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In recent years, the genus *Bombus* commonly known as bumblebees have shown drastic population declines, more than 70% of the *Bombus* species worldwide have suffered from processes of population decline or local extinction in the past 10 years (Potts et al., 2010; Martin & Melo, 2010; Arbetman et al., 2017). These bees are one of the most important pollinator groups due to their indispensable ecosystem service in natural and agricultural landscapes (Garibaldi et al., 2013; Fijen et al., 2018). Bumblebees exhibit a substantial vulnerability, as opposed to other wild bees due to characteristics related to complex life cycles and different degrees of sociality (Samuelson et al., 2018; Piiroinen & Goulson, 2016; Persson & Smith, 2013; Rasmont & Iserbyt, 2012). For this reason, both Europe and North America have begun to study in depth the causes of the decline of these Apidae (Cameron et al., 2011; Williams & Osborne, 2009; Kerr et al., 2015; Rasmont et al., 2015). Although some of the factors related to the decline are still difficult to know (Kent et al., 2018), it seems that different species of bumblebees are mostly affected by changes in the landscape due to agricultural practices and human expansion (Sánchez-Bayo & Wyckhuys, 2019; Jha & Cremen, 2013, Carvalheiro et al., 2013). This factor influences the available resources and nesting places, considerably reshaping important biological aspects of the bees life cycle, promoting the prevalence of parasites and the increase of inbreeding in populations (Goulson, Whitehorn & Fowley, 2012, Woodard et al., 2015).

Despite the interest granted to the conservation of these bees in other countries, in Colombia little is known about the population status of different species of bumblebees, a total of nine species are reported with clearly documented records: *B. excellens* (Smith, 1879), *B. funebris* (Smith, 1854), *B. hortulanus* (Friese, 1904), *B. melaleucus* (Handlirschi, 1888), *B. pullatus* (Franklin, 1913), *B. robustus* (Smith, 1854), *B. rubicundus* (Smith, 1854), *B. transversalis* (Oliver, 1789), and *B. atratus* (Franlin, 1913) (Thompson & Oldroyd, 2004; Parra et al., 2016). Moreover, the dynamics and processes of dispersion of these species in landscapes affected by humans remain unknown (Martins et al., 2015). Colombia display one of the greatest potentials in South America to develop the agricultural landscape (De Jaramillo et al., 2017). However, the use of land, must take in to account, how pollinator groups such as bumblebees cannot withstand drastic habitat modifications (Marshall et al., 2018; Reininghaus et al., 2017; Goulson et al., 2010). Since the change in habitat can increase intrinsic threats to populations, including genetic drift and inbreeding (Darvill et al., 2006; 2012), the use of molecular tools in conjunction with population genetics is essential to understand how the bumblebee species founded in these areas are being affected (Zayed, 2009). Consequently, the study of the genetic variation and genetic structure of bumblebees is called for elucidate the most basic levels of biological diversity and identify the genetic status of populations affected by changes in the landscape (Murray et al., 2009; Lozier & Cameron, 2009).

A clear example in the field of genomic conservation of bees is the greater availability of genomic resources; Currently, 11 species of bees have a sequenced genome (Elsik et al., 2015), within which two species of bumblebees are found*,* *Bombus impatiens* and *B. terrestris*

(Sadd et al., 2015), this last two has given the opportunity to investigate in depth the environmental factors that can affect the structure and genetic diversity of this important group of bees. Next-generation sequencing (NGS) is rapidly changing the field of genetic conservation for bees, allowing non-model species to be studied and helping researchers to test new or previous hypotheses about the meaning of genetic variation and the architecture that underlies several ecological and evolutionary processes (Lozier & Zayed, 2017). The field of genomic conservation allows us to evaluate and quantify the genetic diversity of relevant loci and study how species can respond to different environmental threats (Holderegger et al., 2006; Schoville et al., 2012). Likewise, it allows to accurately estimate the levels of genetic diversity and provide novel and relevant information to delimit significant evolutionary units (Funk et al., 2012, Hoffmann et al., 2015).

Previous studies have investigated intraspecific genetic diversity in wild bees in order to understand how landscape quality, fragmentation, isolation and potential barriers affect the process by which genetic diversity change in a population (Jha, 2015; Darvill et al., 2010; Wilson et al., 2016). These studies have included different estimates of population structure based on; the genetic difference, like Fst (Brown, 1970); distance and resistance isolation (IBD, IBR); tree-based distance and genetic clustering. The incorporation of these estimates has allowed the creation of realistic models, which can identify with greater certainty the factors that influence the structure and genetic variation of bees (Jackson et al., 2018; Kent et al., 2018). Estimates of genetic diversity as effective population size and heterozygosity; give important information about the population's health from genetic and demographic perspectives (Charlesworth, 2009; Boff et al., 2014; Mattila & Seeley, 2007), for this reason these estimates are efficient tools to identify species that may be in danger (Laikere et al., 2010; Cameron et al., 2011; Maebe et al., 2015); and in synergy with NGS, allows to develop optimal investigations, at optimum cost, that contemplate influence of the landscape on genetic diversity understanding how the genetic variation is influenced by environmental patterns (Lozier, 2014; Jaffé et al., 2014; Lozier et al., 2013; Epps & Keyghobadi, 2015).

