared across all four fields. In 2008, because of narrower range in sample size across fields, the number of workers genotyped across the six fields was less variable compared with 2007 samples. In all, 50 colonies were detected foraging in only one field, 66 in two fields only, 18 in three fields, and three in four fields (Table 3). No colonies were detected foraging at five fields. We observed sharing between one to four colonies in 11 of the 20 possible sets of 3-field combinations, and sharing between one colony each in four of the 10 possible sets of 4-field combinations. Colonies that were found at only one field in both years could have shared workers foraging in clover fields not included in the study.

In both years, some *B. vosnesenskii* colonies contributed workers to fields of both arrow leaf and red clover fields (Table 2).

Average Foraging Distance. Based on foragers genotyped in 2007, the distance between two fields that shared colonies ranged from 2.9 to 8.8 km (Table 2). Fields A and D were furthest apart and yet they shared the highest number of colonies (38); however, they also had the largest sample sizes of the 2007 survey and thus the highest likelihood of detecting sisters.

Forager bumble bees genotyped in 2008 documented that the distance between fields that shared colonies ranged from 3.0 to 23.11 km (Table 2). The fields that were most distant, G and J (23.11 km apart) and H and J (23.14 km apart), shared one and two colonies, respectively whereas fields G and H which were closer (3.76 km) shared only one colony. However, <20 workers were collected from fields G and H; with larger sample sizes, greater sharing may have been detected between these fields and with field J.

The average foraging distances of workers contributing to each field ranged from 1.31 to 3.68 km in 2007 and from 3.41 km to 7.28 km in 2008 (Table 4, Column 4). The average foraging distance of workers across all fields sampled in each year was 2.21 km in 2007 and 4.79 km in 2008.

Maximum Foraging Distance. Potential maximum foraging range for *B. vosnesenskii* workers was thus estimated to be at least 11.57 km, which is half the distance between Fields H and J (fields that shared colonies that were furthest apart), assuming that nests are located exactly midway between the fields.

Foraging Area. The foraging areas of bumble bees foraging in each field ranged from 5.42 km^2 to 42.58 km^2 in 2007, and from 36.6 km^2 to 166.3 km^2 in 2008 (Table 4, Column 5). Because the average foraging distance of workers across all fields sampled in 2007 was 2.21 km and in 2008 was 4.79 km, the foraging areas of bumble bees across all field sampled in each year were estimated to be 15.36 km^2 and 72.13 km^2 for 2007 and 2008, respectively.

Nest Density. Estimations of nest density are affected by estimations of foraging ranges and estimations of unsampled colonies used in the calculation (Table 4). Thus, the density of nests contributing workers to each field range from 1.1 km^{-2} to 76.2 km^{-2} in 2007 which included a large sample size from one field (Field A), and 0.1 km^{-2} to 4.2 km^{-2} in 2008, which included one remote site (Field J). Across all fields, the nest density estimates varied from 9.4 to 22.2 in 2007 and 0.8–3.2 in 2008 depending on colony estimation with no estimation of unsampled colonies, with Chao's estimator or with the truncated Poisson distribution.

Discussion

The current study is the first to provide estimates of flight ranges and nest densities of a nearctic bumble

bee species contributing foragers to isolated patches of bloom in an agricultural landscape. It is the first study to examine flight distances of workers foraging on a late-season blooming crop, and to document long distance foraging in a bumble bee belonging to the subgenus *Pyrobombus.* The maximum foraging range reported thus far for a species in this subgenus is 674 m for *B. pratorum* (L.) in an arable farmland landscape (Knight et al. 2005). Our study suggests that *B. vosnesenskii* workers can fly at least 11.57 km in search of forage. This also represents the largest distance reported so far for a nearctic species. Elliott (2009) observed that *B. appositus* Cresson, *B. bifarius* Cresson, *B. californicus* Smith, *B. flavifrons* Cresson, *B. frigidus* Smith, *B. nevadensis* Cresson, *B. occidentalis* Greene, and *B. sylvicola* Kirby foraged <100 m at high elevations in Colorado, whereas in a study by Rau (1924) in Missouri, a single *B. pensylvanicus* (DeGeer) was recorded at a distance of 2.4 km from its nest. Batra (1993) speculated that workers of *B. terricola* Kirby traveled 4 km based on the presence of milkweed pollen on tarsi and distance of closest milkweed plants from where the bees were collected.

