Novel microsatellite markers for *Osmia lignaria* (Hymenoptera: Megachilidae), a North American pollinator of agricultural crops and wildland plants

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Abstract

Comprehensive decisions on the management of commercially produced livestock, including solitary bees, depend largely on associated knowledge of genetic diversity. In this study, we present novel microsatellite markers to support the breeding, management, and conservation of the blue orchard bee, *Osmia lignaria*. Native to North America, *O. lignaria* has been trapped from wildlands and propagated on-crop and used to pollinate certain fruit, nut, and berry crops. Harnessing the *O. lignaria* genome assembly, we identified 59,632 candidate microsatellite loci in silico, of which 22 were tested using molecular techniques. Of the 22 loci, 14 loci were in Hardy-Weinberg equilibrium (HWE) and demonstrated no linkage disequilibrium (LD) in two Intermountain North American wild populations in Idaho and Utah. We found no difference in population genetic diversity between the two populations, but there was evidence for low but significant population differentiation. Also, to determine if these markers amplify in other *Osmia*, we assessed 23 species across the clades *apicata*, *bicornis*, *emarginata*, and *ribifloris*. Nine loci amplified in three species/subspecies of *apicata*, 22 loci amplified in 11 species/subspecies of *bicornis*, 11 loci amplified in seven species/subspecies of *emarginata*, and 22 loci amplified in two species/subspecies of *ribifloris*. Further testing is necessary to determine the capacity of these microsatellite loci to characterize genetic diversity and structure under the assumption of HWE and LD for species beyond *O. lignaria*. These markers will help to inform the conservation and commercial use of trapped and managed *O. lignaria* and other *Osmia* species for both agricultural and non-agricultural systems.

Introduction

The discovery and interpretation of genotypes sequenced from neutrally evolving genetic markers are critical to breeding decisions of managed and wild species in agriculture and biodiversity conservation, respectively. Globally, the genetic diversity of some 7,500 livestock breeds is decreasing, putting significant pressure on economic markets and food availability (Boettcher et al., 2010). Supporting genetically diverse populations of agriculturally significant species allows for breeds to remain resilient in the face of climate change, changes in market needs, management practices, husbandry practices, parasites, and pathogens (Boettcher et al., 2010). Likewise, the conservation of genetic diversity is critical for the conservation of declining wildlife to buffer against comparable environmental stressors such as parasites, pathogens, and climate change (DeWoody, Harder, Mathur, & Willoughby, 2021; Teixeira & Huber, 2021). To this end, identifying and employing genetic markers such as microsatellites in guiding the management of livestock and wildlife is important to agricultural and non-agricultural ecosystems (Abdelmanova et al., 2021; Beacham et al., 2008; Ginja et al., 2013; Guichoux et al., 2011; Pham et al., 2013; Strange, Delaney, Tarpy, & James, 2017), especially when

combined with additional biological and ecological data (Addis, Lowe, Hossack, & Allendorf, 2015; Koch, Vandame, Mérida-Rivas, Sagot, & Strange, 2018).

Bees (Hymenoptera: Anthophila) are a unique group of animals as they serve as couriers of pollen across flowering plants more often than any other taxa, thereby supporting plant reproduction. Globally, there are more than 20,000 described bee species (Ascher & Pickering, 2020). The most intensively managed bee species is the European honey bee, *Apis mellifera* L. (Hymenoptera: Apidae) due to its reputation as a productive honey-maker and crop pollinator. Within the last century, the critical role of managed honey bees in supporting industrial scale crop production has been demonstrated worldwide, well outside their endemic range of Europe and North Africa. The identification and interpretation of genetic markers have been critical in the development of effective honey bee breeding and management strategies (Bourgeois, Sylvester, Danka, & Rinderer, 2008; Delaney, Meixner, Schiff, & Sheppard, 2009; Jensen et al., 2006; Paál et al., 2021; Parejo, Henriques, Pinto, Soland-Reckeweg, & Neuditschko, 2018). More recently, environmental pressures such as increased use of pesticides, increased susceptibility to and transmission of disease, changes in diet quality, and land-use change, have become major stressors that imperil the commercial supply of honey bees (Shanahan, 2022). The recent losses of managed honey bee colonies in some parts of the world have emphasized the need to develop breeding and management programs for additional bee species that can be used for pollination of commercial crops (National Research Council, 2007).

