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RESEARCH ARTICLE

High levels of male diploidy but low levels of genetic structure characterize *Bombus vosnesenskii* populations across the Western US

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Abstract Patterns of genetic structure for some bee species suggest that gene flow may be limited across natural and human-created barriers and that local dispersal or natal site fidelity may be common. Interestingly, this past work has primarily focused on female bees, despite the fact that males may differ substantially in their dispersal processes. By examining genetic structure and diploidy in males, it is possible to gain insight into potential barriers to gene flow and drivers of inbreeding. In this study, we examine diploidy as well as regional and local spatial genetic structure using males of *Bombus vosnesenskii,* a stable bumble bee species found across western North America. Specifically, we investigate patterns of genetic structure in both island and mainland populations, across spatial scales, and over a range of natural and human-altered habitats. We document high levels of male diploidy, with significantly higher levels in mainland populations compared to island populations and increasing diploidy in areas with poor nesting habitat. Interestingly, we also find evidence of significant spatial genetic structure from 0 to 10 km and 0 to 5 km on island and mainland populations, respectively. Finally, we document low but significant genetic differentiation across the region ($\Phi_{ST} = 0.049$). Overall, this work reveals the unique potential for biogeographic context and local habitat composition to drive male diploidy patterns in bumble bees.

Keywords Bumble bee · Dispersal · Landscape genetics · Resistance · Pollinator · Island

Introduction

Pollinators play a major ecological and economic role in terrestrial ecosystems by supporting global crop yields (Klein et al. 2007; Gallai et al. 2009) and by facilitating reproduction for the majority of native plant species (Ollerton et al. 2011). One of the most critical pollinators for temperate plants are bumble bees (genus *Bombus*, family: Apidae), large-bodied species that visit a variety of plant species (Goulson et al. 2008). The abundance and generalist foraging behavior of most bumble bees make them particularly important for stabilizing plant-pollinator networks (Memmot et al. 2004); additionally, their large body size and buzzing abilities are valued traits for agriculture, as they are among the most effective pollinators for a variety of crops that benefit from buzz pollination (Kremen et al. 2002; Dogterom et al. 1998). Unfortunately, bumble bees are also one of the most threatened bee taxa, with evidence of decline across North America (Cameron et al. 2011; Bartomeus et al. 2013) and Europe (Williams 1982; Williams and Osborne 2009), likely due to habitat loss, disease, and pesticide exposure, among other factors (reviewed in Goulson et al. 2015).

One of the primary threats to bumble bees is habitat alteration, which can lead to losses in nesting habitat and food resources (Goulson et al. 2008; Williams and Osborn 2009); despite this fact, much remains unknown about the impacts of landscape composition on ecological and evolutionary processes for bumble bees. Studies of pollinator community ecology across human-altered landscapes reveal that as land use intensification or disturbances

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increases (e.g., increased tilling and decreased semi-natural habitat), bumble bees tend to become less abundant (Larsen et al. 2005). Molecular-based studies examining bumble bee nesting densities likewise reveal that wooded areas, including oak woodland and chaparral (Jha and Kremen 2013), as well as woodland and backyard gardens (Goulson et al. 2010) tend to have higher nesting densities than heavily urbanized or agriculturally managed areas. These studies suggest that human-mediated features of the landscape could be influencing not only the population dynamics of bumble bees but potentially population-level genetic variation and distribution.

Population genetic structure characterizes the distribution of genetic variation across populations and can provide insight into the demographic history and gene flow processes occurring over space. Using pairwise population comparisons, population genetic structure is often examined across Euclidean geographic distances (isolation by distance, Wright 1943; reviewed in Jenkins et al. 2010) and more recently across land-use based resistance distances (isolation by resistance, e.g., McRae 2006). Previous bee studies examining pairwise population comparisons at continental spatial scales (~1000 km) have documented a wide range of genetic differentiation patterns, ranging from relatively low levels of differentiation (Cameron et al. 2011; Lozier et al. 2011) to relatively high levels of differentiation (Jha 2015); studies conducted at smaller spatial scales (<600 km) for other species have revealed relatively high levels of differentiation (Goulson et al. 2011; Davis et al. 2010). While few studies have examined spatial genetic structure across multiple spatial scales for the same species, previous work suggests that genetic structure can indeed be distinct across spatial scales and populations (Jha and Kremen 2013; Lozier and Cameron 2009; Lozier et al. 2011). Distinct spatial genetic structure patterns across populations and spatial scales could be due to a variety factors, including differences in effective population sizes or differences in dispersal and philopatry. Additionally, previous work has shown that poor nesting habitat, including intensive urban cover (e.g., Davis et al. 2010), intensive crop cover, and open water can significantly explain patterns of genetic differentiation for some bee species (Jha and Kremen 2013) but not others (Jaffe et al. 2016), revealing a need for further study of land use impacts across species and spatial scales.

