

## RESEARCH ARTICLE

# Think globally, breed locally: Limited genetic impact of management in solitary bees (*Osmia bicornis* and *Osmia cornuta*)

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## Abstract

1. An increasing number of pollinator species are being reared by humans to ensure crop pollination. The release of reared pollinators can lead to genetic admixture with wild conspecifics, potentially disrupting local adaptations. While such admixture has been reported in bumblebees, respective data for solitary bees are lacking.
2. We investigated whether the release of locally reared solitary bees (*Osmia* spp.) influences genetic diversity and population structure of wild conspecifics. *Osmia bicornis* and *Osmia cornuta* females were sampled from two commercial breeders as well as from high, mid and low *Osmia* spp. management intensity areas in Switzerland. Museum specimens of *O. bicornis* sampled from the same areas before commercial rearing were used to control for the effect of management. Genetic diversity, population structure and admixture between managed and wild bee populations were investigated across sampling areas and time periods using mitochondrial and nuclear DNA markers.
3. In the contemporary period, both species showed no differences in genetic diversity and low genetic differentiation between sampling areas and breeder stocks, indicating high gene flow despite *Osmia* spp. being considered philopatric and relatively short-distance flyers.
4. Similar levels of gene flow were found in *O. bicornis* before and after management.
5. Genetic diversity of *O. bicornis* declined over time, which is probably independent of management.
6. *Synthesis and application.* The data suggest that the release of locally reared *Osmia* spp. is unlikely to interfere with the genetic structure of wild conspecifics. This contrasts with bumblebees, where the use of non-local colonies has led to significant genetic introgression with local conspecific populations. Our results emphasize the importance of using locally reared pollinator populations in order to minimize the risks associated with genetic pollution. Further investigation into the population genetics of pollinators is essential to identify the main drivers of genetic erosion.

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**KEYWORDS**

conservation genetics, genetic diversity, *Osmia* spp., pollinator management, population admixture, solitary bees

## 1 | INTRODUCTION

Recent insect declines have resulted in shortages of pollination services, thereby creating more demand for managed pollinators (Mashilingi et al., 2022; Osterman et al., 2021). However, such management may pose a threat to wild pollinators via resource competition and host shifts of pathogens (Beaurepaire et al., 2025). In addition, the rearing, transportation and release of managed individuals into areas where wild conspecific populations occur may lead to genetic admixture (Beaurepaire et al., 2024), with potential impact on local adaptations (Garant et al., 2007).

Given that increasing numbers of pollinator species are being reared for crop pollination (Osterman et al., 2021), genetic admixture with wild conspecific populations is likely to increase as well. For instance, the release of commercial buff-tailed bumble bee (*Bombus terrestris*) has led to introgression with wild conspecifics (Bartomeus et al., 2020; Cejas et al., 2021). However, variation in physiology and behaviour among bee species may modulate how reared populations impact wild conspecifics (Leroy et al., 2023). For instance, philopatric bee species (i.e. with a tendency to stay in their birth area) may display stronger levels of local adaptation and may therefore be more strongly impacted by introgression with managed populations than other species (Bonte & Dahirel, 2017).

Solitary mason bees, *O. cornuta* and *O. bicornis*, are increasingly used in Europe since the 1990s as managed pollinators of fruit and berry crops (Gruber et al., 2011). Rearing of *Osmia* spp. is usually based on a combination of indoor and outdoor practices (Bosch et al., 2008). Indoor facilities are used to optimize pupation while the breeding and nesting phases usually occur outdoors (Bosch et al., 2008). Consequently, wild conspecifics can access the solitary bee breeder nests, which may lead to genetic admixture between wild and managed populations and introgression. *O. cornuta* and *O. bicornis* typically exhibit nest fidelity (Splitt et al., 2022) and may therefore be particularly sensitive to genetic admixture (Danckwerts et al., 2021). However, the potential population genetic consequences of management have not yet been investigated in solitary bees and it remains unclear whether management facilitates admixture among wild mason bee populations, thereby contributing to their genetic homogenization.

To better understand the potential risks associated with the management of solitary bees for wild conspecific populations, we investigated (1) if genetic admixture between sympatric wild and managed mason bees varies across regions displaying different management intensities (very strong to low), and (2) if the genetic structure of *Osmia* populations has changed before and after management started. We expected that solitary bee populations from areas with more intense bee management would display higher

levels of genetic diversity and admixture levels than areas where these pollinators are not introduced because commercial breeders mix cocoons from different populations and origins. Moreover, we expected that historical populations collected before the onset of bee management display lower genetic diversity and higher genetic differentiation levels compared to contemporary populations. To test these predictions, we used mitochondrial and microsatellite DNA markers to compare the genetic diversity and structure of *O. cornuta* and *O. bicornis* populations over space (three regions reflecting a gradient of pollination demand) and time (pre- and post-management periods).

## 2 | MATERIALS AND METHODS

### 2.1 | Sampling

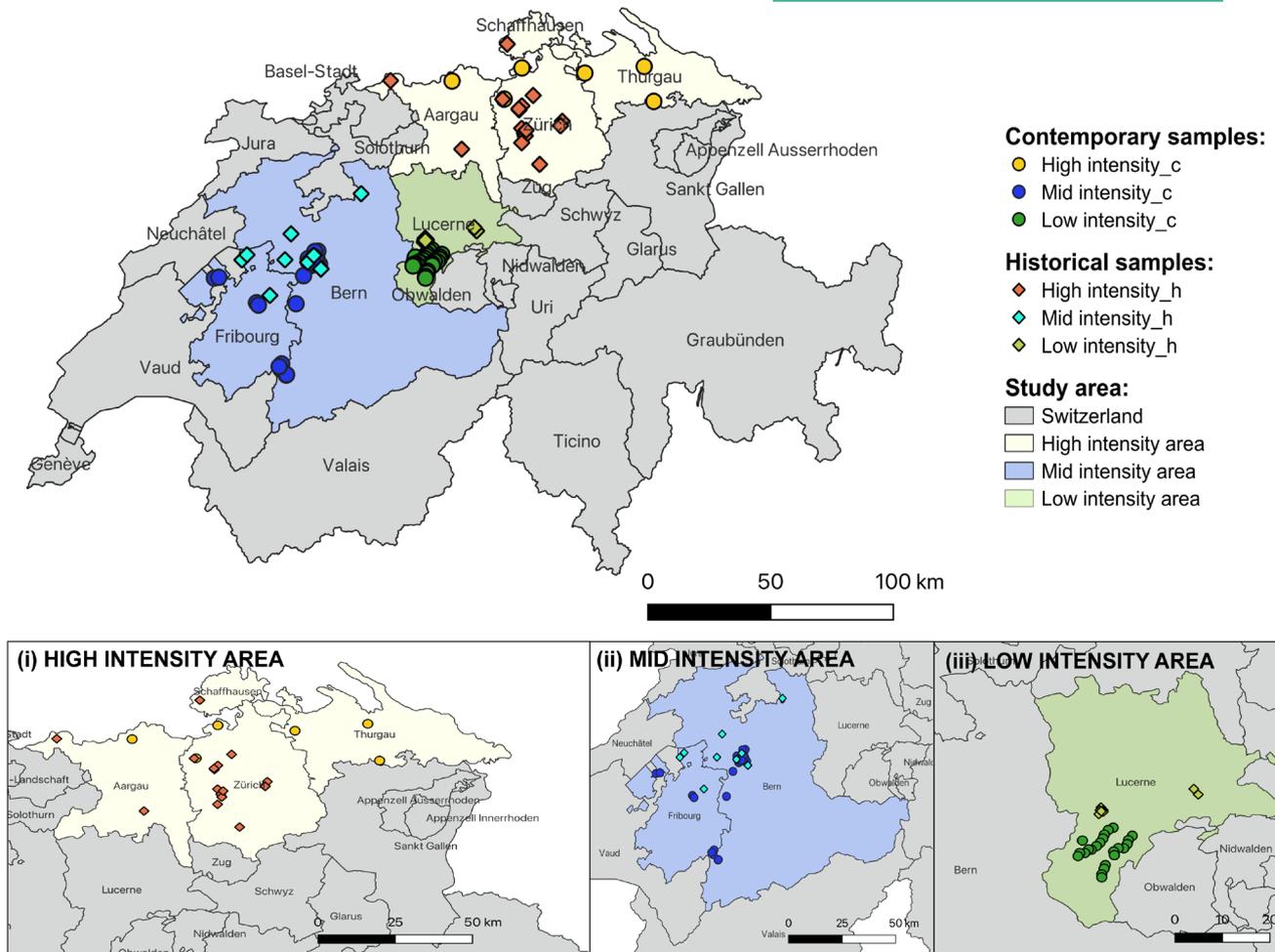
*Osmia cornuta* and *O. bicornis* adults were sampled in three areas of Switzerland reflecting different intensities of *Osmia* spp. management defined by the combination of breeding activities and the level of demand for pollination services from managed pollinators (Figure 1):

- (i) high intensity area: predominantly rural sites in fruit and berry production areas in which both species are used and bred as managed pollinators in the cantons of Zurich (ZH), Thurgau (TG), Aargau (AG) and Schaffhausen (SH) (i.e. the main fruit and berry production regions of Switzerland);
- (ii) mid-intensity area: predominantly (peri-)urban sites in the cantons of Bern (BE) and Fribourg (FR) where there is no *Osmia* breeding and a moderate introduction of managed populations;
- (iii) low intensity area: semi-natural sites located within the Entlebuch UNESCO Biosphere Reserve, canton of Lucerne (LU) where there is no *Osmia* breeding and a limited introduction of managed populations.