The blue orchard bee, *Osmia lignaria* Say (Hymenoptera: Megachilidae) is a solitary, cavity-nesting mason bee native to North America (Bosch & Kemp, 2001; Branstetter, Müller, Griswold, Orr, & Zhu, 2021). Starting in the 1970s, pioneering work by Philip Torchio and his colleagues demonstrated the usefulness of *O. lignaria* to support the pollination of certain agricultural crops (Fig. 1) (J. Bosch & Kemp, 2001). It is an effective pollinator of fruit trees, especially almonds, apples, cherries, nectarines, and plums (Bosch & Kemp, 1999; Bosch, Kemp, & Peterson, 2000; Bosch, Kemp, & Trostle, 2006; Boyle & Pitts-Singer, 2019; Sheffield, 2014; Torchio, 1976), among other rosaceous and berry crops (Bosch & Kemp, 2001). The capacity of these bees to forage for pollen and nectar under cool and wet spring weather conditions, their tendency to move between trees and tree rows, and their collection of pollen as loose, dry granules on the underside of the abdomen lends to their efficiency as pollinators, especially in orchard crops (Bosch & Kemp, 2001). For example, when *O. lignaria* are deployed alone or as co-pollinators with honey bees into almond and cherry orchards, an increase in fruit set and yield have been observed (Pitts-Singer, Artz, Peterson, Boyle, & Wardell, 2018). Thus, the inclusion of *O. lignari* a into integrated crop pollination management strategies provides pollination insurance and supports sustainable yields of important pollinator-dependent crops (Isaacs et al., 2017).

The commercialization of native and introduced solitary bee species to deliver pollination services has occurred across the globe (Osterman et al., 2021), and includes several megachild species, like *O. lignaria* , that readily build nests in provided artificial tunnels. *Osmia lignaria* is a member of the *bicornis* clade within the subgenus *Osmia* (*Osmia*), a group that includes a number of managed pollinator species, and it is composed of two named subspecies: *O. lignaria lignaria* Say and *O. lignaria propinqua* Cresson (Branstetter et al., 2021). The two subspecies are geographically separated into eastern and western ranges approximately by the 100th Meridian (Bosch & Kemp, 2001; Rust, 1974). In the eastern portion of its native range, *O. l.lignaria* is distributed from Georgia north to Nova Scotia, and west to Texas and Michigan. In the western portion of its distribution, *O. l. propinqua* is distributed from southern California north to British Columbia and east to South Dakota and Texas (Bosch & Kemp, 2001). However, the validity of the subspecies is uncertain and morphological and genetic data is needed to test the subspecies hypothesis.

Microsatellites are useful and affordable genetic markers that have the capacity to capture multilocus genotype information for estimating genetic diversity and structure (Guichoux et al., 2011). In this study, we use a recently developed genome assembly of *O. lignaria* to identify novel microsatellite markers that can support population genetic analysis of *O. lignaria* . Next, we used the markers to estimate sib-ship relationships among two Intermountain North America *O. l. propinqua* (hereafter stated as *O. lignaria*) populations and determined basic population genetic diversity metrics. Furthermore, we estimated population structure with

an analysis of molecular variance to test for differences in genetic variance in the *O. lignaria* populations. Finally, we test the novel microsatellite loci on other *Osmia* species within the *apicata* , *bicornis, emarginata* , and *ribiflorisclades*. Our overall goal is to demonstrate the utility of these new microsatellite markers in *O. lignaria* as well as their potential use in other agriculturally important *Osmia* species.

Materials and Methods

Specimen collection. To evaluate marker amplification and sequencing capacity, we sampled bees from populations of *O. lignaria* from California (El Dorado, Fresno, and Placer Counties) and Idaho (Franklin County) sourced from commercial suppliers who trap bees on private lands. However, the sex of these specimens was not recorded. Thus, material derived from these specimens were only used for method development as they are inappropriate for population genetic analysis due to constraints of haplodiploidy on our analytical methods.

Additional collections of *O. lignaria* were later achieved by placing wooden blocks ($n = 5$) with paper straws inserted into drilled tunnels in two canyons in the Intermountain West of North America: Birch Creek, Utah (41.852556, -111.775259, 1620 m) and Cub River, Idaho (42.138868, -111.667406, 1678 m). These two trapping sites were ˜30 km apart and both located in canyons in the Bear River Mountains (Fig. 1). The nesting blocks were deployed in April 2018, retrieved in May 2018, and brought to the U.S. Department of Agriculture - Agricultural Research Service - Pollinating Insect Biology, Management, Systematics Research Unit (USDA-ARS-PIBMSRU). The straws were removed so that contents of nest cells inside could be determined from x-radiological images. In October 2018, cells containing cocoon diapausing adults were excised from nests and stored at - 80°C until needed for species identification, sex determination, and molecular testing. The pharates were easily sexed based on dimorphic characteristics from dissected cocoons (males have an abundance of setae on their clypeus and frons whereas females lack this trait) and their position in the straw nest. In general, the first laid cells in a nest develop into female bees (distal end of straw), whereas the later laid cells develop into male bees (proximate end of straw). All *O. lignaria* individuals captured in the nesting blocks were used for population genetic analysis.