Specifically, patterns of relatedness, which represent measures of shared genotypes over distance (e.g., Loiselle et al. 1995), and are typically measured across small spatial scales (1–25 km), could be critical tools for understanding land use impacts on spatial genetic structure; however, they are not well understood across bee species and sexes. The study of male relatedness is particularly important in the case of social bees because, while the non-reproductive female (workers) are usually the individuals collected for study, it is only the males and queens that reproduce and thus mediate gene flow. Female-based genetic studies indicate significant levels of relatedness at 0–9 km scales (Jha and Kremen 2013) and genetic recapture-based dispersal studies similarly estimate queen dispersal up to ~5–8 km (Lepais et al. 2010); yet little is known about males. Interestingly, a recent study of the solitary bee *Colletes inaequalis* within a heavily urbanized area revealed that females exhibit greater genetic relatedness than males in nest aggregations (Lopez-Uribe et al. 2015). This may be indicative of greater male dispersal, which has indeed been suggested as a mechanism to reduce inbreeding in ground-nesting bees (Smith 1983).

Levels of male relatedness are particularly important as they may regulate population-level inbreeding, which can be apparent in the form of ‘diploid males’ (Zayed et al. 2004). Diploid males in haplo-diploid species are a result of mating between closely related queens and drones which are homozygous at the sex determining locus, giving them the physical appearance of being male despite being diploid (Owen and Packer 1994). Because most diploid males are sterile and do no forage as workers, their production reduces the fitness of the colony (Whitehorn et al. 2009), thus they serve as an indicator of inbreeding as well as a potential source of decline in colony-level fitness. Diploid males have been recorded in Euglossine bees (Roubik et al. 1996; Souza et al. 2010; Suni and Brosi 2012), halictid bees (Zayed and Packer 2001), and a few bumble bee species, including *B. muscorum* (Darvill et al. 2006), *B. occidentalis, B. perplexus* (Whidden and Owen 2011), and *B. terrestris* (Whitehorn et al. 2009). Interestingly, past studies have not examined male diploidy across time periods, or landscapes, despite the likelihood that male diploidy levels may differ across the season and could be impacted by environmentally-driven nesting, colonization, and extinction patterns (Zayed and Packer 2001).

In this study, we investigate the population genetics of the yellow-faced bumble bee, *B. vosnesenskii,* at three different spatial scales (site, landscape, and regional scales) and two distinct bioregions (island and mainland). Specifically, at the site scale, we investigate diploid male frequencies for insight into the drivers and patterns of inbreeding. At the landscape scale, we quantify fine scale relatedness to illuminate potential dispersal patterns for bumble bees across islands and mainlands. Finally, at the regional scale, we investigate genetic differentiation in the context of isolation by distance (IBD) and isolation by resistance (IBR). We hypothesize that populations exhibit higher male diploidy in areas with limited nesting habitat, higher male diploidy on islands, and low levels of spatial genetic structure, IBD, and IBR.

Methods

Study region and species

Male *B. vosnesenkii* ($n = 582$) were collected in June and early July of 2012 in 18 sampling sites across California, USA (mean 32.33 bees per site ± 3.23 SE), spanning 687 km. Of these 18 sites, 11 were located on the mainland and 7 were located on islands, specifically on Santa Catalina (194 km^2), Santa Cruz (250 km^2), and Santa Rosa (215 km^2) islands (Table 1). Bees were collected by hand netting within a 25 m radius from the sample point between 8:00 and 17:00. These 18 sites were used for male diploidy and landscape-scale spatial genetic structure analyses. Since many sites were sampled in clusters, of the 18 study sites, 7 were separated enough (by more than 8 km in any direction) to be treated as separate populations and used in the regional-level population genetic structure (IBD and IBR) analyses (Table 1). For the entire region, land use was classified using the National Land Cover Database in 2011 at 1 km resolution, and for the male diploidy analyses, low quality nesting habitat was quantified as the proportion of land use including intensive crop cover, urban cover, or open water (as per Jha 2015) within a 2 km radius around each site.