Landowners were contacted by phone and individual permissions to place the sampling units in their land were obtained prior to starting fieldwork. No ethical approval was required for this study.

Stocks of managed populations of both species were also acquired from two commercial breeders (hereinafter BREEDER1 and BREEDER2) as positive controls. The breeding sites of BREEDER1 are located near Constance, Germany (DE), whereas BREEDER2 has multiple breeding locations across Switzerland, including the sampling site (i). Both breeders supply mason bees in Switzerland, and both species are purchased for pollination purposes by fruit and berry producers.

Between January and February 2022, six nest blocks (20×22×17cm) each with 100 cavities (∅=8–11mm) were placed on 1m poles in the rural, high *Osmia* management intensity area and 26



**FIGURE 1** Map of Switzerland displaying cantonal borders as well as the locations of the trap nests for contemporary sampling of solitary bees *Osmia bicornis* and *Osmia cornuta* (blue, green and yellow circles) and the sampling location of the *O. bicornis* museum specimens (historical samples: Light blue, light green and orange diamonds). (i), (ii) and (iii): The three sampling areas with higher resolution.

trap nests consisting of 10–12 bamboo sticks (cavities  $\varnothing=8\text{--}11\text{ mm}$ ; length=20cm) (Seidelmann et al., 2016) were installed in the (peri-) urban, medium management intensity area (see coordinates in Tables S1 and S2). The nests were collected in January and February 2023, stored at 0–10°C in constant darkness (Beer et al., 2019) and dissected to retrieve *Osmia* spp. cocoons in March 2023. In March 2023, 24 trap nests consisting of nest blocks (16×16×9cm) each with 60 rounded cavities ( $\varnothing=8\text{--}11\text{ mm}$ ) and 10 reed stacks on top (12cm long) were placed on 1m wooden poles in the semi-natural (low management intensity) area (Table S2). The nests were placed 1km from each other, a distance higher than the known flight distance of *Osmia* spp. (Splitt et al., 2022) (see coordinates Table S1). Nests were collected in November 2023, stored at 0–10°C under constant darkness (Beer et al., 2019) and dissected in December 2023. All cocoons sampled were placed into individual Eppendorf tubes and stored at –25°C for the genetic analyses. Only innermost cocoons of each trap nest cavity were selected to ensure sampling of diploid female offsprings ( $N=568$ ) (Ivanov, 2006). The sex of each bee was confirmed based on dimorphism in phenotypes (Giejdasz et al., 2016; Table S2). In addition, cocoons of both species were provided by BREEDER1 and BREEDER2

between 2022 and 2023 and 104 *O. cornuta* and 93 *O. bicornis* were randomly selected (Table S2).

To further test the effect of bee management on population genetics of wild populations, historical bee specimens collected between 1990 and 2000 (i.e. before local management of *Osmia* spp. started) were selected. Using the Swiss Information Centre for Species (<http://www.infofauna.ch/>), 50 museum specimens of *O. bicornis* females collected in the same three sampling areas were sampled. A single mid-leg was sampled from each specimen for DNA analyses (Table S3). No museum specimens of *O. cornuta* were included due to their scarcity in Swiss collections. From hereinafter, the samples from 2022 to 2023 will be referred to as 'contemporary' samples (C) while the 1990–2000 *O. bicornis* samples are referred to as 'historical' samples (H).

## 2.2 | DNA isolation

The DNA of contemporary samples was extracted from individual mid legs using Chelex© 5% and following standard methods (Evans et al., 2013). Mid legs of historical samples were homogenized in

200  $\mu$ L TN buffer using one metallic bead (3 mm  $\varnothing$ ) and DNA was then extracted using the NucleoSpin® Tissue extraction kit following the instructions of the manufacturer (Macherey-Nagel, Oensingen, Switzerland).

### 2.3 | Mitochondrial haplotypes sequencing and genotyping

Contemporary *O. cornuta* ( $N=28$ ), and contemporary ( $N=29$ ) and historical ( $N=22$ ) *O. bicornis* samples were selected among sample groups (Table S2). Individual mid legs were dissected, and DNA was extracted using the NucleoSpin® Tissue extraction kit (Macherey-Nagel, Oensingen, Switzerland) as described above. For the contemporary samples, a 670 bp fragment of the mitochondrial Cytochrome Oxidase I gene (*cox1*) was amplified and sequenced using BeeCox1F1 and BeeCox1R2 (Bleidorn & Henze, 2021). Since DNA was degraded in the historical specimen, amplification of a 670 bp fragment from *cox1* was not feasible. Therefore, two new primers R1 and F2 (Table S4) were designed using Primer-BLAST (Ye et al., 2012) to divide the *cox1* fragment in two regions (Figure S1). In 13 of the 22 historical samples, sequencing with the second primer set (F2-BeeCox1R2) failed, resulting in data for only the first subregion, while both subregions were successfully sequenced in the remaining nine individuals (Table S5).

To amplify the mtDNA sequences, PCR reactions were performed in 10  $\mu$ L using 1  $\mu$ L of extracted DNA, 0.3  $\mu$ L for forward and reverse primer, 5  $\mu$ L of 5X buffer, 0.05  $\mu$ L of Taq polymerase and 6.35  $\mu$ L of MilliQ water as follows: initial denaturation for 5 min at 94°C, 40 cycles of denaturing at 94°C for 30s, annealing at 50°C for 30s for the primer set BeeCox1F1-BeeCox1R2 (Bleidorn & Henze, 2021) and 53°C for 30s for the novel primer sets BeeCox1F1-R1 and F2-BeeCox1R2 designed for historical samples, elongation at 72°C for 1 min and the final extension for 10 min at 72°C. Sequencing of the final PCR products was carried out by Microsynth AG (Balgach, Switzerland) and the sequences were manually checked using Chromas software v.2.6.6 (Technelysium Pty Ltd).

### 2.4 | Mitochondrial haplotype analyses

The sequences were aligned with the software MEGA v.11 (Tamura et al., 2021). A maximum likelihood tree was constructed with MEGA v.11 excluding missing data though pairwise deletion of 95%. In parallel, the distance between sample groups as well as between haplogroups (here defined by a minimum cut-off pairwise  $p$ -distance of 0.025), was calculated using 1000 bootstraps in MEGA v.11. MtDNA diversity was inferred using Fu  $F_s$  and the Fu and Li's  $F$  tests (Fu & Li, 1993) using DnaSP v.6 (Rozas et al., 2017).  $F_s$  is designed to detect recent signatures of demographic events such as population expansions ( $F_s < 0$ ) and contractions ( $F_s > 0$ ) by evaluating the

distribution of haplotype frequencies.  $F$  detects mutation deviations from neutrality, and it is sensitive to recent demographic expansion ( $F < 0$ ). Additionally, a median-joining network was constructed using NETWORK v. 10.2.0.0 (Bandelt et al., 1999). To infer populations structure at a larger scale, additional *O. cornuta* and *O. bicornis cox1* sequences from other European countries were retrieved from NCBI database (Table S6).