Microsatellite mining and assessment. To identify microsatellite loci, we surveyed the publicly available annotated genome assembly of *O. lignaria* (USDA_OLig_1.0), available on NCBI (https://www.ncbi.nlm.nih.gov/assembly/GCF_012274295.1/). Using Krait v1.3.3, we queried the genome assembly for microsatellite markers across 147 scaffolds and developed primers to be tested using PCR amplification (Du et al., 2018). In this study, we identified perfect, imperfect (*i.e.* contain indels or substitutions), and compound tandem repeats *in silico* . Microsatellites were filtered using custom scripts in the R programming language (R Core Team, 2020). Microsatellites that were di-, tri-, and tetra-nucleotide repeats were targeted. Products that were 100 – 500 nucleotides (nts) long were selected. Primer pairs that annealed to multiple sites across the genome were excluded. Loci that have primer pairs not exhibiting between 40 and 60% GC content were excluded. Only primers that have an annealing temperature near 55°C (54.5 - 55.4°C) were included. A more detailed narrative of the conditions used to filter microsatellites and design primers is provided in the Supplementary Material 1. Of the loci that fit our search criteria, we randomly selected 22 loci for further testing with molecular methods. We used NCBI Genome Data Viewer to examine the placement of the novel loci across intergenic (*i.e.*exon) and intragenic (*i.e.* intron) space in the annotated *O. lignaria* genome assembly, as well as proximity and placement near or within a gene, respectively.

Genomic DNA was extracted from the legs of 124 adult specimens with a Quick-DNA Miniprep Plus Kit (Zymo Research, Irvine, CA, U.S.A.) following the manufacturer's protocol, except that the Proteinase K incubation step with the following modifications: Proteinase K digestion incubated 12-16 hours, DNA elution buffer was warmed to 60 °C prior to addition, and elution buffer was added in two steps to increase DNA yield. The 22 novel loci were amplified with extracted DNA from a haphazardly selected sample of eight specimens, PCR amplified, and confirmed on 1.2% agarose gel alongside a 100 bp ladder (GeneRuler DNA Ladder, ThermoFisher Scientific). PCR reactions were performed using the following methods: 1-2 µl extracted DNA, 1x Promega (Madison, WI) reaction buffer, 0.6 mM dNTP mixture, 0.1-0.4 µM primer, 0.001 mg BSA, 0.4 units Taq polymerase (Promega, Madison WI), and 1.4 mM $MgCl_2$. The PCR conditions

for multiplex reactions were: one 4 min cycle at 95 °C, 30 cycles of 95 °C for 30 sec, and annealing at 55 °C for 75 sec, then 72 °C for 45 sec. The cycles were followed by a final extension period of 15 min at 72 °C.

Once a product was confirmed at the expected length in the PCR reactions, the 5' ends of each forward primer were fluorescently labeled as either 6-FAM, VIC, NED, or PET and combined in a four-marker multiplex reaction. Each multiplex reaction represented one of the dye-labeled conditions, which were then separated on an ABI PRISM 3730 DNA Analyzer along with a dye-labeled size standard (GeneScan 500 LIZ dye size standard, Applied Biosystems^{TM}, ThermoFisher Scientific) at the Utah State University Center for Integrated Biosystems (Table 1). Allele sizes were scored using Geneious Prime 2021.0.1 (Kearse et al., 2012). Twenty percent of the specimens underwent additional PCR amplification to determine genotype error rates. We tested for differences in genotype error rates across the different microsatellite types with a Wilcoxon Rank-sum test.

Sibship identity and population genetic analysis. We first identified sibship relationships at the Birch Creek and Cub River field sites by assigning individuals to families using full-pedigree likelihood methods implemented in Colony v2.0 Linux software (Jones & Wang, 2010). We set the mistyping error rates of each locus based on the genotype error rates for the 20% of specimens re-genotyped (Table 1). We set the sex-determination system to 'haplodiploid' and mating system I to "Polygamous" for both males and females based on research by Bosch and Kemp (2001). Colony analyses were conducted in Ceres, a high-performance computing cluster maintained by the Agricultural Research Service SCINet (*https://scinet.usda.gov/*