DNA extraction, amplification, and genotyping

Post-collection, whole bees were stored at room temperature (23 °C) in 95% ethanol. DNA was extracted from one leg of each specimen using the HotShot Protocol (Truett et al. 2000) and stored at −20 °C. Samples were screened at 12 microsatellite loci: B96, B100 and B119 (Estoup et al. 1995), and BT33, BT43, BT65, BT124, BT125, BT128, BT131, BT132 and BT136 (Stolle et al. 2009). BT136 and BT128 were eliminated due to poor allelic yield, reducing the number to 10 loci. Loci are located on 10 different chromosomes based on the sequencing of the *B. terrestris* genome v1.1 (Stolle et al. 2009). Multiplex polymerase chain reactions (PCRs) were conducted in a final volume of 20 µL, with approximately 2 ng DNA, 2.5 µL 10X Buffer, 1 µg/mL BSA, 2 mM MgCl$_2$, 200 uM of each dNTP, 2 U of Taq Polymerase and 0.25 µM of each primer. The thermal cycle procedure commenced with a 4-min 95 °C denaturation step, followed by 37 cycles of the following: 45-second denaturation 95 °C, 1-min annealing at locus-specific temperatures, and a 30-s extension at 72 °C, ending with a 20-min 72 °C extension. For all bee samples, each multiplexed PCR set of screening primers contained at least one primer labeled with FAM, NED or VIC. All samples were genotyped on an Applied Biosystems 3730

Table 1 List of 18 study sites, all of which were analyzed for male diploidy and landscape-level spatial genetic structure analyses, and seven of which were separated enough to be included in the regional-scale IBD and IBR analyses (marked with an asterisk)

Site	Region	Site type	Lat.	Lon.	Nest limit.	Indiv.	Hap.	Dip.	Prop. Dip.	Nef (SE)	Div (SE)
BerryA*	Berry	M	38.57	−122.24	27.72	32	20	12	0.38	3.85 (0.56)	0.73 (0.06)
Holl25*	Holl	M	36.82	−121.35	11.61	58	50	8	0.14	4.28 (0.56)	0.73 (0.05)
Pope2	Pope	M	38.64	−122.35	0.08	42	16	26	0.62	3.94 (0.49)	0.75 (0.06)
Pope3	Pope	M	38.64	−122.35	0.08	28	4	24	0.86	2.13 (0.3)	0.63 (0.12)
Pope4	Pope	M	38.65	−122.36	0.08	35	15	20	0.57	3.44 (0.47)	0.70 (0.07)
PopeA*	Pope	M	38.63	−122.34	0.09	48	19	29	0.60	3.81 (0.49)	0.73 (0.06)
PopeB	Pope	M	38.64	−122.38	0.31	19	8	11	0.58	3.13 (0.37)	0.73 (0.09)
SantaCata18	SantaCata	I	33.44	−118.48	54.85	29	16	13	0.45	2.60 (0.27)	0.63 (0.04)
SantaCata19*	SantaCata	I	33.35	−118.42	0.82	47	40	1	0.02	3.21 (0.43)	0.66 (0.04)
SantaCata20	SantaCata	I	33.46	−118.53	30.30	13	8	5	0.38	3.18 (0.56)	0.69 (0.08)
SantaCata21	SantaCata	I	33.47	−118.54	46.36	25	18	7	0.28	3.26 (0.52)	0.66 (0.06)
SantaCruz14*	SantaCruz	I	34.03	−119.69	41.49	43	38	3	0.07	2.96 (0.35)	0.63 (0.05)
SantaCruz7	SantaCruz	I	34.00	−119.71	0.10	12	12	0	0.00	2.90 (0.35)	0.68 (0.05)
SantaRosa*	SantaRosa	I	33.99	−120.05	22.13	51	40	12	0.24	3.52 (0.41)	0.69 (0.05)
Second2	Second	M	38.66	−122.28	30.19	20	13	7	0.35	3.20 (0.36)	0.73 (0.06)
Second3	Second	M	38.66	−122.28	24.36	29	22	7	0.24	3.75 (0.51)	0.72 (0.07)
SecondA	Second	M	38.65	−122.29	41.76	15	8	7	0.47	2.89 (0.41)	0.71 (0.10)
SecondB*	Second	M	38.68	−122.27	12.26	36	27	9	0.25	3.95 (0.59)	0.72 (0.06)

