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Fantastic bees and where to find them: locating the cryptic overwintering queens of a western bumble bee

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Abstract. Bumble bees are among the best-studied bee groups worldwide, yet surprisingly we know almost nothing about their overwintering habitats nor the microsite characteristics that govern selection of these sites. This gap represents a critical barrier for their conservation, especially if preferred overwintering habitats differ from foraging and nesting habitats. Current conservation plans focus on foraging habitat, potentially creating a problem of partial habitats where improved forage might fail to prevent population declines due to limited overwintering sites. We provide the first data on the overwintering habitat for any western North American bumble bee. Our data suggest that overwintering and foraging habitats are likely distinct, and queens' selection of overwintering sites may be shaped by environmental stressors of the year. In our study area, queens overwintered in litter beneath cypress trees, where no floral resources exist. Whether this separation of overwintering and foraging habitat holds for other bumble bee species remains to be discovered. Our data highlight the need to consider the whole life cycle for understanding population dynamics and conservation planning. This need is underscored by growing evidence for the decline of multiple North American bumble bee species.

Key words: *Bombus vosnesenskii;* bumble bee; diapause; habitat selection; hibernation; life cycle; overwintering; partial habitat; queens.

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Bumble bees are among the best-studied bee taxa worldwide, yet certain stages of their annual social life cycle remain unknown for nearly all species. In spring, a mated queen emerges from her overwintering site, searches for a spot to nest, and works alone to raise a first cohort of worker daughters. The colony grows over several months, producing successive cohorts of workers before switching to produce males and new queens. In mid- to late summer, newly mated queens seek sheltered sites where they overwinter. We know a great deal about bumble bee foraging and some about nest-searching queens and nest establishment in spring (Kells and Goulson 2003, Suzuki et al. 2009, Lye et al. 2012, O'Connor et al. 2017). We know almost nothing about overwintering queens (Alford 1969, Liczner and Colla 2019). Overwintering sites have been described for only a handful of species (Sladen 1912, Plath 1927, Alford 1969), and hibernating biology is poorly understood even in commercially raised species (but see

Hobbs 1967). This knowledge gap is alarming, given bumble bees' importance as pollinators and well-documented declines of species from multiple geographic regions (Williams and Osborne 2009, Cameron et al. 2011).

Our understanding of bumble bee habitat needs is primarily shaped by distributions of easily observed foraging workers during periods of colony growth and reproduction (Goulson 2003, Williams et al. 2012, Rundlöf et al. 2014). Bumble bees certainly benefit from abundant forage habitat (Goulson et al. 2010, Crone and Williams 2016). However, these partial habitats provide an incomplete picture of the total habitat needed to complete a complex life cycle, when different life stages require different resources and conditions (Wilbur 1980, Williams and Osborne 2009) distributed across a landscape (Mandelik et al. 2012). For bees, recognizing that nesting habitat may be separate from foraging habitat is a key component of predictive models of abundance in different landscapes (Lonsdorf et al. 2009). The same separation likely applies to overwintering habitats. Explicitly recognizing the importance of non-forage habitat and its impact at other life stages is a critical shift in how we approach pollinator conservation. Suitable overwintering sites are unknown and currently omitted from bumble bee habitat models, despite calls for their inclusion in conservation planning (USFWS 2018). This research gap is its own barrier: It is difficult to learn about overwintering queens without knowing how to find them. To date, there are no published descriptions of overwintering habitats for any of the ~30 bumble bee species in western North America. Descriptions of queen behavior come solely from confined cage trials (Hobbs 1967). Furthermore, we suspect that overwintering queens may be disproportionately important to demography. At this stage, the queen is unbuffered by the colony and thus is likely to be especially sensitive to environmental conditions.

We looked for overwintering queens on California's central coast. We spent ~80 person-hours searching different ground covers around abandoned military barracks at former Fort Ord Military Base where we previously observed large numbers of nest-searching queen *Bombus vosnesenskii* Radoszkowski and *B. melanopygus* Nylander. Our approach was guided by three reports from England and the eastern United States (Sladen 1912, Plath 1927, Alford 1969) and by anecdotal reports from the Pacific Northwest of the United States (S. Rao, *personal communication*). Given the near absence of existing knowledge, our goals were to (1) determine overwintering site preference among local candidate ground covers, (2) describe overwintering queen substrates, and (3) quantify the search effort needed to locate overwintering queens.

Ground cover at Fort Ord is typical of the central California coast: a patchwork of grassy meadow, mats of non-native ice plant (*Carpobrotus edulis*), and small stands of Monterey cypress (*Cupressus macrocarpa*) and Monterey pine (*Pinus radiata*). In December 2018, we searched for bumble bee queens by carefully digging the vegetative, litter, and soil strata of grassy meadow, ice plant mat, and the needle litter under two cypress and two pine trees (Fig. 1). We found one *B. melanopygus* and four *B. vosnesenskii* queens, all in cypress needle litter. We returned in February 2019 to conduct a more systematic search, stratifying effort among cypress needle litter, ice plant mat, and grassy meadow. We chose two areas of each habitat to establish six 0.5-m^2 quadrats (Fig. 2A–C). Under cypress, quadrats were placed to span the distance from trunk to canopy margin (0–3 m, Appendix S1: Fig. S1). At each quadrat, we measured physical characteristics of the ground substrate (Appendix S1: Table S2), then cleared the surface vegetation, and hand-sifted litter, duff (decomposed needle fragments), and loose soil layer by layer to a depth of 20 cm or until we reached compact soil. It took 12 person-hours to search the total 6 m^2 per cover type (~20 min for one 0.5 m quadrat). This systematic search yielded three *B. vosnesenskii* queens, only in cypress litter. We found two additional *B. vosnesenskii* queens in three opportunistic cypress litter quadrats.