To determine population genetic diversity within and across the two *O. lignaria* populations, we first identified whether the loci were in Hardy-Weinberg equilibrium (HWE) and that the loci were in random association (*i.e.,* linkage disequilibrium [LD]). Deviations from HWE and LD resulted in the removal of the loci for population genetic analysis. HWE and LD tests were conducted with the Genepop algorithm (Raymond & Rousset, 1995). To account for multiple comparisons inherent to HWE and LD analyses, sequential Bonferroni corrections were applied to the HWE and LD P values to minimize type I errors across population by marker combinations. Following the HWE and LD analyses, we calculated observed and Nei's expected heterozygosity ($H_o$and Nei's H_e , respectively), Simpson's Index of diversity, allelic richness, number of alleles, and private alleles for the Birch Creek and Cub River study populations.H_o is the observed number of loci that are heterozygous for a sampled individual whereas Nei'sH_e is the expected number of loci that are heterozygous for a sampled individual. Simpson's Index of diversity (D) measures the probability that individuals randomly selected from a sample will belong to the same allele class. In our study, we present the index 1-D which represents the probability that two individuals randomly selected from a population will belong to a different allele class. Thus, the greater the 1-D index, the greater the diversity of alleles (Magurran, 2003). Allelic richness is calculated as the number of alleles at a locus divided by the number of specimens without data missing at the target locus. Number of alleles is the number of alleles at a locus, and private alleles is the number of alleles that were only detected in one population. We tested for differences in these genetic diversity indices between Birch Creek and Cub River with a two-sample t -test or Wilcoxon rank-sum test. The latter non-parametric test was used if the genetic diversity indices demonstrated unequal variances between the two populations.

To characterize population genetic structure, we conducted an analysis of molecular variance (AMOVA). This approach mirrors the analysis of variance approach based on Wright's fixation indices (Weir & Cockerham, 1984). We conducted a hierarchical analysis of molecular variance where variance was partitioned to covariance components based on variation within sample, variation between samples, and variation between populations. These covariance components were calculated as Φ fixation indices (Grünwald & Hoheisel, 2006). We further tested for the significance of Φ through comparison of 999 nonparametric permutations of the data (Kamvar et al. 2015) . We conducted additional independent tests for structure between the two populations by calculating the following indices:F_{ST} , F_{IS} ,G_{ST} , and Jost's D. Definitions of these different fixation indices are articulated in Meirmans and Hedrick (2011). In general, higher values of F_{ST} ,G_{ST} , and Jost's D implicate higher fixation of alleles at a locus in a population, whereas lower values of these indices suggest lower fixation of alleles at a locus in a population.F_{IS} is a measure of inbreeding, where high values

of F_{IS} are evidence for inbreeding. In these tests of population structure, we calculated theF_{IS} based on a global comparison between populations also with 999 permutations of the data (Archer et al., 2017).

Osmia **subspecies, species, and clade microsatellite marker amplification.** We aimed to determine the capacity for the novel microsatellite markers to amplify in taxa other than *Osmia lignaria* . We explored marker amplification for the *apicata* ,*bicornis* , *emarginata* , and *ribifloris* clades following Branstetter et al. (2021). We included three species/subspecies of *apicata* , 12 species/subspecies of *bicornis* (including *O. lignaria*), seven species/subspecies of *emarginata* , and two species/subspecies of *ribifloris* in our assessment. DNA was sampled from a specimen library described in Branstetter et al. (2021).

Except for the Colony analysis conducted on SCINet, all population genetic analyses were conducted with the base, strataG (Archer, Adams, & Schneiders, 2017), poppr (Kamvar, Brooks, & Grünwald, 2015), and adegent (Jombart, 2008) libraries in R version 4.0.3 (R Core Team 2020). Data and R scripts of the described analysis are available at*https://github.com/jbkoch/OligMsatStudy.*

Results

Specimen Collection. For initial microsatellite PCR amplification and gel electrophoresis assessments, we sampled 48 adult specimens from populations of *O. lignaria* collected in California (El Dorado County [n = 16], Fresno County [n = 11], Placer County [n = 13]) and Idaho (Franklin County [n = 8]). Using other wild-caught specimens, we conducted a population genetic analysis of 35 *O. lignaria* female specimens from Birch Creek and 41 *O. lignaria* female specimens from Cub River.

Microsatellite mining and assessment. Based on the filtering criteria we defined for our microsatellite query with the *O. lignaria* genome assembly, we identified 48,816 imperfect microsatellites, 10,553 perfect microsatellites, and 263 compound microsatellites. Average imperfect microsatellite product size is 153 nts ± 0.14 SE (maximum = 500, minimum = 100); average perfect microsatellite size is 148 nts ± 0.27 SE (maximum = 241, minimum = 100); and average compound microsatellite size is 171 nts ± 2.35 SE (maximum = 353, minimum = 100). We provide the filtered imperfect, perfect, and compound microsatellite primer sets in Supplementary Tables 1, 2, and 3, respectively.