From left to right, the Site name (Site), Region (Region), Site Type (*I* Island, *M* Mainland), Latitude (Lat.), Longitude (Lon.), proportion low quality nest habitat (Nest. Limit.), total number Individuals sampled (Indiv.), number of Haploids (Hap.), number of Diploids (Dip.), Proportion of Male Diploid individuals in each site (Prop. Dip.), number of Effective Alleles (Nef), and Nei's unbiased haploid gene diversity (Div), with SE representing Standard Error sequencer. GENEMARKER (Softgenetics) was used to manually score all genotypes. All samples had ≥4 scored loci.

Site-scale analyses: establishing male diploidy and sibships

Using all 582 males, diploidy was estimated as the proportion of males that were diploid per site (reviewed in Packer & Owen 2001). We used a microsatellite-based assignment that assigned an individual as diploid if any locus exhibited heterozygosity (as per Giangarelli et al. 2015), a method which is considered more conservative than previous approaches. Because the probability of assigning a male as diploid (heterozygous) should correlate with locus variability, we conducted two follow-up analyses: (1) we examined our data for correlations between allelic richness and diploidy and (2) we re-analyzed our data with a more conservative approach requiring two heterozygous loci for diploidy. Overall we found the expected positive correlation between allelic richness and diploidy and we found similar results for our diploidy analyses when utilizing the more conservative method of diploid designation (Supplementary Materials). We calculated the effective number of alleles (Nef) (Kimura and Crow 1964), and Nei's unbiased haploid genetic diversity (Div) for each population, using GenAlEx (Peakall and Smouse 2006). Sibship was established via COLONY 2.0 (Jones and Wang 2010) where genotyping error was set to 0.001. The haplo-diploid option was used to compensate for mixed ploidy in the dataset given the presence of male diploids; the monogamous option was implemented given that monandry is likely for bumble bees (Estoup et al. 1995). Individuals with exclusion probability of 0.95 and above were assigned to a colony.

We examined the response of the dependent variable, proportion male diploidy, to the independent variables island vs mainland site type, percent low quality nesting habitat, and sampling date (Julian date) via a generalized linear mixed effects model with binomial distribution (comparing male diploidy to haploidy) and region as a random effect using the 'lme4' package in R. Likewise, within lme4, in two separate models, we examined the response of the dependent variables, Nef and Div, to the independent variables Island versus Mainland site type, proportion low quality nesting habitat, and sampling date via a linear mixed effect model with a normal distribution and region as a random effect. Finally, we compared % male diploidy between island and mainland sites using a Kruskal–Wallis rank sum test and compared Nef and Div using ANOVA. All independent variables were centered before site-scale analyses.

Landscape-scale analyses: spatial genetic structure

To conduct population genetic analyses on just the haploid individuals and to ensure that colony mates were not duplicated in the population genetic analyses, one haploid individual per colony was randomly chosen to represent the colony in the landscape-scale and regional-scale data set ($N = 374$, 20.78 ± 3.09 haploid individuals per site). Utilizing the haploid data, spatial genetic structure was examined using SpaGeDi (Hardy and Vekemans 2002). Pairwise geographic distance between individuals in island and mainland sites were calculated and binned from 0 to 25 km in intervals of 5 km. The number of pairs in each interval was >2500. Standard errors (SE) were computed by jackknifing over loci and 95% confidence intervals (CI) were computed by permuting over geographical location and multiloci genotypes (nperm = 10,000).