The long foraging distance reported for *B. vosnesenskii* here is in line with the estimates by Cresswell et al. (2000) based on the foraging economics of workers which suggest that bumble bee flight distances may well exceed 10 km, and the observations by Hedtke (1996 from Walther-Hellwig and Frankl 2000) which indicated that bumble bees can return to nests when released 11.5 km away. In addition, Kraus et al. (2009) reported male flight distances of 9.9 km. In general, as indicated earlier, flight distances refer to foraging trips, not necessarily a single take-off and landing.

The current study questions the assumption that workers will always forage close to the nest to minimize time loss and to maximize net energy gain (Pyke et al. 1977, Heinrich 2004). Bumble bee flight distances of European species based on estimates made in both arable and natural landscapes in Europe have been largely found to be <1,000 m (Osborne et al. 1999, Walther-Hellwig and Frankl 2000, Chapman et al. 2003, Darvill et al. 2004, Wolf and Moritz 2008). A recent study by Lepais et al. (2010) documented apparent worker movement >8 km, but this was attributed to the detection of false sister pairs and not to actual foraging activity and this also may, in part, explain the foragers we detect at such remote fields. However, studies on multiple species in the same environment, where resource availability was equal for all species, have found foraging distances to be species-specific (Walther-Hellwig and Frankl 2000), suggesting that each species is responding to the environment in a unique manner.

Several factors could influence foraging distance of bumble bee workers, including duration of their life cycles, location, and quality of food resources relative to nest location. In the agricultural habitats in the Willamette Valley in Oregon, the presence of sequential bloom in crops such as blueberries in spring and red clover in late summer are believed to have enabled several bumble bee species to thrive in the region, and

these in turn have led producers to achieve record high crop yields (Rao and Stephen 2010). Of these, *B. vosnesenskii* is the dominant species. Its life cycle lasts longer than bloom in any one crop. Queens emerge in late April and May in the Willamette Valley, and workers forage from May through September, whereas new queens and drones appear from August to September. Thus, food may be abundant in the vicinity of the nest at certain periods, whereas at others workers may have to travel farther to collect adequate resources. Foraging behaviors of bumble bee workers have been examined in natural landscapes, whereas workers of *B. vosnesenskii* had been reported as being away from the colony in excess of 2 h (Allen et al. 1978) thus displaying a potential for long distance foraging trips. We believe that the presence of the fragmented mass flowering clover patches at the end of the summer is the driving force for the long distance flight exhibited by *B. vosnesenskii* in this region. It is well known that red clover is highly valuable as a pollen source for bumble bees (Carvell et al. 2006). In fact, declines in red clover are believed to have had a negative impact on bumble bee populations in the United Kingdom (Carvell et al. 2006), and studies have examined the influences of bumble bee species abundances on red clover yield (Wermuth and Dupont 2010; Bommarco et al. 2011, Dupont et al. 2011). Thus, individual *B. vosnesenskii* may pass over less nutritious pollen sources while foraging long distances to access the high quality food sources in clover fields at the end of summer. Osborne et al. (2008) documented that *B. terrestris* workers traveled 1.4 km to forage on borage, a highly rewarding resource for bumble bees, even though food resources were available closer to the nest. It is possible that in the Willamette Valley, *B. vosnesenskii* workers may fly long distances only when red clover is in bloom. In spring, flight ranges to spring blooming crops in the same landscape may be considerably shorter than estimations in the current study, and this needs to be examined.