From the queried loci, we haphazardly selected 22 microsatellites for testing, 13 of which were perfect microsatellites and nine of which were imperfect microsatellites (Table 1). We did not include compound microsatellites in our study due to their small sample size of 263 and product size overlap with perfect microsatellites. In the 76 *O. lignaria* specimens tested from Cub River and Birch Creek, average amplification success of the 22 markers was 95% ± 1% SE (maximum = 100%, minimum = 80%) (Table 1). Oli61901 demonstrated the least amplification success with 20% of specimens not amplifying at this locus. Oli064 demonstrated 100% amplification success in all tested specimens, whereas Oli160, Oli84207, Oli119, Oli131, Oli216, Oli127, Oli076, Oli053, Oli76146, and Oli03483 demonstrated 99% amplification success in all tested specimens. Average genotyping error rate was 11% ± 3% SE (Median = 3%; maximum = 42%, minimum = 0%). Eight markers had a genotyping error rate of 0% (*i.e.,* sampled specimens were genotyped consistently at these loci), and included Oli156, Oli105252, Oli104572, Oli963, Oli144609, Oli76146, Oli141, Oli103483. Markers with a genotyping error rate > 30% included Oli053, Oli076, Oli107, and Oli127 (Table 1). Furthermore, a Wilcoxon Rank-sum test found that perfect microsatellites (M = 0.11, n = 13) had significantly larger genotype error rates than imperfect microsatellites (M = 0, n = 9) (W = 20.5, P = 0.01).

The 22 novel microsatellite loci tested in our study were distributed across 16 scaffolds of the *O. lignaria* genome assembly (Supplementary Table 4). Oli127 and Oli84207 were placed in scaffold NW_023009259.1; Oli107, Oli119, Oli131, and Oli216 were placed in scaffold NW_023009260.1; and Oli104572, Oli105252, and Oli156 were placed in scaffold NW_023009264.1. Based on the start position of each locus, mean pairwise nucleotide distances between loci co-located on a scaffold exceed a nucleotide distance of 500,000 nts: NW_023009259.1$_{\mathrm{DIST}}$ = 559,304 nts (n = 1), NW_023009260.1$_{\mathrm{DIST}}$ = 2,369,751 nts ± 514,195 SE (n = 6), and NW_023009264.1$_{\mathrm{DIST}}$ = 6,599,034 nts ± 3,062,327 SE (n = 3). Comparison of the loci with the annotated*O. lignaria* genome assembly suggests that four of the tested microsatellite loci are in an intergenic space, whereas 18 of the loci are in an intragenic space of a predicted gene. The average estimated distance of the

intergenic loci to the nearest gene was 8,803 nts. Additional information, including gene identity, of the 22 novel microsatellites examined in this study are available in Supplementary Table 4.

Sibship identity and population genetic analysis. Of the 35 *O. lignaria* individuals surveyed in Birch Creek, 21 full-sibling families were identified. Family size of the surveyed specimens in Birch Creek range from one to three individuals (mean = 1.67 ± 0.16 SE). Of the 41 *O. lignaria* individuals surveyed in Cub River, 24 full-sibling families were identified. Family size of the surveyed specimens in Cub River ranged from one to five individuals (mean = 1.70 ± 0.19 SE) (Table 2).

Seven loci, Oli160, Oli053, Oli064, Oli076, Oli127, Oli61901, and Oli84207, were not in HWE after Bonferroni correction (Table 1). Of the markers tested for LD and not in HWE, Oli053 was in LD with Oli076. Conversely, the following LD marker comparisons had at least one marker that was in HWE: Oli216 (in HWE) was in LD with Oli053 (not in HWE); Oli131 (in HWE) was in LD with Oli076 (not in HWE); Oli101 (in HWE) was in LD with Oli156 (in HWE) and Oli119 (in HWE); and Oli119 (in HWE) was in LD with Oli127 (in HWE) (Table 1). For the final analysis, we elected to remove all seven loci that were not in HWE and removed Oli101 as it was in LD with two other markers (Oli156 and Oli119). The loci either in HWE or demonstrating LD also demonstrated high genotype error rates (Supplementary Tables 5, 6). These loci are unlikely to be reliable markers for *O. lignaria* population genetic analysis due to phenomena like null alleles or primers binding to multiple annealing sites. Removal of these eight loci resulted in 14 loci suitable for further population genetic analysis: Oli021, Oli107, Oli119, Oli131, Oli141, Oli144609, Oli156, Oli216, Oli963, Oli74400, Oli76146, Oli103483, Oli104572, Oli105252.

We found no significant difference between mean H_O and Nei's H_E in the Birch Creek population (mean H_E = 0.63 ± 0.05 SE, mean H_O = 0.60 ± 0.07 SE; $t = 0.32$, $df = 26$, $P = 0.75$) or Cub River population (mean H_E = 0.60 ± 0.06 SE, mean H_O = 0.53 ± 0.06 SE; $t = 0.71$, $df = 26$, $P = 0.48$). Furthermore, we found no significant difference in average H_E between the Birch Creek or Cub River populations ($t = 0.38$, $df = 26$, $P = 0.71$). In addition to heterozygosity comparisons across populations, we found no significant difference between the Birch Creek and Cub River populations in Simpson's Index of Diversity (1 - D) (Birch Creek mean [1-D] = 0.61 ± 0.05 SE; Cub River mean [1-D] = 0.58 ± 0.06 SE; t = -0.35, $df = 28$, $P = 0.73$), allelic richness (Birch Creek mean AR = 0.40 ± 0.06 SE; Cub River mean AR = 0.33 ± 0.07 SE; $t = 0.79$, df = 26, $P = 0.44$), the number of alleles (NA) (Birch Creek mean NA = 6.86 ± 0.94 SE; Cub River mean NA = 7.36 ± 1.51 SE; t = -0.26, $df = 26$, $P = 0.79$), or private alleles (PA) (Birch Creek M PA = 2; Cub River M PA = 1.5; Wilcoxon rank-sum test, W = 112.5, $P = 0.51$). All population genetic diversity and richness statistics and indices are described in Table 3.