### 2.5 | Microsatellite genotyping

Twenty-three DNA microsatellite loci of *O. bicornis* were selected (Neumann & Seidelmann, 2006; Van Eeckhoven et al., 2022), of which 11 loci were polymorphic, amplified and scored in both species (Table S4). Multiplex PCR reactions were performed in 10  $\mu$ L using 1  $\mu$ L of extracted DNA, 0.2  $\mu$ L for each forward and reverse primer, 5  $\mu$ L of 2X KAPA2G Fast Multiplex Mix (Sigma Aldrich) and MilliQ water as follows: initial denaturation for 5 min at 94°C, 35 cycles of denaturing at 94°C for 30s, annealing at 57°C for 30s for Obic primers (Van Eeckhoven et al., 2022) and 50°C for 30s for Oru primers (Neumann & Seidelmann, 2006), elongation at 72°C for 45s and final extension for 5 min at 72°C. Microcapillary electrophoresis of the final PCR products was carried out by Microsynth AG (Balgach, Switzerland) and the genotypes were manually scored using the Peak Scanner software v1.0 (Applied Biosystems®). Only individuals successfully genotyped at more than six loci were retained for subsequent analyses.

### 2.6 | Microsatellite analyses

A test for Hardy-Weinberg equilibrium (HWE) was performed by locus with GENEPOP v.4.7.5 (Rousset, 1995) using 1000 dememorization steps, 100 batches and 1000 interactions per batch. The same software was used to test the linkage disequilibrium between pairs of markers. In parallel, a rarefaction-based estimation of allelic richness using ADZE v.1.0 (Szpiech et al., 2008) was performed on overall markers to evaluate if the sample size of the different sample groups was sufficient to accurately represent the levels of genetic diversity of populations.

To compare genetic diversity across groups, the number of alleles ( $N_A$ ) and observed heterozygosity ( $H_o$ ) were measured with GenAlex v6.5 (Peakall & Smouse, 2006). Allelic richness (AR) and inbreeding index ( $F_{is}$ ) were estimated using Fstat v. 2.9.4 (Goudet, 2002). Null alleles were assessed with Microchecker v2.2.3 (Van Oosterhout et al., 2004). Private alleles richness ( $N_{pr}$ ) was measured through rarefaction method in ADZE v.1.0 (Szpiech et al., 2008), trimming sample groups to a standardized sample size ( $g$ ) equal to the smallest sample group size of each species. AR,  $H_o$  and  $F_{is}$  between sample groups were compared with Kruskal-Wallis using R v4.3.2 (R Core Team, 2023). If significant, post hoc Dunn tests applying Bonferroni corrections were conducted. The genetic differentiation between sample groups

was assessed through population pairwise FST and Dest using GenAlex v6.5. Dest is usually used to complement FST because it better represents the differences between alleles in polymorphic loci characterized by a high mutation rate, basing the calculations on the effective number of alleles instead of the expected heterozygosity (Whitlock, 2011). The same software was used to test the effect of genetic drift (i.e. the accumulation of random mutations through time). To do so, Dest levels were calculated by comparing two historical *O. bicornis* subgroups (Historical1:1990–1993,  $N=17$ ; Historical2:1998–2000,  $N=15$ ) to assess the accumulation of genetic differentiation over time. The same index was used to estimate differentiation between these two groups and contemporary samples (2022–2023,  $N=176$ ) to test whether genetic differentiation levels increased constantly over time (i.e. suggesting genetic drift alone caused the changes) or increased more substantially after management (i.e. suggesting other factors caused the changes).

The genetic structure of the different sample groups was then analysed to test potential admixture, given by the mixing of genetic material between managed bee and conspecific wild bee populations, and introgression, as well as gene flow, represented by the movement of genes between regions through individuals migration, leading to homogenization, which constitute the reduction of genetic differentiation between populations. First, a Principal Component Analysis (PCA) was executed using *adegenet* package (Jombart, 2008) in R v4.3.2 to identify genetic divergence among sample groups based on their allele frequencies. A Molecular Variance Analysis (AMOVA) was performed to estimate the proportion of variance explained by different sampling levels (i.e. within and among populations) using GenAlex 6.5 (Peakall & Smouse, 2006). In addition, the model-based Bayesian clustering method using the software STRUCTURE (Pritchard et al., 2000) was used to illustrate potential changes in the allelic composition over space and time. The admixture model and correlated allele frequencies among populations were used to analyse the dataset for a numbered clusters ( $K$ ) ranging from 1 to 10. The software performed 10 independent runs, each consisting in a Burnin period of 50'000 followed by 100'000 MCMC repetitions and the best number of clusters was determined according to the  $\Delta K$  method (Evanno et al., 2005). CLUMPP v1.1.2 (Jakobsson & Rosenberg, 2007) was used to find the optimal cluster membership alignment and the results were then plotted by sample group with Distruct v1.1 (Rosenberg et al., 2002). A  $\chi^2$  test was performed on R v4.3.2 using a contingency table to compare the proportion of individuals per inferred subpopulation and identified significant shifts in subpopulation assignment across space and time in the sampling groups.

Finally, isolation by distance was tested with the software GenAlex v.6.5. Mantel tests were conducted in historical *O. bicornis* and both *Osmia* spp. contemporary sample groups using respectively the coordinates of *O. bicornis* specimens and of the nests sampled and the pairwise genetic distances. The significance of the Mantel test was assessed with 999 permutations.

### 3 | RESULTS

Both *O. cornuta* and *O. bicornis* were found in the high and mid intensity areas, but only *O. bicornis* colonized trap nests in the low intensity area.