Regional-scale analyses: genetic differentiation, IBD, and IBR

To calculate pair-wise differentiation across loci between all populations, we used the Φ_{ST} parameter as it is most appropriate for haploid data (Owen and Packer 1994; Holsinger and Weir 2009). We used GenAlEx to calculate Φ_{ST} and the 95% CI were estimated by bootstrapping over loci. We analyzed both IBD and IBR to examine the correlation between genetic differentiation, geographic distance, and resistance distance. Resistance surfaces were created to quantify the barrier that low quality nesting habitat may pose to gene flow. Resistance values ranged from 0 (no resistance)—1 (highest resistance), providing a narrow range of resistance and a more intuitive scale for comparative analysis. Open water, highly impervious land, and crops were assigned 0.9 resistance value as they have been shown to correlate with increased genetic structure for the species, whereas grassland, suburban, and forested areas were assigned 0.1 (Jha and Kremen 2013; Jha 2015). The resistance distance matrix was calculated via CIRCUITSCAPE V3 (McRae 2006) which is based on electronic circuit theory and evaluates contributions of multiple dispersal pathways. We used a raster map at 1 km resolution, chosen due to the multi-kilometer dispersal ability of bumble bees and the large size of our study region (Jha 2015).

Both geographic distance and resistance distance matrices were examined for co-linearity via the R package USDM (Babak 2013). As the variance inflation factor (VIF) was >5, we decided to compare the effect of the two distances on genetic differentiation separately. Specifically, we used multiple regression on distance matrices (MRDM) conducted using the R package ECODIST

(Goslee, and Urban 2007) where we examined differentiation (Φ_{ST}) as a function of log10 geographic distance (IBD) and then separately as a function of resistance distance (IBR). All independent variables were centered before regional-scale analyses.

Results

Site scale

We found that proportioin male diploidy was significantly greater in mainland sites than island sites (z-value = 2.100, p = 0.036) and increased significantly with the proportion of low quality nesting habitat (z-value = 3.889, p = 0.0001), though it did not show a response to date (z-value = 0.972, p = 0.331). Likewise, Div was significantly higher in mainland sites (t-value = 3.14, p = 0.025) but unaffected by nesting habitat (t-value = −0.723, p = 0.488) and date (t-value = −0.451, p = 0.663). Nef was not significantly different between island or mainland sites (t-value = 1.316, p = 0.245) and was not affected by nesting habitat (t-value = −0.549, p = 0.596) or by date (t-value = −0.563, p = 0.586) (Fig. 1; Table 1).

Landscape scale

At the landscape scale we found evidence of significant spatial genetic structure for male *B. vosnesenskii* at the 0–5 km ($F_{ij} = 0.050$, $p < 0.001$) and 5–10 km ($F_{ij} = 0.063$, $p = 0.065$) scales in island populations and at the 0–5 km ($F_{ij} = 0.010$, $p = 0.012$) in mainland populations. As distance increased, average relatedness decreased in both island and mainland sites (Fig. 2). Full sibs (not included in this analysis) were found between 25 m and 11.92 km apart.

Regional scale

Global differentiation, though low, was significant ($\Phi_{ST} = 0.049$, $p = 0.001$). Examination of pairwise Φ_{ST} values across sites indicated significant pairwise differentiation ($p < 0.1$ for all comparisons) (Table 2). The MRDM analysis demonstrated no significant IBD nor IBR for Φ_{ST} (Table S1).

Discussion

We present one of the first analyses to measure diploidy levels as well as landscape-scale and regional-scale patterns of genetic structure assessed from wild male bumble bees. At the site scale, across mainland and islands, we reveal high proportions of male diploidy for *B. vosnesenskii*. At the landscape scale, we find strong evidence of local dispersal or natal site fidelity for males, with greater and more extended spatial genetic structure in island sites. At the regional scale, we document low but significant levels of genetic differentiation, though not significantly explained by IBD or IBR.