AMOVA found evidence for genetic differentiation between the two Intermountain North America populations (Table 4). Specifically, 1.87% of the genetic variance was partitioned between populations, 4.83% of the genetic variance was partitioned between individuals within a population, and 93.3% of the genetic variance was partitioned within individuals across populations (Table 4). While high variance across individuals in both populations was observed, we found that Φ_{ST} is highest in variance comparisons between populations ($\Phi_{ST} = 0.07$) as opposed to between individuals within populations ($\Phi_{ST} = 0.04$) (Table 4). Furthermore, based on 999 permutations of the data, we present statistical inference to support the conclusion that these populations are differentiated at all population stratifications (Table 4). While population structure estimates are low, estimates of Φ_{ST} between the two populations is further supported by analogs of this index in F_{ST} (1.66 X 10^{-2}, $P = 0.01$), G_{ST} (8.39 X 10^{-3}, $P = 0.01$) and Jost's D (1.18 X 10^{-2}, $P = 0.05$). Finally, an assessment of the inbreeding with F_{IS} implicates no evidence of inbreeding across populations (F_{IS} = 4.20 x 10^{-3}, $P = 0.99$).

Osmia **subspecies, species, and clade marker amplification.** In total, eight loci consistently amplified in all four clades: Oli101, Oli84207, Oli131, Oli216, Oli127, Oli076, Oli156, and Oli105252. Nine loci amplified in three species/subspecies of *apicata* , 11 loci amplified in seven species/subspecies of *emarginata* , and all 22 loci amplified 11 species/subspecies of *bicornis* and two species/subspecies of *ribifloris* (Table 5, Supplementary Table 7). Average marker amplification rate across specimens was 21% ± 6% ($n = 3$), 51% ± 5% ($n = 27$), 22% ± 6% ($n = 7$), and 66% ± 7% ($n = 11$) in the clades *apicata* , *bicornis* , *emarginata* ,

and*ribifloris,* respectively.

Discussion

Microsatellites are a useful tool to rapidly acquire genetic information that informs conservation, breeding, and management decisions. They are especially informative for identifying population genetic structure among subpopulations and have the capacity to infer sibship relationships among individuals within a subpopulation (Koch, McCabe, Love, & Cox-Foster, 2021; Van Eeckhoven et al., 2022). While the front-end of microsatellite development can be expensive, using established microsatellites is a cost-effective method for population genetics studies relative to reduced representation genome sequencing on a per-specimen basis in the market today (Guichoux et al., 2011). Microsatellite analyses have uncovered cryptic genetic diversity among different bee species (Koch, Rodriguez, Pitts, & Strange, 2018), and have been useful markers for correlating biological phenomena such as population declines and genotype-by-environment associations (Cameron et al., 2011; Koch, Vandame, et al., 2018; Pitts-Singer, Cane, & Trostle, 2014). Furthermore, microsatellite marker development is on the rise across agriculturally important solitary bee species (Neumann & Seidelmann, 2006; Strange et al., 2017; Van Eeckhoven et al., 2022). In this study, we expand microsatellite marker availability and utility in the solitary bee genus *Osmia* , with special focus on *O. lignaria* .

Our identification and filtering strategy using the *O. lignaria*genome assembly uncovered a high volume of candidate microsatellite loci that can be tested using additional molecular techniques. We present those candidate loci in Supplementary Tables 1 - 3. In our study, we failed to find many perfect microsatellites that resulted in a product size greater than 241 nts under our filtering strategy. However, we were able to identify sizes exceeding 241 nts when we further examined imperfect microsatellite loci. An imperfect microsatellite is the result of a mutation at a microsatellite locus that often creates an imperfect motif (Behura & Severson, 2015). Imperfect microsatellites are suspected to be more stable than perfect microsatellites as they are less likely to incur slippage mutations (Sturzeneker, Haddad, Bevilacqua, Simpson, & Pena, 1998). Interestingly, in our study of *O. lignaria* , we found significantly more genotyping error in the perfect microsatellites relative to the imperfect microsatellites. Based on this result, we suggest that further microsatellite development studies should examine genotype error in the context of microsatellite type when identifying loci for population genetic studies. Markers that succumb to high genotyping error will likely result in deviations from HWE and should be removed from population genetic analyses (Hoffman & Amos, 2005; Morin et al., 2009).