Information on nest density is valuable for insights on the population size of bumble bee species in an area, and potential for intra-specific competition. However, it is a challenge to estimate nest density because of the influence of several parameters that are integral to its calculation, and this is well highlighted in the current study. In 2007, the sample size was larger but the fields were closer compared with 2008. Hence, estimated average foraging distance (2.21 km for 2007 and 4.79 km in 2008) and the foraging area (15.36 km^2 in 2007 and 72.13 km^2 in 2008) were smaller in 2007 than in 2008. This variation coupled with differences in numbers of colonies detected or estimated using Chao and the truncated Poisson, yielded average nest density estimates ranging from only 0.76 km^{-2} to 1.85 km^{-2} in 2008, because of the larger sampling area across which sister pairs were detected, to 9.37 km^{-2} to 22.16 km^{-2} in 2007 when a higher number of bees were included in the study (Table 4). More studies on the forage range are required to refine the forage range so that nest density estimates can be made more accurately. Nonetheless, based on this study, large num-

bers of colonies are providing the foragers that are pollinating the isolated patches of late season blooming clover in the Willamette Valley in Oregon.

Issues with Microsatellite Analyses. Several factors influence the reconstruction of sister pairs from microsatellite genotypes and, subsequently, nest density and forage range estimates (Chapman et al. 2003, Wang 2004). Queen multiple mating poses a primary challenge in determining kinship, resulting in half sister pairs in colonies that would lead to an overestimation of the number of colonies in given area (Wang 2004). However, polyandry in bumble bees is not believed to be common (Estoup et al. 1995, Payne et al. 2003, Takahashi et al. 2008). In addition, this type of error would, in fact, result in an underestimation of sister pairs foraging on different fields and could lead to an underestimation of forage distance (Wang 2004). Another potential error occurs by incorrectly assigning sister pair status to unrelated individuals that nonetheless share a genotype. Using multiple highly variable loci reduces that risk, but as the study area increases, the number of nests increases as do the chances of this type of error (Chapman et al. 2003). Misassigning unrelated individuals as sisters would lead to an underestimation of nest density and an overestimation of flight distance in forage range studies. Finally, the low allelic richness of the species (Lozier et al. 2011) may be contributing some falsely assigned sister pairs.

For large sample sizes, caution is needed while estimating the number of unsampled colonies. The Field A sample differed significantly from the truncated Poisson distribution and thus the predicted number of colonies in the zero category (250 colonies) more than doubled the observed number of colonies. Chao's equation provided a more conservative estimate as it added only 24 colonies to the zero category. However, the truncated Poisson method performed fairly well when sample sizes were <100. The goodness-of-fit test revealed that two of the seven smaller samples tested were significantly differently distributed from the Poisson distribution; however, these two samples were only marginally significant. The high correlation between detected colonies and the number of colonies predicted using this method is expected (the number of detected colonies is a factor in both methods of estimation; however, this linear relationship may be leading to overestimation in large samples. Similarly, Chao's estimate and Ne both have a significant correlation to the number of detected colonies. Given that Ne is not a measure of the actual number of colonies it is probably not the best method for determining colony contributions to field crops. Chao's estimate resulted in generally higher numbers of estimated colonies than the truncated Poisson, but also seems a bit more robust to sample size. One revealing aspect of this study is that estimation of the zero category becomes less relevant with an increase in sample size, and larger samples appear to be warranted in studies of this nature.

Conclusion

Based on the current study, bumble bees workers from the same colony can forage in isolated patches of a late season blooming crop at considerable distances from each other. Hence, although past studies on foraging behaviors of bumble bees have been designed based on the assumption of short flight distances, future studies should include longer transects and larger sample sizes. In particular, studies in which correlations are made between bumble bee abundances and resource availability in surrounding areas should consider areas in the range of several km^2 not just several m^2.

Habitat fragmentation because of agricultural intensification has led to declines in certain bumble bee species. However, the western U.S. *B. vosnesenskii* is thriving in an agricultural landscape. Its success could be because of the ability of workers to forage over distances >10 km to reach the fragmented patches of the highly rewarding clover crops in the region. While, ultimately, flight distances could affect the ability of a species to survive, especially in fragmented landscapes. other species besides *B. vosnesenskii* could fly long distances and this needs to be determined. Meanwhile, conservation programs that target bumble bees should take into consideration their potential long flight distances when determining the scale for habitat management.

Acknowledgments

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