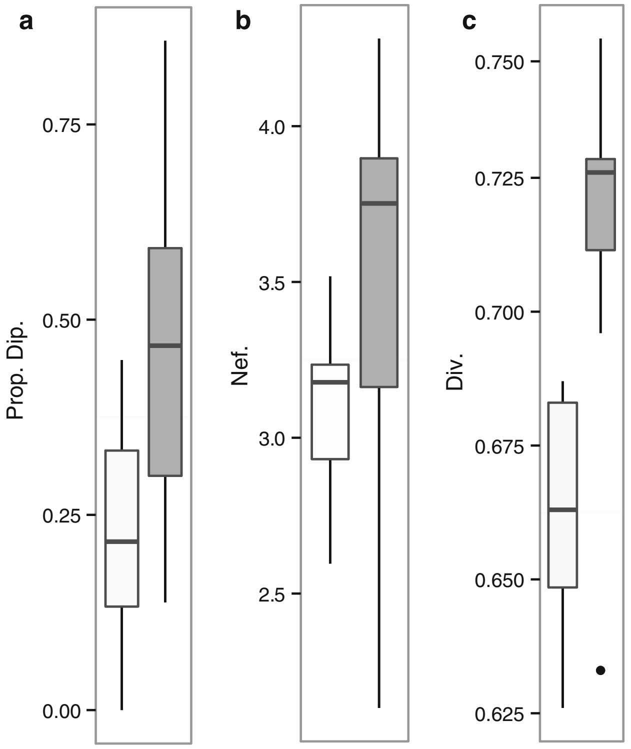

Fig. 1 *Box and Whisker plots* for island (*white*) and mainland (*grey*) sites comparing **a** proportion diploid (Pro. Dip) (p = 0.085), **b** number of effective alleles (Nef) (p = 0.701), and **c** Nei's unbiased haploid gene diversity (Div) (p = 0.024)

Site scale: sibship and male diploidy

Our results indicate highly variable levels of male diploidy in wild populations (range 0–86%) of *B. vosnesenskii,* at rates much higher than those reported for other *Bombus* species. Specifically, past studies in Scotland and the southern UK have shown that *B. muscorum* exhibits rates of 5% male diploidy (Darvill et al. 2006), and in Canada, *B. occidentalis* and *B. perplexus* exhibit male diploidy frequencies of 6 and 3% respectively (Whidden and Owen 2011), though these studies examined fewer males per site and surveyed bees later in the season compared to the present study. In a lab study of *B. terrestris*, where brother-sister individuals were mated, 50% of diploid offspring produced by the colony were male (Duchateau et al. 1994),

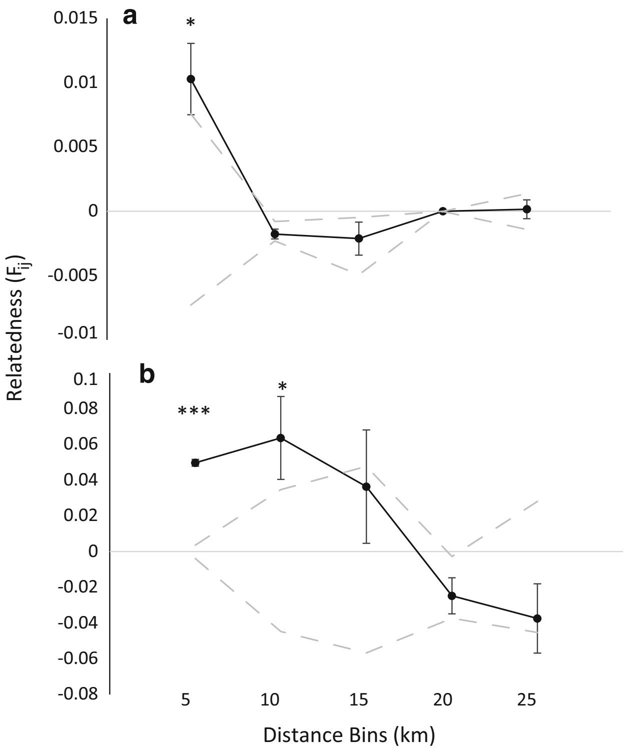

Fig. 2 Loiselle's Relatedness index (F_{ij}) estimated for pairwise individuals in Euclidean distance bins for **a** mainland and **b** island sites. *Dashed lines* represent permuted 95% CI for the null hypothesis ($F_{ij} = 0$) and significance * indicates significance at $p < 0.1$, and *** indicates significance at $p < 0.001$. Estimates and SD are mapped to the upper boundary of the distance bin such that 0–5 km is depicted at 5 km

revealing a critical role for sib-mating to promote male diploidy. Euglossine bees have also been documented to exhibit substantial variation in male diploidy ranging from 0.5% (Souza et al. 2010) to up to 11.3% (Giangarelli et al. 2015). Studies on Halictine bees have recorded some of the highest diploid male frequencies, similar to levels documented in this study, where *Halictus poeyi* exhibited 9.1–50% male diploidy within their populations (Zayed and Packer 2001) and *Lasioglossum zephyrum* exhibited 1.6–100% male diploidy (Kukuk and May 1990).