Our characterization and assessment of novel *O. lignaria*microsatellites underscores the importance of including genotype error rates in population genetic studies. Genotyping error can significantly impact estimates of genetic diversity, population structure, and sibship relationships (Hoffman & Amos, 2005). In turn, these errors impact the interpretation of genetic data and can lead to poor management decisions for livestock and wildlife if not controlled. Genotyping error in a microsatellite analysis is the product of diverse phenomena including poor quality of template DNA, allelic dropout, and misprinting (Hoffman & Amos, 2005). Even in cases where well established microsatellite markers are used for population genetics studies (Hoffman & Amos, 2005), poor DNA template quality has caused 50% of the genotyping error (Morin et al., 2009). This phenomenon underscores the importance of performing pilot studies and strategic re-amplification of samples when conducting genetic analyses (Hoffman & Amos, 2005).

The goal of this study was to present novel microsatellite markers for*O. lignaria* and characterize their utility. Application of the microsatellite markers on two Intermountain North America populations of*O. lignaria* in Idaho found no differences in population genetic diversity across populations and low, but significant, population structure. Furthermore, we found no significant difference betweenH_O and Nei's H_E in both populations, and thereby, provide no evidence to suggest inbreeding is occurring within either population. This inference is further supported by low $F_{I\ S}$ values and permutation tests. We estimated that ˜60% of the alleles (1 - [Uniq. alleles/No. Alleles]) identified in the study are shared between both populations. Combined, these results suggest that it is likely that contemporary dispersal (*i.e.,* gene flow) is taking place across populations. This is not surprising as the populations studied are ˜30 km apart and native residents of the Bear River Mountains (Fig. 1). Furthermore, sibship analysis provides evidence for full sibling families

within each population. Thus, the novel microsatellites have the capacity to identify genetic lineages/families within a population, which is important information for biological research such as the characterization of nest founding behaviors and offspring survival (Tepedino & Torchio, 1994).

Our assessment on the utility of the novel microsatellites in other *Osmia* species supports future population genetic studies of the genus. To date, microsatellite markers have been developed for *O. bicornis* (Neumann & Seidelmann, 2006; Van Eeckhoven et al., 2022), which is in the same clade (*bicornis* clade) as *O. lignaria* . The subgenus *Osmia* , especially the clade *bicornis* , possess a diversity of *Osmia* species that are important pollinators of crops including *O. cornuta* , *O. cornifrons* , and *O. taurus* (Branstetter et al., 2021; Osterman et al., 2021). We found that the novel microsatellite loci amplified in up to 18 of the 22 in one specimen of *O. cornuta* (mean marker amplification = 9.9 ± 2.0 [$n = 9$]) (Supplementary Table 7). *Osmia cornuta* is native to north Africa and Europe and was introduced to the U.S. from Spain to pollinate crops in 1984 (Torchio & Asensio, 1985). While *O. cornuta* has not been established in the U.S. due to biological limitations (Torchio, Asensio, & Thorp, 1987; Torchio & Asensio, 1985), female *O. cornuta* have been found to visit between 9,500 and 23,600 almond flowers implicating high pollination efficiency (Bosch, 1994). Given the significance of *O. cornuta* to agriculture particularly in Europe, the novel microsatellite markers will have the capacity to characterize population genetic structure and diversity of managed populations to guide future management and breeding strategies. Finally, the invasion of the intentionally introduced *O. cornifrons* and accidentally introduced *O. taurus* to North America may benefit from population genetic study (LeCroy, Savoy-Burke, Carr, Delaney, & Roulston, 2020). Specifically, we expect that the novel microsatellites to answer questions concerning the colonization timing of these non-native bees, underlying genetic diversity and structure, and potentially their rate of expansion throughout North America.