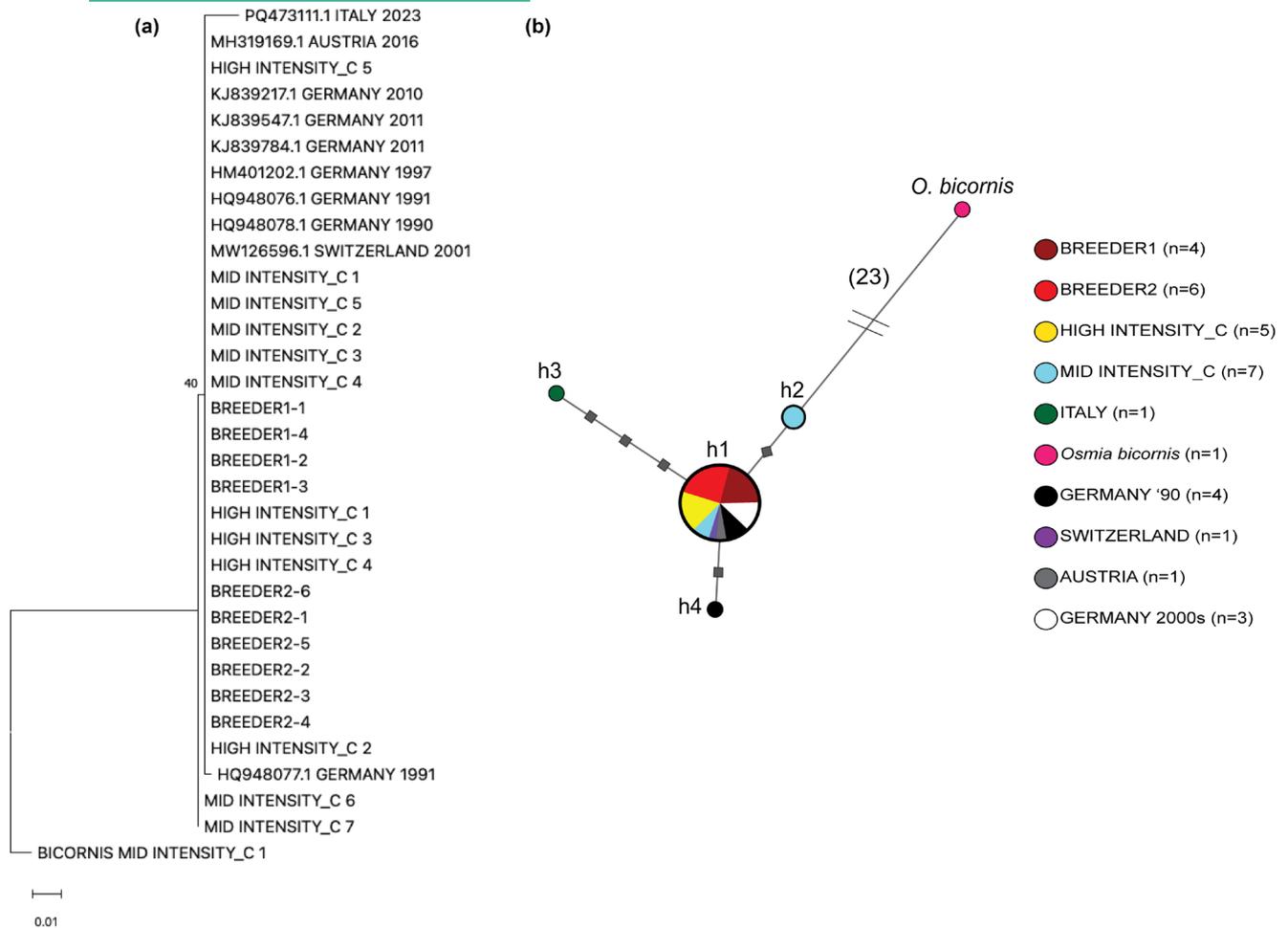
#### 3.1 | Mitochondrial DNA

Most of the *O. cornuta* mitochondrial sequences clustered in one haplogroup (Figure 2a). In total, four haplotypes were identified (Table S7) and most samples (87.5%) harboured the same haplotype (h1) (Figure 2b). The remaining samples differed from h1 by one up to three substitutions. Accordingly, low distances were detected between most sample groups (Table S8). Haplotype diversity among *O. cornuta* sample groups was  $0.403 \pm 0.016$  (variance) and both  $F_s$  and  $F$  suggested recent demographic expansion ( $F_s = -1.781$ ,  $F = -0.519$ , n.s.). The sequences from Germany, Italy, Austria and Switzerland clustered with the sequences from managed and wild Swiss *O. cornuta* populations and harboured the same haplotype. The analyses also revealed no significant difference between sequences from Germany in the 1990s and the other sequence group.

The sequences from contemporary ( $N=29$ ) and historical ( $N=22$ ) *O. bicornis* encompassed 15 haplotypes (Table S7). Contemporary samples clustered in one haplogroup together with some ( $N=14$ ) of the historical sequences (Haplogroup1), while the remaining historical sequences from the three sampling areas ( $N=8$ ) clustered separately (Haplogroup2) with a pairwise  $p$ -distance of  $0.030 \pm 0.007$  (SE) from Haplogroup1. The third haplogroup (Haplogroup3) included sequences from Spain and Portugal (OR796206.1; OR796571.1), with a distance of  $0.028 \pm 0.007$  to the two previous haplogroups (Figure 3a). High genetic distances were detected between historical sample groups as well as between historical and contemporary sample groups, while low genetic distance between contemporary sample groups was detected (Table S9). Historical populations showed a haplotype diversity of  $0.658 \pm 0.013$  and both  $F_s$  and  $F$  suggested a demographic contraction ( $F_s = 0.370$ ,  $F = 0.393$ , n.s.), while contemporary populations had a haplotype diversity of  $0.522 \pm 0.012$  and negative  $F_s$  and  $F$  (respectively  $-2.296$  and  $-1.435$ , n.s.) suggesting expansion of populations. Except for two sequences from Spain and Portugal (Haplogroup3, haplotype h15), the other sequences from France, Germany, Spain and Portugal obtained from NCBI resulted identical to the sequences sampled from the managed and the wild Swiss *O. bicornis* populations.

#### 3.2 | Microsatellites

The rarefaction analysis showed that the allelic richness of most sample groups reached a plateau at 30–40 individuals for both *O. cornuta* (Figure S2) and *O. bicornis* (Figure S3), indicating that the sampling



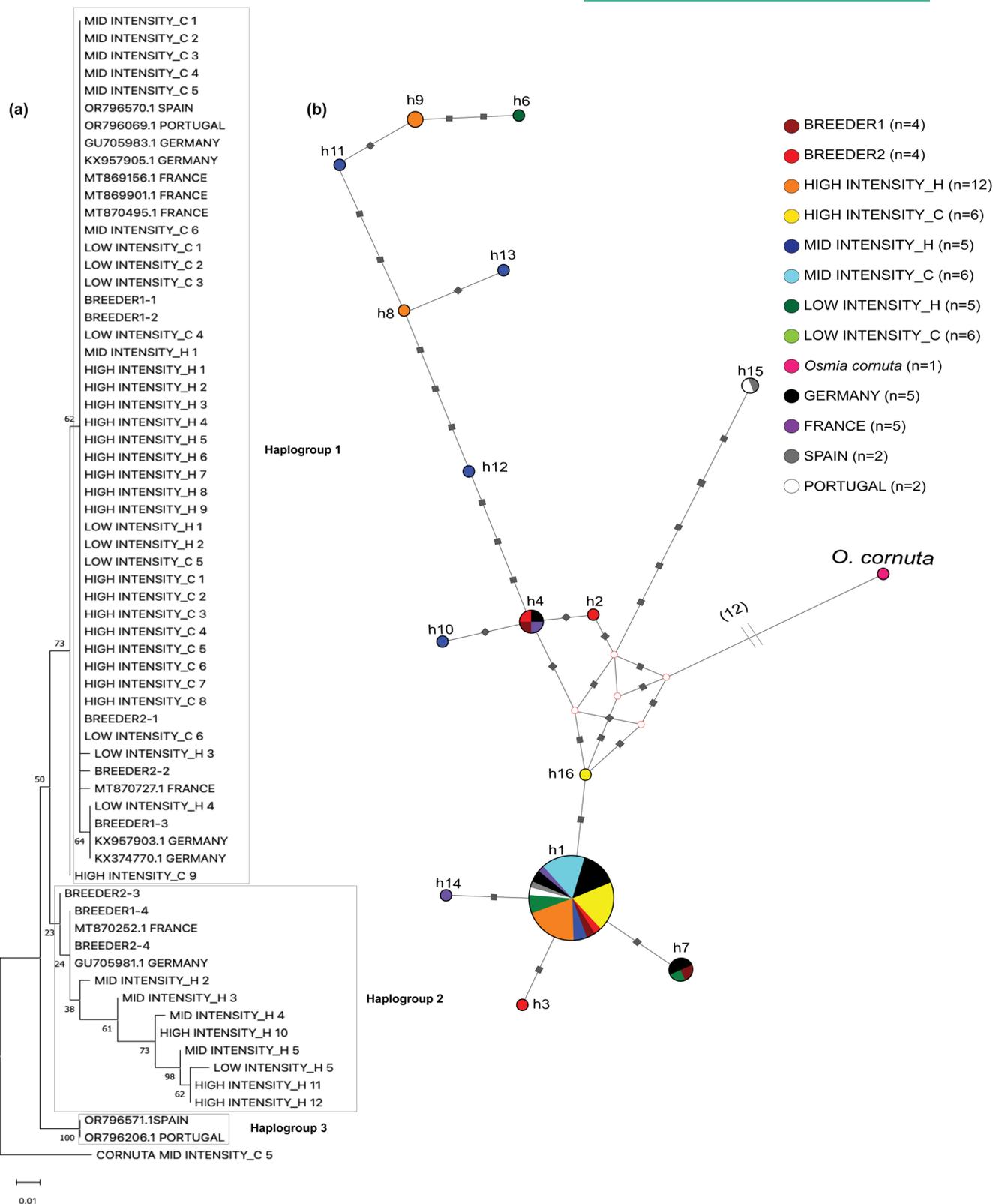
**FIGURE 2** Diversity of mitochondrial haplotypes in *Osmia cornuta*. Maximum likelihood phylogenetic tree (a) and haplotype network (b) representing the diversity of, and distance between different *cox1* haplotypes obtained by sequencing *O. cornuta*. The genetic distance is represented by the length of branches in (a) or by the number of grey squares (nucleotide substitutions) separating them in (b). A single *Osmia bicornis* sequence was used to root the tree in (a). The colours in the haplotype network indicate the origin of the samples used (legend on the right), while the size of the coloured circles represents the number of samples matching the haplotype (h1–h4).

effort was sufficient to capture most of the genetic diversity present in those populations, and that adding more individuals would likely not have revealed many additional alleles. Only the curve representing the historical low intensity area group in *O. bicornis* did not reach a plateau due to the smaller sample size available ( $N=7$ ). In *O. cornuta*, null alleles were detected in less than half of the loci (Table S10). In *O. bicornis*, except for BREEDER1 where nine loci showed null alleles, a maximum of four loci per sample group showed null alleles (Table S11). In *O. cornuta*, no significant linkage disequilibrium was found among locus pairs (Table S12) while most of the loci resulted in linkage disequilibrium in *O. bicornis* (Table S12). In both species, significant deviations from HW equilibrium as well as heterozygosity deficiency were identified in most of the sample groups (Table S13).