All 10 queens (both *B. vosnesenskii* and *B. melanopygus*) were located separately but in remarkably consistent conditions, burrowed beneath 3.5–5 cm of cypress litter in a thin layer of duff between needle litter and mineral soil (Fig. 2D, E). Queens were within short distances of the trunk (0.1–1.5 m, Appendix S1: Table S1) and were shaded from direct sun, as reported for several other British species overwintering

Fig. 1. Team excavating for overwintering bumble bee queens under cypress along the Monterey coast, February 2019. Dry and shaded litter layer under the canopy was preferred by queens in this area.

beneath trees (Alford 1969). Other aspects of our data contrast with previous descriptions of overwintering queens. Most species appear to rest in distinct hibernacula (chambers/pockets within soil where queens have been reported to overwinter; Sladen 1912, Plath 1927, Alford 1969); the queens we found did not. Several species burrow considerably deeper (~8 cm) in soil (Alford 1969). The only previously reported North American bumble bee, the eastern *B. impatiens*, was found overwintering 7 cm beneath sod in loose aggregations of several to dozens of individuals (Plath 1927). Although we found only one *B. melanopygus* queen, it was found in the same condition as the *B. vosnesenskii* queens, suggesting that this overwintering strategy may be common among other western bumble bees.

Queens had pristine coats and no wing wear. They roused quickly when uncovered, vibrating their wings, walking, and striking defensive postures. When we attempted to rebury two queens, they re-emerged and flew away. Winter temperatures at Fort Ord average between 5° and 17°C. Whether queens here ever enter a heavy torpor, as described in reports from colder regions (Sladen 1912, Alford 1969), and whether they resettle after disturbance, warrants further study.

Our observations provide insight about the factors that produced the strong microhabitat selectivity we report (Table 1) and suggest the types of environmental drivers that influence bumble bee overwintering more broadly. In our region, overwintering bumble bee queens must first endure a hot, dry late summer followed by a cold, wet winter. The needle litter beneath shady cypress canopies likely buffers against both of these extremes. Litter and canopies, particularly of larger trees, have been shown to effectively moderate temperature and moisture fluctuations for soil organisms (Vetaas 1992, Owens et al. 2006, Fekete et al. 2016), including hibernating queen bumble bees (Sladen 1912). Anecdotally, our February search followed nine consecutive days of rain with 7.4 cm accumulation; the sandy soils beneath ice plant, grass, and pine were at field capacity, but cypress litter and duff were slightly moist to dry.

The ground at Fort Ord is dotted with active and abandoned rodent burrows of various sizes. The importance of rodent burrows for bumble bee nest sites (Free and Butler 1959, Kells and Goulson 2003) may contribute to the high densities of nest-searching bumble bees previously observed here. Rodents are also important

Fig. 2. Potential overwintering microhabitats for *Bombus vosnesenskii* and *B. melanopygus* along coastal California. Sampled microhabitat types: (A) cypress needle litter; (B) grassy meadow; (C) ice plant mat; (D) cross section of needle litter and duff illustrating depth of *B. vosnesenskii* overwintering; (E) disturbed overwintering queen *B. vosnesenskii* within cypress litter at the boundary of duff–soil layer.

predators of bumble bees, early noted by Darwin: "The number of humble-bees in any district depends in a great degree on the number of field-mice, which destroy their combs and nests; and Mr. H. Newman, who has long attended to the habits of humble-bees, believes that 'more than two thirds of them are thus destroyed all over England'" (Darwin 1859). We found many active rodent burrows in the grassy meadow, ice plant mat, and under pines, but not a single rodent burrow under cypress. Perhaps these cypress understories represent a relatively predator-free microhabitat for queens when they are dormant and immobile.

Cypress understories are cool and shady and tend to suppress ground cover. Prior to this project, we would have overlooked these cypress stands as critical bee habitat. If overwintering microhabitat typically contrasts with foraging and nesting habitat, bumble bee conservation will require that we look beyond flowering meadows, not just in this site but throughout the ranges of different species. At Fort Ord, these habitats are adjacent, but this may be atypical,

Table 1. Total search effort by seven people in two days searching different ground covers for hibernating queen bumble bees

Ground cover	Person × hours (by date)	Area (m^2)	Queens
Cypress litter	15 (December 8.5, February 6.5)	18.5	10
Ice plant	6 (December 2.0, February 4.0)	10	0
Grassy meadow	6 (December 2.0, February 4.0)	9†	0
Pine litter	2 (December 1.5, February 0.5)	2	0

† An additional 2 m^2 were excavated 50 cm in an attempt to find queens deeper in soil.

and wide dispersion of foraging and overwintering habitats would further increase the challenges for conserving bumble bees and other species (Sardinas et al. 2016) in fragmented and changing landscapes. Different partial habitats may also respond differently to disturbances: For example, wildfires often have a positive effect on bees via increased flower abundance (Mola and Williams 2018) but could kill queens overwintering near the surface. More surveys of overwintering and nesting are needed to understand and conserve the landscapes of partial habitats that support complete bumble bee life cycles.

We are confident that the current lack of knowledge is due to the cryptic nature of the hibernation stage and the risk of undertaking research where the study organism might not be found. Therefore, we are excited to have demonstrated that hibernating queens can be found with feasible search effort. We hope this report encourages a flourish of explorations of the overwintering sites for bumble bee queens in western North America and elsewhere and allows this critical life stage to be better understood and included in conservation efforts.

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Supporting Information

Additional Supporting Information may be found online at: http://onlinelibrary.wiley.com/doi/10.1002/ecs2.2949/full