In this study, we use nextRAD to examine the genetic diversity and population structure of the bumblebee *Bombus atratus*, in a fine geographic scale. This species is widely distributed in South America, presenting one of the largest ranges of distribution compared to other bumblebees (Abrahamovivich et al., 2007). In Colombia, this species has an altitudinal range distribution from 1500 to 3800 meters above sea level, however, they are mostly between 1800 and 2900 m. a. s. l. (Lievano et al., 1991). *B. atratus* is one of the few species of bumblebees that overlap their range of distribution with areas of extensive agriculture in Colombia (Gonzalez et al., 2004). This makes this bumblebee an appropriate model to understand the processes of genetic variation and isolation due to changes in the landscape. Few studies have examined the processes of genetic variation in high Andean bumblebees at local spatial scales through heterogeneous landscapes (Françoso et al., 2019).

Our objectives in this study were: 1) Evaluate, if habitat loss produced by agricultural growth, is affecting the genetic diversity of *B.atratus*. We measured land use, heterogeneity and plant richness and dominance to quantify habitat status and determine whether or not these variables are affecting the process that underlies the genetic variation in our bumblebee. We expected to find a negative relation between genetic diversity and habitats with high agricultural use, low heterogeneity and higher plant dominance, as found in other species of bumblebees in danger or decay (Cameron & Sad, 2019); 2) To estimate the effective population size (N_e) of *B. atratus* in different temporal periods, an compared the values with recent ecological evidences of bees decline (Ferreira et al., 2015; Vickruck et al., 2017). 3) Based on the environmental variables that model the distribution of our study species and the factors that shape the habitat loss, we presume to find genomic signatures of adaptation related to temperature and land use, that will be relevant in decision making regarding conservation strategies. This is the first study in Colombia that uses NGS to understand the patterns of genetic variation based on environmental variables in a species of bumblebees of high importance, both for its ecosystem service and for its intrinsic diversity.

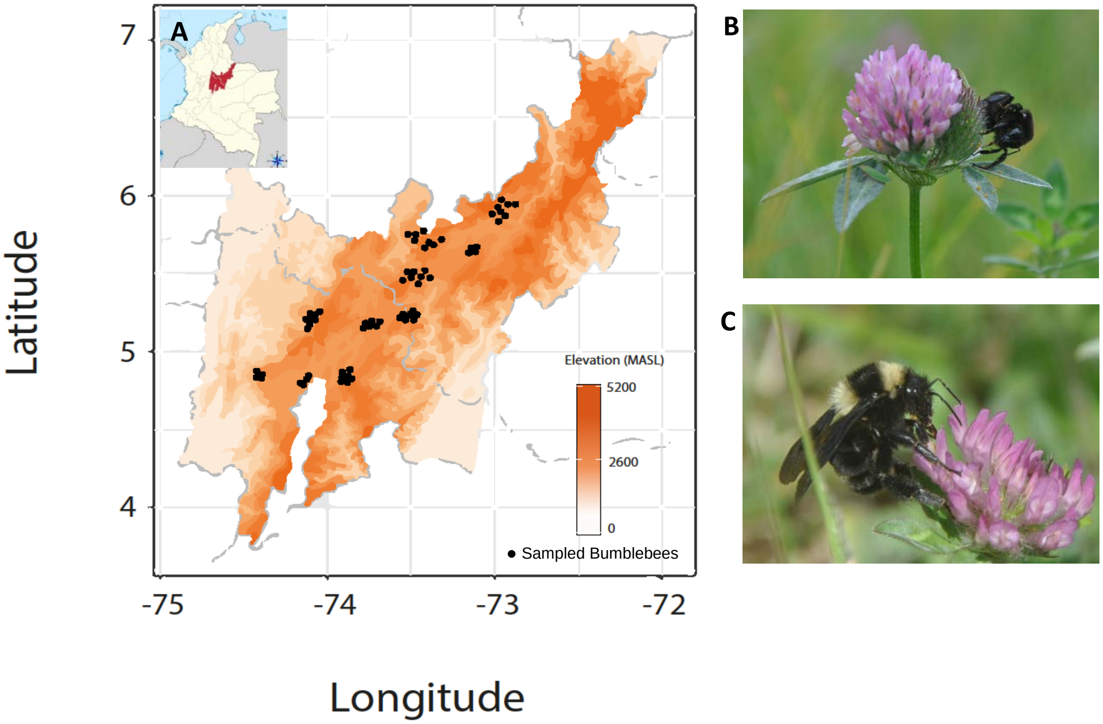

Figure 1. A. Map of the sampled area, the map was produced using R (Murell, 2018) from the BioClim data base (WorldClim: http://www.worldclim.org) for the raster of altitude, the points show the samples collected. B, C, Predominantly black and yellow respectively forms of *B.atratus*.