Contemporary samples of *O. cornuta* from the high and mid intensity areas possessed higher levels of observed heterozygosity ( $H_o$ ), allelic richness (AR) as well as the number of alleles ( $N_a$ ) compared to the two breeder sample groups (Figure 2, Table 1). However, none of these differences were statistically significant (Kruskal–Wallis,  $p>0.05$ ). The group from the high intensity area showed similar private alleles

values to BREEDER2, while the mid intensity area group showed the highest level of private allele richness ( $g=29$ ). Both *O. cornuta* and *O. bicornis* sample groups showed signs of inbreeding ( $F_{IS}>0$ ) (Table 1). None of the differences in  $F_{IS}$  values between sample groups were significant in *O. cornuta* (Kruskal–Wallis,  $p>0.05$ ), while significant differences (Kruskal–Wallis,  $p<0.001$ ) were detected in *O. bicornis* between BREEDER1 and the historical and contemporary sample groups from the low intensity area (post hoc Dunn test, Bonferroni corrected  $\alpha=0.0018$ ). Even though not significant (Kruskal–Wallis,  $p>0.05$ ), an increasing trend in  $F_{IS}$  from the historical to the contemporary period was detected across all *O. bicornis* sample groups.

In *O. bicornis*, there were no significant differences in allelic richness between sample groups (Kruskal–Wallis,  $p>0.05$ ) while differences in observed heterozygosity between BREEDER1 and historical high intensity area were significant (post hoc Dunn test, Bonferroni corrected  $\alpha=0.0018$ ) (Table 1, Figure 4). Although statistically not significant, a decrease in allelic richness and observed heterozygosity was observed between historical and contemporary samples from high and mid intensity areas (Figure 4). A decrease in private alleles richness



**FIGURE 3** Diversity of mitochondrial haplotypes in *Osmia bicornis*. Maximum likelihood phylogenetic tree (a) and haplotype network (b) representing the diversity of and distance between different *cox1* haplotypes obtained by sequencing *O. cornuta*. The genetic distance is represented by the length of branches in (a) or by the number of grey squares (nucleotide substitutions) separating them in (b). A single *Osmia bicornis* sequence was used to root the tree in (a). The colours in the haplotype network indicate the origin of the samples used (legend on the right), while the size of the coloured circles represents the number of samples matching the haplotype (h1–h4).

Population	N	Na	Ho	AR	NpR	$F_{IS}$
<i>Osmia cornuta</i>						
BREEDER1	30	3.364 (2.461)	0.342 (0.294)	3.198 (2.413)	0.087 (0.039)	0.028 (0.184)
BREEDER2	44	4.455 (3.012)	0.393 (0.260)	3.728 (2.362)	0.369 (0.105)	0.007 (0.279)
HIGH INTENSITY_C	144	5.545 (4.298)	0.316 (0.219)	3.818 (2.901)	0.341 (0.116)	0.183 (0.153)
MID INTENSITY_C	39	5.364 (3.613)	0.354 (0.254)	4.482 (3.025)	0.998 (0.209)	0.137 (0.275)
<i>Osmia bicornis</i>						
BREEDER1	32	6.182 (2.183)	0.484 (0.104)	4.779 (1.500)	0.130 (0.032)	0.312 (0.108)
BREEDER2	45	8.273 (2.796)	0.690 (0.137)	5.236 (1.646)	0.295 (0.065)	0.053 (0.100)
HIGH INTENSITY_H	18	6.636 (2.248)	0.749 (0.203)	5.378 (1.622)	0.299 (0.075)	0.014 (0.179)
HIGH INTENSITY_C	56	7.000 (2.366)	0.568 (0.116)	4.903 (1.193)	0.184 (0.035)	0.088 (0.107)
MID INTENSITY_H	25	7.727 (2.832)	0.666 (0.157)	5.323 (1.458)	0.292 (0.067)	0.111 (0.182)
MID INTENSITY_C	21	6.364 (1.912)	0.568 (0.125)	4.948 (1.155)	0.310 (0.062)	0.200 (0.118)
LOW INTENSITY_H	7	5.091 (1.514)	0.608 (0.210)	4.842 (1.300)	0.366 (0.117)	0.077 (0.313)
LOW INTENSITY_C	99	7.909 (2.256)	0.667 (0.117)	4.796 (1.233)	0.185 (0.037)	0.071 (0.070)

Note: Means over all loci ( $\pm$  standard errors), number of individuals (N), number of alleles (Na), observed (Ho) heterozygosity, allelic richness (AR) inbreeding index ( $F_{IS}$ ) and private alleles richness (NpR) of *O. cornuta* and *O. bicornis* sample groups of historical (H) and contemporary (C) time periods are shown.

( $g=7$ ) was also observed between historical and contemporary sample groups from high and low intensity areas; however, no significant differences were detected (Mann-Whitney  $U$  test,  $p>0.05$ ; Table 1).

In *O. cornuta*, both  $F_{ST}$  and  $Dest$  (Table 2) revealed low genetic differentiation between sampling areas. For *O. bicornis* (Table 3),  $F_{ST}$  showed comparable genetic distances between wild populations in both historical and contemporary periods, while  $Dest$  indicated greater differentiation in contemporary populations. Both indices also indicated significant but low levels of genetic differentiation between historical and contemporary populations within the same sampling area, with the highest divergence observed in the semi-natural area. For both species, genetic distances between contemporary populations and those of the two breeders were similarly small.

The PCA analysis shows no segregation between sample groups of both species (Figure 5). Despite haplotype distances, most of the microsatellite genotypes of historical and contemporary *O. bicornis* samples sequenced at the *colx* gene overlapped in the PCA (Figure S4).

TABLE 1 Overview of genotyped solitary bees *Osmia cornuta* and *Osmia bicornis*.

AMOVA revealed that in both species most contemporary genetic variation occurred within individuals ( $>50\%$ ,  $p=0.001$ ) rather than among nests ( $<10\%$ ,  $p=0.001$ ) or sampling areas ( $<5\%$ ,  $p=0.001$  in *O. cornuta* and  $p>0.05$  in *O. bicornis*), revealing low population structure (Table S14). Similarly in the historical time period, 90% of the genetic variation of *O. bicornis* was within individuals ( $p=0.001$ ),  $<10\%$  among individuals ( $p=0.001$ ) and 1% among sampling areas ( $p=0.025$ , Table S14). The most likely number of genetic clusters in *O. cornuta* inferred by the STRUCTURE software was  $K=7$  ( $\Delta K_7=9.02$ , Figure S5). All the identified clusters were found in all sample groups, each of them being represented by  $14\% \pm 4\%$  (mean  $\pm$  SD) of individuals in each sample group (Figure 6). The chi-square test showed no significant differences between the observed clusters contribution compared to a random contribution ( $\chi^2=11.256$ ;  $df=18$ ,  $p=0.88$ ). Thus, no significant genetic structure was identified for *O. cornuta*.

For *O. bicornis*, the most likely number of clusters inferred was  $K=7$  ( $\Delta K_7=7.38$ , Figure S6). All clusters were found in the different sample groups, but a change in the probability of cluster membership

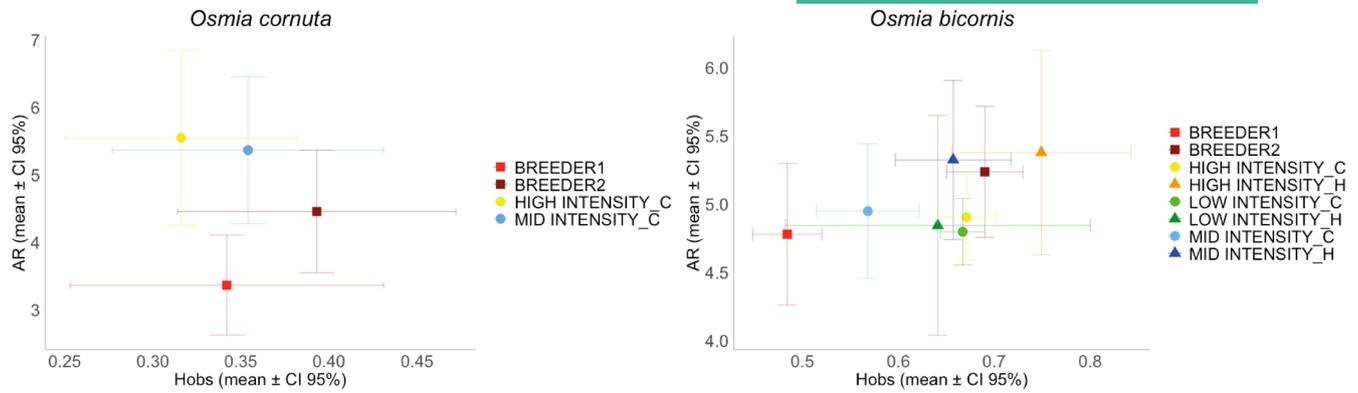


FIGURE 4 Allelic richness and observed heterozygosity in solitary bees *O. cornuta* and *O. bicornis*. Allelic richness (AR) and observed heterozygosity (Ho) (mean  $\pm$  CI 95%) for *O. cornuta* and *O. bicornis* for each sample group over all markers are displayed.