The use of microsatellites to characterize population genetic diversity in bee pollinators has a long history. Based on Google Scholar (*https://scholar.google.com/*), the first peer-reviewed paper that characterizes microsatellites in bees (Hymenoptera: Anthophila) was on *A. mellifera* and *Bombus terrestris* by Estoup et al. (1993). Over the last 29 years, microsatellites continued to be developed for honey bees (*Apis* spp.) (Estoup, Garnery, Solignac, & Cornuet, 1995; Solignac et al., 2003), bumble bees (*Bombus* spp.) (Estoup, Scholl, Pouvreau, & Solignac, 1995; Reber Funk, Schmid-Hempel, & Schmid-Hempel, 2006; Stolle et al., 2009), mason bees (*Osmia*spp.) (Neumann & Seidelmann, 2006; Van Eeckhoven et al., 2022), stingless bees (*Melipona* spp.) (Peters, Queller, Imperatriz Fonseca, & Strassmann, 1998), orchid bees (*Euglossa* spp.) (López-Uribe, Santiago, Bogdanowicz, & Danforth, 2013; Paxton, Zobel, Steiner, & Zillikens, 2009), and alfalfa leafcutting bees (*Megachilerotundata* F.; Megachilidae) (Strange et al., 2017), among others. The development of microsatellite markers in bee pollinators has been critical in supporting research studies of bee evolution and ecology and in making informed decisions on their management in agricultural and wildlife conservation settings. For example, microsatellites have proven instrumental in informing honey bee breeding decisions (Bourgeois et al., 2008; Delaney et al., 2009; Jensen et al., 2006), estimating bumble bee decline (Cameron et al., 2011; Lozier & Cameron, 2009), and determining the impacts of habitat fragmentation on gene flow in orchid bees (Soro, Quezada-Euan, Theodorou, Moritz, & Paxton, 2016; Suni & Brosi, 2011).

Osmia lignaria has been heavily adopted by producers to pollinate a diversity of orchard and berry crops that span across a broad geographic range with diverse climates (Bosch & Kemp, 1999; Boyle & Pitts-Singer, 2019; Sheffield, 2014; Torchio, 1976). Furthermore, populations endemic to the continental U.S. lends to both genetic and physiological regional differences (Branstetter et al., 2021; Pitts-Singer et al., 2014). Commercial suppliers in the western U.S. are sourcing *O. lignaria* from different parts of their native range including Washington, Utah, Idaho, and California, but may sell such bees directly to customers or to other distributors across the U.S. As new *O. lignaria* producers enter the market in response to the growing demands for integrated crop pollination (Isaacs et al., 2017), the sustainability of *O. lignaria* sourcing, management, and breeding would benefit from knowledge on underlying population genetic diversity and structure. For example, illegal trap nesting of *O. lignaria*and other solitary bees on public lands is commonplace throughout the Intermountain West. In fact, the Cub River field site is associated with a history of illegal trap nesting for *Osmia* species (Tepedino & Nielson, 2017). If left unchecked, trap-nesting could decimate endemic *O. lignaria* populations, ultimately reducing the genetic diversity available to commercial enterprises. Application of

population genetic diversity data with microsatellites could uncover resilient or imperiled populations and ultimately guide sustainable trap-nesting pursuits. In conclusion, we anticipate the novel markers developed in our study to support critical investigations of *O. lignaria* evolution, ecology, conservation, and livestock development.

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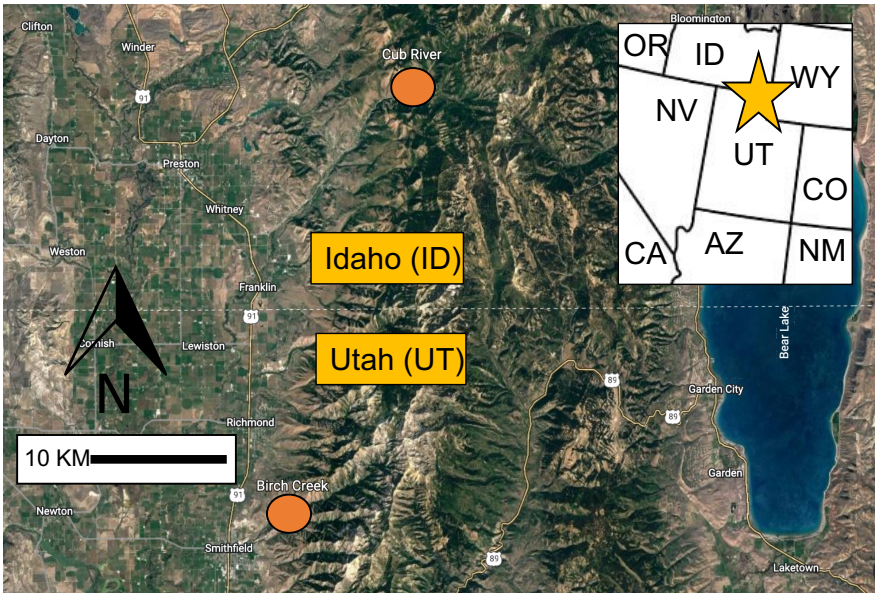
Data Accessibility and Benefit-Sharing

Data and R scripts of the described analysis are available at*https://github.com/jbkoch/OligMsatStudy.*

Author contributions

J. Koch led project development, data generation, analysis, and writing. J. Strange and T. Pitts-Singer conceived the project idea and obtained funding. M. Branstetter and D. Cox-Foster provided resources and specimens. T. Lindsay, J. Knoblett, A. Rohde, and K. Tobin conducted preliminary analysis and generated data. All authors contributed to the final version of the manuscript.



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