We suggest four potential reasons for the high frequencies of male diploidy observed in this study. First, male frequencies may simply vary across *Bombus* species, as documented for Euglossine bees (Giangarelli et al. 2015); low male and queen frequency or reduced male and queen dispersal may lead to increased reproduction with closely related individuals. Specifically, if bumble bee queens in these study sites exhibit strong natal site fidelity or dispersal limitation, as previously documented for *B. vosnesenskii* (Jha and Kremen 2013) then this local dispersal may promote male diploidy. Second, past studies on other bee species have suggested that variation in male diploidy may be due to limited nest availability and frequent extinction and colonization events (Zayed and Packer 2001). This latter argument is supported in our study given that we document significantly higher male diploidy in sites with limited nest availability. Third, male diploidy could be driven by seasonality, where diploidy may be highest when diploid (queen and worker) production is highest. Diploid males are believed to be common when queens are producing many diploid workers (Zayed and Packer 2001). For *B. vosnesenskii* both worker and queen production peak in June and July (Thorp et al. 1983), during the time of our sampling. Interestingly, we did not find a pattern between date and male diploidy level, perhaps due to the short and early time span of our sampling (June–July), as males are likely to have been present until September (Thorp et al. 1983). A fourth potential explanation is that, because male diploidy often manifests itself in reduced physiology (Cook and Crozier 1995), it is also plausible that diploid *B. vosnesenskii* males may have reduced flight capacity, facilitating capture in surveys like this study, and potentially biasing our sampling as a result. However, this does not explain the documented correlation between male diploidy and low quality nesting habitat, unless these habitats also facilitate the capture of more physiologically challenged males, which we do not believe to be the case.

Whatever the cause, diploidy in males is an indicator of local inbreeding and is likely detrimental to the colony

Table 2 Pairwise Φ_{ST} differentiation located below diagonal and p values located above the diagonal

	BerryA	Holl25	PopeA	SantaCata19	SantaCruz14	SantaRosa	SecondB
BerryA		0.001*	0.001*	0.001*	0.056	0.009*	0.001*
Holl25	0.051		0.100	0.001*	0.001*	0.001*	0.025*
PopeA	0.076	0.012		0.001*	0.001*	0.001*	0.004*
SantaCata19	0.071	0.055	0.054		0.001*	0.001*	0.001*
SantaCruz14	0.014	0.053	0.088	0.094		0.070*	0.001*
SantaRosa15	0.030	0.042	0.062	0.051	0.011		0.002*
SecondB	0.074	0.015	0.028	0.039	0.082	0.048	

* Indicates significance at $p < 0.05$

(Cook and Crozier 1995; Zayed et al. 2004). Past research conducted on laboratory bumble bees show that colonies with diploid males experience lower colony growth rates and reduced offspring production compared to colonies without diploid males (Whitehorn et al. 2009). Further, a reduced production of foraging workers due to diploid male production decreases the overall resources that the colony obtains and limits colony growth. If diploid males are reproductive, the genetic repercussions of diploid male mating may include sterile triploid (female) worker production (Darvill et al. 2012). The fact that triploid female worker frequencies are inversely correlated with population size (Darvill et al. 2012) further suggests that mating with diploid males may be likely when males are rare or when males and new queens are dispersal limited.