TABLE 2 Genetic distances between *O. cornuta* sample groups.

FST	BREEDER1	BREEDER2	HIGH INTENSITY_C	MID INTENSITY_C
BREEDER1				
BREEDER2	0.021**			
HIGH INTENSITY_C	0.013*	0.008		
MID INTENSITY_C	0.024**	0.014*	0.010*	
Dest	BREEDER1	BREEDER2	HIGH INTENSITY_C	MID INTENSITY_C
BREEDER1				
BREEDER2	0.015**			
HIGH INTENSITY_C	0.009*	0.003		
MID INTENSITY_C	0.019**	0.008*	0.006*	

Note: Dest and FST values based on 999 permutations for pairwise *O. cornuta* sample groups. Significant values are indicated as: \* $p < 0.05$  and \*\* $p < 0.001$ .

was observed between historical and contemporary sample groups. In fact, over all historical groups,  $57\% \pm 16\%$  of the individuals were assigned to one of the clusters whereas only  $8\% \pm 3\%$  of the individuals of the contemporary sample groups belonged to that cluster (Figure 7). Accordingly, the chi-square test revealed significant differences in the clusters' contribution across all sample groups compared to the random contributions ( $\chi = 110.91$ ,  $df = 42$ ,  $p < 0.001$ ).

For *O. cornuta* as well as the historical *O. bicornis* samples, the Mantel test used to assess the correlation between genetic and geographical distances showed no significant correlation between the two distances, indicating that populations farther apart were not genetically more different than those closer together. For contemporary sample groups of *O. bicornis*, there was a significant but negative correlation between the two distances suggesting that the genetic differentiation may not be caused by geographical isolation due to distance (Figure S7).

The genetic drift approximation between the two historical *O. bicornis* microsatellite subgroups showed low, non-significant differentiation over time (Dest:  $-0.012$ ,  $p > 0.05$ ). Assuming constant genetic drift through time, Dest values between historical and contemporary groups highlighted that the changes that occurred over

more than 20 years were greater than changes that would occur solely due to genetic drift (Dest Historical1-contemporary: 0.043,  $p < 0.05$ ; Dest Historical2-contemporary: 0.027,  $p < 0.05$ ).

## 4 | DISCUSSION

The comparison of wild and managed mason bees (*O. bicornis* and *O. cornuta*) across three regions revealed similar levels of genetic diversity and admixture despite different levels of management intensity, suggesting that management intensity does not affect the genetics of wild populations. In addition, despite dissimilar environments and relatively long geographical distances, high gene flow within and between the three considered regions was detected, suggesting that these species were less philopatric than expected. All populations sampled in the wild were genetically identical to stock populations of two local breeders. In *O. bicornis*, for which historical specimens from before the onset of management were also analysed, similar levels of gene flow were found over time and a temporal decline in genetic diversity was found. Since the levels of gene flow between the studied regions were similar before the onset of management,

TABLE 3 Genetic distances between *O. bicornis* sample groups.

	Population1	Population2	FST	Dest
_H versus _H	HIGH INTENSITY_H	MID INTENSITY_H	0.014	0.004
	HIGH INTENSITY_H	LOW INTENSITY_H	0.046*	0.097*
	MID INTENSITY_H	LOW INTENSITY_H	0.043*	0.084*
_C versus _C	HIGH INTENSITY_C	MID INTENSITY_C	0.033**	0.121**
	HIGH INTENSITY_C	LOW INTENSITY_C	0.016**	0.065**
	MID INTENSITY_C	LOW INTENSITY_C	0.031**	0.111**
_H versus _C	HIGH INTENSITY_H	HIGH INTENSITY_C	0.021*	0.065
	MID INTENSITY_H	MID INTENSITY_C	0.033**	0.100**
	LOW INTENSITY_H	LOW INTENSITY_C	0.057**	0.174**
BREEDER1 versus _C	HIGH INTENSITY_C	BREEDER1	0.018**	0.049**
	MID INTENSITY_C	BREEDER1	0.031**	0.083**
	LOW INTENSITY_C	BREEDER1	0.015**	0.040**
BREEDER2 versus _C	HIGH INTENSITY_C	BREEDER2	0.013**	0.043**
	MID INTENSITY_C	BREEDER2	0.019*	0.042*
	LOW INTENSITY_C	BREEDER2	0.010**	0.031**

Note: Dest and FST values based on 999 permutations for pairwise *O. bicornis* sample groups. C indicates contemporary sample groups while H refers to historical sample groups. Significant values are reported as \* $p < 0.05$  and \*\* $p < 0.001$ .

the relatively recent local breeding and transportation of these managed solitary bees for pollination seems to be unlikely to interfere with the genetics of wild conspecifics.

#### 4.1 | Comparison of regions with different degrees of *Osmia* spp. management

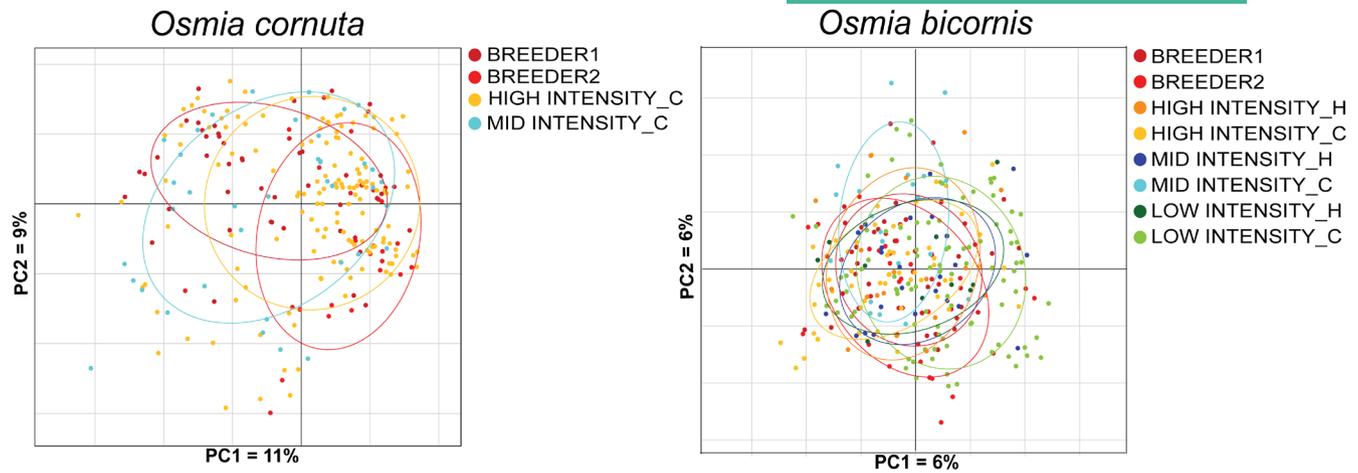
Both the mitochondrial and microsatellite marker analyses conducted in this study showed that contemporary *O. cornuta* and *O. bicornis* populations did not differ significantly in genetic diversity or genetic structure levels. These results are surprising, as they suggest high dispersal levels between *Osmia* populations, even though both species are generally considered to be philopatric (Splitt et al., 2022). This high dispersal of *Osmia* across landscapes is likely the result of an interplay between natural migration over short distances and human-mediated dispersal over long distances. Over short distances, areas with suitable foraging and nesting sites may act as geographical corridors, thereby connecting solitary bee populations (Suní & Hernandez, 2023). Likewise, the widespread provisioning of different nesting aids for cavity-nesting bees such as *O. cornuta* and *O. bicornis* in urban environments may lead to genetic homogenization of solitary bee populations (Rezende et al., 2025). Over longer distances, the unintentional movement of bees and colonized nesting material by humans may have enhanced gene flow and genetic homogeneity between bee populations before active management of these bees started (Ballare & Jha, 2021). Given that mason bees can utilize various nesting structures (Splitt et al., 2022), road transportation of materials with suitable nesting sites may have also facilitated dispersal and homogenization of *Osmia* spp.

While dispersal between neighbouring populations could enhance genetic diversity and reduce inbreeding, long-distance dispersal across dissimilar environments may alter local genetic makeups and introduce non-local genes, potentially disrupting local adaptations (Garant et al., 2007) or leading to outbreeding depression (Leung et al., 2025). However, despite strong differences in intensity of *Osmia* spp. management between the regions studied, admixture with managed populations was consistent across wild populations, suggesting that management intensity does not drive the genetic structure of wild populations.

The genetic homogeneity between wild and managed populations found in this study likely reflects the fact that, in Switzerland, the managed *Osmia* populations were reared and released locally using an open-breeding system (TM, Strobl, 2023, pers. comm.). This contrasts with bumblebees (*B. terrestris*) management, where indoor rearing and the release of non-local commercial bees have led to hybridization between local and non-local subspecies (Kraus et al., 2011; Bartomeus et al., 2020; Cejas et al., 2021). In other contexts, for example in the USA, the lack of trading regulations and the use of long-distance mason bee management have led to the widespread mixing of subspecies and the introduction of non-native species, with currently unknown consequences for the genetic integrity of wild populations (Heiner et al., 2025; MacIvor & Irwin, 2025).

#### 4.2 | Pre- and post-management comparisons in *O. bicornis*

While management was expected to increase genetic diversity and admixture (Harpur et al., 2012), both mtDNA and nuclear DNA markers revealed losses of genetic diversity through time in *O.*



**FIGURE 5** Genetic structure of *O. cornuta* and *O. bicornis*. PCA figures illustrate differences among individuals (dots) from each sample group (circles) based on their microsatellite data. Proximate or overlapping dots and circles indicate greater genetic similarity. The plot is displayed according to the two first Principal Components (PC1-PC2) and illustrates, respectively, 21.3% and 12% of variability between sample groups of the two species.

*bicornis*. The presence of multiple mtDNA haplotypes in historical populations contrasted with the predominance of a common haplotype (h1) shared across all wild and managed contemporary populations. Diversity and heterozygosity decreases were also detected using microsatellite markers, although these changes were not statistically significant. Genetic diversity levels were generally lower in the contemporary sample groups even though one historical sample group had a relatively low sample size ( $N=7$ ). Increasing sample size in that group would likely have amplified the observed genetic losses as the rarefaction analysis showed that not all alleles were captured with the sample size used. The diversity and heterozygosity declines observed are most likely related to other factors not accounted for in this study rather than bee management. Globally, bee diversity is declining due to multiple anthropogenic pressures, including agricultural intensification, urbanization, climate change and pollution (Wagner et al., 2021). In Switzerland, climate and regional land-use changes have impacted numerous insect species over the past 40 years (Neff et al., 2022). A follow-up study aiming at assessing genetic diversity fluctuations across varying levels of these different parameters may help better understanding the factors driving the observed declines.

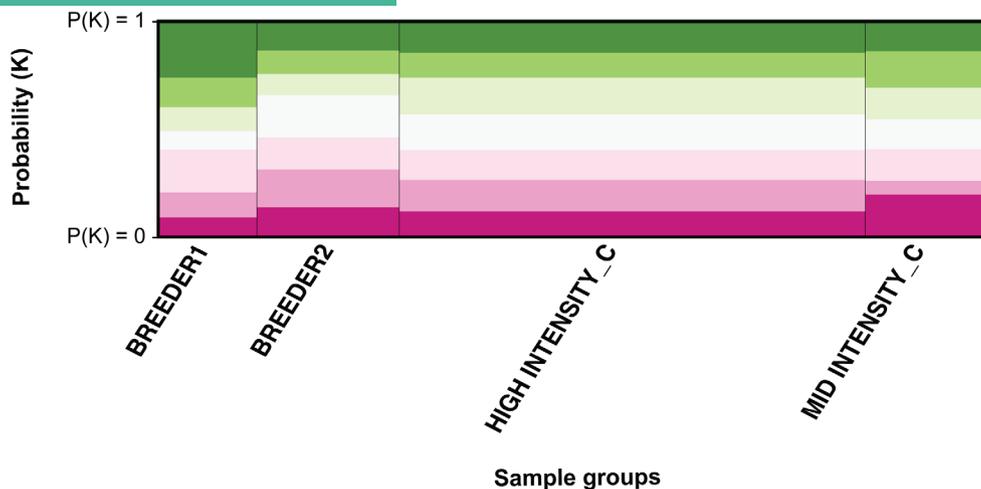
In addition to the genetic diversity losses detected in this study, significant temporal changes in allele frequencies between pre- and post-management populations, and novel (private) nuclear DNA alleles, were found in the contemporary wild *O. bicornis* populations. Splitting the historical samples in two subgroups (i.e. 1990–1993 and 1998–2000) showed that these changes were not likely to be caused by genetic drift alone. Genetic differentiation levels obtained when comparing these two historical groups were null. In contrast, pronounced levels of genetic differentiation were found when comparing pre- and post-management *O. bicornis* populations, suggesting that the changes may have been caused by factors other than drift, such as selection or the introduction of non-local genotypes. Using more detailed genomic approaches to study selection signals

on the genome of these bees may help understanding the factors driving these changes, as previously observed in *B. terrestris* (Hjort et al., 2025).

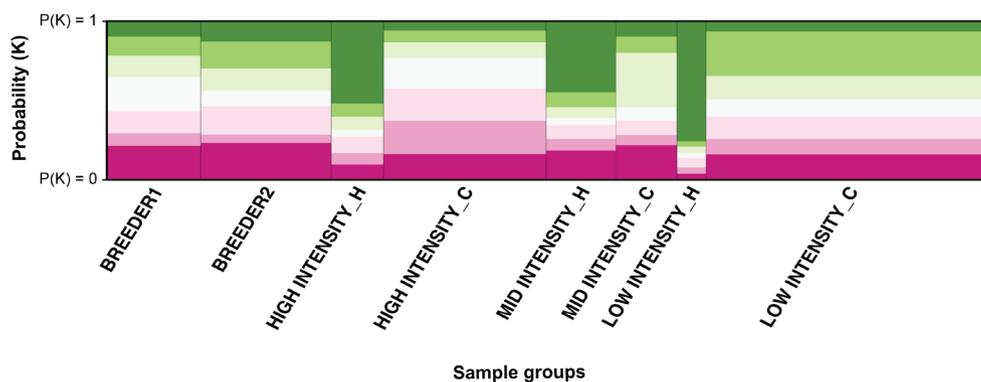
Historical *O. bicornis* populations showed high genetic similarity between the three sampling areas, highlighting the putative role of long-distance transportation in enhancing the connectivity and homogenization of bee populations. However, the presence of haplotype h1 in some historical samples suggests that managed populations may have acquired h1 through open breeding practices and facilitated its spread across the country. The movement of managed populations between the regions studied here might have additionally contributed to the genetic homogenization of wild populations, but was not a main driver of the dispersal of mason bees, which had already been taking place before the beginning of management.

## 5 | CONCLUSIONS

Our results indicate that the management of *O. bicornis* and *O. cornuta* populations has not significantly altered the genetics of wild conspecific populations under the conditions studied. This contrasts with patterns observed in bumblebees, where hybridization between commercial and local subspecies and the spread of non-local haplotypes have been documented. These discrepancies may be related to differing management practices: while bumblebee management often involves the release of non-local bees, the managed populations considered here were bred, sold and released locally, thereby minimizing genetic impacts on wild populations. According to our results, policies on bee pollinator management should encourage the use of locally reared populations and avoid long-distance transportation of managed populations for pollination services to preserve the genetic makeup of wild conspecific populations, limit the mixing of subspecies and the potential introduction of non-native species.



**FIGURE 6** Genetic structure analysis of *O. cornuta*. STRUCTURE bar plot representing the genetic structure of *O. cornuta* across sample groups. The different colours reflect the most likely number of genetic clusters inferred by the software ( $K=7$ ,  $\Delta K=9.02$ ) based on individual microsatellite genotypes. The proportion of the different colours in each sample group reflects the proportion of sample group belonging to an inferred cluster.