Landscape-scale analyses: relatedness

Within island sites, our analyses of male *B. vosnesenskii* reveal high levels of relatedness from 0 to 5 km and 5 to 10 km scales, similar to patterns seen in female *B. vosnesenskii* (Jha and Kremen 2013) and similar to recapture-based estimates of queen dispersal extending up to ~5–8 km for *B. lapidarius* and *pascuorum* queens (Lepais et al. 2010). This contrasts the findings of a recent study that compared relatedness levels in male and female bees for the solitary ground-nesting species *C. inaequalis*, which revealed lower relatedness in males compared to females, likely driven by greater male dispersal (Lopez-Uribe et al. 2015). Similarity in the relationship between relatedness and distance for males and females for *B. vosnesenskii* may be due to the fact that, unlike many solitary bees, the reproductive females in *Bombus* (queens) tend to be much larger-bodied than males and workers. Though it is unknown how bee body size correlates with dispersal ability, meta-analyses reveal a strong positive relationship bee between body size and foraging ability (Greenleaf et al. 2007), suggesting that dispersal may be likewise related to body size, as seen in other taxa (Stevens et al. 2014). However, it must be noted that we do not know the reproductive status of the males in this study, thus signatures of relatedness are likely derived from a combination of individuals engaging in reproduction and subsequent movement process, and those that do not.

Interestingly, we show that the male bumble bees exhibit varying levels of relatedness, with higher levels at larger spatial scales (from 0 to 10 km) in island sites compared to mainland sites (0–5 km). This can be explained by three factors: (1) island sites have lower levels of genetic diversity and this could drive patterns of greater relatedness at larger scales, (2) island males exhibit philopatry at larger scales due to behavioral or physiological differences in male dispersal patterns, (3) islands have more suitable nesting options within a 10km range, promoting larger-scale philopatry. Overall, this high relatedness suggests that many males are staying close to their natal colonies, which is unexpected given the long distance foraging abilities of some male bumble bees (9.9 km for *B. terrestris*) compared to female workers (2.4 km) (Kraus et al. 2009). In another example, Wolf et al. (2012) used relatedness patterns of males and workers to estimate that male dispersal may exceed workers by a factor of 1.66 in *Bombus terrestris* and a factor of 1.74 in *Bombus lapidarius*. We suggest the high levels of landscape-level relatedness in our system indicate that while males are capable of dispersing across long distances, many are staying close to their natal colonies. Studies on the physical and behavioral attributes of male flight, such as degree of philopatry, dispersal duration, and impact of local flight conditions on movement would provide further insight into the patterns we observed.

Regional-scale analyses: genetic differentiation

The differentiation patterns documented in this study are similar to those for conspecific workers collected in a similar study region ($F_{ST} = 0.019$, $D_{EST} = 0.054$ in Jha and Kremen 2013). This is expected, to some degree, since microsatellites are nuclearly inherited and represent both male and female migration and drift processes. Overall, we show that *B. vosnesenskii* males exhibit low levels of genetic structure, a pattern seen in studies conducted on the workers of other Bombini species, such as studies of *B. muscorum* ($Fst = 0.13$), *B. jonellus* ($Fst = 0.034$) (Darvill et al. 2010), and *B. hortorum* ($Fst = 0.16$) (Goulson et al. 2011). While not significant, male *B. vosnesenskii* isolation by resistance (IBR) explained differentiation patterns to a similar degree as isolation by geographic distance (IBD), though these patterns were not as strong as those documented in past studies, where intensively managed crops, urbanized habitat, and ocean cover significantly explained differences in genetic differentiation in workers of *B. vosnesenskii* (Jha and Kremen 2013; Jha 2015). Past studies on other *Bombus* species have also shown that incorporating landscape features, like open water, can improve model fit for predicting bumble bee differentiation patterns (Goulson et al. 2010).

In this study, we document that high levels of male diploidy can exist in non-declining species, especially in sites with poor nest availability. Further, we show that males exhibit similar levels of relatedness as documented in the past for workers. Interestingly, we also document low genetic differentiation across the region, and thus propose that male diploidy levels represent largely local processes and may not limit the reproductive capacity or dispersal ability of the species at large spatial scales.

Indeed, male diploids, if not sterile, have reduced reproductive capability, limiting their contribution to gene flow, and potentially preventing them from impacting regional-scale gene flow patterns. We note that our survey was conducted across just one year, and thus represents a snapshot of male diploidy, relatedness, and differentiation patterns; the long-term patterns and impacts of high diploidy and relatedness on population processes remain an important understudied aspect of bee biology. Overall, while the high male diploid frequencies in this study are a cause for concern, examination of genetic structure across the study region suggests that many reproductive males and queens are still engaging in long-distance dispersal.

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