**FIGURE 7** Genetic structure analysis of *Osmia bicornis*. The STRUCTURE bar plot represents the genetic structure of *O. bicornis* across sample groups. The different colours reflect the most likely number of genetic clusters inferred by the software ( $K=7$ ,  $\Delta K=7.38$ ) based on individual microsatellite genotypes. The proportion of the different colours in each sample group reflects the proportion of the sample group belonging to an inferred cluster.

Management alone may not allow to counter the losses of genetic diversity caused by the multiple stressors affecting wild pollinators. It appears important to better understand the drivers of losses and to address them subsequently. Despite being common and abundant in Europe, our study revealed progressive losses of genetic diversity in *O. bicornis* over time, highlighting the importance of using molecular tools to inform conservation efforts. As more wild pollinators will be domesticated in the future to provide commercial pollination services, it seems essential to recognize the risks associated with genetic admixture and to implement strict legislations promoting the use of local stocks to safeguard the genetic makeup of wild populations. Additionally, an assessment of the genetic diversity and population structure of wild populations before starting the management of a new species would enable better quantification of potential impacts, for example, allowing adjustments to be made according to the natural diversity and dispersal abilities of the target species.

#### AUTHOR CONTRIBUTIONS

Asia Piovesan, Alexis Beaufreire and Peter Neumann conceived and designed the study, Asia Piovesan collected and analysed the data with the advice of Alexis Beaufreire; Matthias Albrecht and Florian Knaus supported the development of the sampling design and the placement of trap nests respectively in the high intensity area and in the Entlebuch Biosphere; Asia Piovesan wrote the first draft of the manuscript with contributions from Peter Neumann and Alexis Beaufreire. All authors contributed critically to the drafts and gave final approval for publication.

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### CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest. Wildbiene+partners company provided information and cocoons for the project, but they were not involved in the project development.

### DATA AVAILABILITY STATEMENT

Data are available from the Dryad Digital Repository: <https://doi.org/10.5061/dryad.sf7m0cgn7> (Piovesan et al., 2026).

### STATEMENT OF INCLUSION

The sampling design of our study was developed through a citizen science framework that combined the expertise of local researchers—whose knowledge on *Osmia* spp. and Swiss territory was essential to project design—with the active participation of stakeholders who hosted trap nests on their properties. By including specimens from museum collections in our analyses, we also engaged several national museums and highlighted the value of Swiss natural history collections and the contributions of Swiss entomologists to long-term biodiversity research.

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## SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

**Table S1.** GPS coordinates of trap nests. For each sampling area the list of the trap nests placed (nest number), the GPS coordinates of the placed trap nests (GPS coordinates) and information about the nests status are reported. O=occupied nest, E=empty nest and NC=not collected nest.

**Table S2.** Information on the genotyped and sequenced individuals. Number of trap nest placed and occupied by each *Osmia* spp. The number of samples collected and genotyped per sampling area and the mean value  $\pm$ SD of individuals genotyped per nest in each sampling site as well the number of historical specimens genotyped are shown. For each species, the number of samples per species,

sampling area and time period that were sequenced at the *colx* gene region is also reported. F = females.

**Table S3.** Museum *O. bicornis* specimens. List of the museum specimens that were sampled and genotyped. For each specimen the GBIF identification number, the sampling location and sampling area, the time of collection (Year) and the housing museum (Museum). NMBE = Natural History Museum of Bern (Naturhistorisches Museum Bern), NMLU = Natural History Museum of Luzern (Natur-Museum Luzern) and ETH = ETH Zurich is reported. If the specimen is included in mitochondrial DNA analyses and an mtDNA sequence is available for it, the ID used to identify that sequence is also reported (mtDNA\_ID).

**Table S4.** Information about the mitochondrial and microsatellite primers used in this study. In the table are indicated the primers used (Name), their reference (Ref) and their size reported as average or range size in bp (Size). The \* mark indicates the primers that were considered unsuitable for the genotyping and excluded after being tested on both species.

**Table S5.** Historical *O. bicornis* mtDNA sequencing. The table shows which subregions have been sequenced for each historical *O. bicornis* specimens sequenced.

**Table S6.** List of NCBI *coxI* sequences of *O. cornuta* and *O. bicornis* from Europe. For each sequence is reported the species, the access number in NCBI database, the country and eventually the year if there are sequences from different time periods and the reference where the sequence has been published.

**Table S7.** *Colx* gene haplotypes. List of haplotypes and corresponding samples where they were found.

**Table S8.** Distance between *O. cornuta* sample groups. Pairwise distance levels between *O. cornuta* sample groups reported as mean  $\pm$  standard deviation calculated after 1000 bootstraps.

**Table S9.** Distance between *Osmia bicornis* sample groups. Pairwise distance levels between *O. bicornis* sample groups reported as mean  $\pm$  standard deviation calculated after 1000 bootstraps. Heat map colours gradient ranges from green, representing low pairwise distances, to red, indicating high pairwise distances.

**Table S10.** Null alleles in *O. cornuta* sample groups. For each sample group, the presence or absence (yes/no) of null alleles (Na) is reported for each locus as well as the null alleles estimated frequency (Freq.).

**Table S11.** Null alleles in *O. bicornis* sample groups. For each sample group, the presence or absence (yes/no) of null alleles (Na) is reported for each locus as well as the null alleles estimated frequency (Freq.).

**Table S12.** *O. cornuta* and *O. bicornis* linkage disequilibrium. *p*-value of linkage disequilibrium per locus (11) overall sample groups of *O. cornuta* (*p O. cornuta*) and *O. bicornis* (*p O. bicornis*) obtained through Fischer's exact test. The Bonferroni adjusted *p*-value for 5% nominal level is 0.0009.

**Table S13.** *O. cornuta* and *O. bicornis* Hardy-Weinberg equilibrium. In the table are reported deficiency or excess of heterozygosity (H) and *p*-values of deviation from HW equilibrium for each sample group overall loci of both species calculated through Fischer's exact test.

The Bonferroni adjusted *p*-value for 5% nominal level is 0.0046.

**Table S14.** Results of the analysis of the molecular variance (AMOVA) based on 999 permutations and performed on the samples groups of *O. cornuta* and *bicornis*.

**Figure S1.** location and combination of the mitochondrial primers R1 and F2. The primers were designed using Primer-BLAST to be respectively combined with BeeCox1F1 and BeeCox1R2 as BeeCox1F1-R1 (in red) and F2-BeeCox1R2 (in green), dividing the *coxI* fragment in two regions of around 330bp each.

**Figure S2.** *O. cornuta* rarefaction analysis.

**Figure S3.** *O. bicornis* rarefaction analysis. Starting from the number of alleles in a given sample group, rarefaction analysis estimates the expected number of alleles in different sample sizes of the same group. Using ADZE software on 11 microsatellite loci, the curves show how allele richness increases with sample size up to 99 individuals (max sample size overall sample groups) for each for *O. bicornis* sample group. When the curves tend to the plateau, it indicates that most of the genetic diversity has been captured and increasing sample size is unlikely to reveal many new alleles.

**Figure S4.** Principal Component Analysis (PCA) of *O. bicornis* samples selected for mtDNA analyses. PCA plot based on microsatellite data of *O. bicornis* contemporary and historical samples amplified at the *coxI* and grouped by haplogroup. The plot is displayed according to the two first Principal Components (Factor 1 on X-axis and Factor 2 on the Y-axis) and illustrates, respectively, 8.91% and 7.69% of variability between haplogroups.

**Figure S5.**  $\Delta K$  values *O. cornuta*. Plot of  $\Delta K$  values of *O. cornuta* calculated according to Evanno et al. (2005) in STRUCTURE software.

**Figure S6.**  $\Delta K$  values *O. bicornis*. Plot of  $\Delta K$  values of *O. bicornis* calculated according to Evanno et al. (2005) in STRUCTURE software.

**Figure S7.** Mantel test of *O. cornuta* and *O. bicornis*. The Mantel test measures the correlation between genetic distances and geographical distance (in km) among sample groups of *O. cornuta* and historical and contemporary *O. bicornis*. A significant positive correlation suggests that genetic differences between individuals increase with the geographic distance, indicating isolation by distance. A flat or non-significant correlation suggest no clear spatial pattern, suggesting ongoing gene flow.

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