Sample Collection

Bombus atratus (Franklin, 1913), is a species found within the subgenus Fervidobombus and is widely distributed in south America having one of the highest ranges of distribution compared to other bumblebees (Abrahamovich et al. 2007). Current distribution includes Venezuela, Colombia, Ecuador, Perú, Uruguay, Bolivia and Argentina (Abrahamovich et al. 2004). In Colombia they can be found from 1500 to 3800 meters m. a. s. l. (Lievano et al. 1991). They are also one of the few species whose range of distribution overlaps with highly agricultural landscapes (Riaño et al., 2014), which makes it a suitable model to recognizes the processes of genetic variation and isolation due to habitat loss.

We sampled 90 female workers of *Bombus atratus* from ten localities across Cundinamarca and Boyacá, Colombia (Figure 1, supplementary Table 1). Bumblebee collection were made actively searching in different types of flowers, always seeking the independence of the samples, to avoid catching individual from the same nest, each sample was collected with a minimum distance of 3 km due to the displacement patterns of the workers of this species of bumblebees (Krauss et al., 2009; Pardo & Jimenez, 2006). Once collected, the samples were stored in 97% alcohol and placed at -20 ° C until DNA extraction.

DNA Extration

DNA was extracted from the third pair of bumblebees legs with punctured metacoxa, using the DNeasy Blood & Tissue Kit from Qiagen (Hilden, Germany). All the extractions were checked by 2.5% agarose gels and then quantified with Qbit 2.0 Fluorometer (Invitrogen). The selected samples had a concentration of ~10ng/ul. After, all the samples were sent to SNPsaurus (SNPsaurus, LLC) for nextRAD sequencing. Voucher specimens were housed at the *Museo de Historia Natural ANDES*, Universidad de los Andes, Bogotá, Colombia.

NextRAD Sequencing

Genomic DNA was converted into Nextera-tagmented reductively-amplified DNA sequencing (NextRAD), and then genotyping-by-sequencing libraries as described by Russello, Waterhouse, Etter, and Johnson (2015). Briefly, genomic DNA was made into whole genome genotyping libraries with Nextera DNA Flex reagent (Illumina, San Diego, CA, USA), this one ligates short adapter sequences to the ends of the fragments. The Nextera reaction was scaled for fragmenting 10 nanograms of genomic DNA. Fragmented DNA was then amplified, with one of the primers matching the adapter and extending nine nucleotides into the genomic DNA with the selective sequence. Therefore, only fragments starting with a sequence that can be hybridized by the selective sequence of the primer were efficiently amplified by PCR. The WGS libraries were sequenced on a HiSeq X with one lane of paired-end 150 bp reads. Custom scripts (SNPsaurus, LLC) were used to assembly a *de novo* low quality reference genome using the reads from four samples, of different localities, with abyss-pe and a k of 86. The resulting contigs were length filtered to a minimum of 250 bp, then cleaned of contaminating species by blastn to the NCBI nt database and removing blast hits to bacteria, plants and fungi. The gene annotation was created with augustus using species=*Bombus terrestris* (Stanke & Morgenstern, 2005).

The genotyping analysis used custom scripts (SNPsaurus, LLC) that trimmed the reads using bbduk (BBMap tools, http://sourceforge.net/projects/bbmap/): All reads were mapped to the reference genome, with an alignment identity threshold of .95 using bbmap (BBMap tools). Genotype calling was done using callvariants (BBMap tools).

SNP Filtering

We used VCFtools (0.1.6; Danecek *et al.* 2011) for final filtering of variants and ensure that VCF data set contained high quality, single-copy loci. We exclude genotypes with genotype quality scores < 30. Sites with > 5% missing data were removed. Next, we calculate average sequencing depth per site (~66), however a small fraction of loci had much higher coverage; these could indicate paralogous or multi-copy regions (Koch et al., 2014), therefore sites with mean sequencing depth > 132 were removed from the data. Then we filter the loci for strong deviations from Hardy-Weinberg equilibrium (*hwe,* 0.001). The remaining sites, those with minor allele frequency of 0.025% across all individuals were removed. After, due to the reproduction patterns of the bumblebees were sex determination typically leads to males arising from unfertilized eggs (haploid) and females from fertilized eggs (diploid), we exclude organisms from the same colony in our data through a kinship analysis performed using the function of Plink1.9

rel-cutoff (Purcell, 2007), this excludes one member of each pair of samples with observed genomic relatedness greater than the given cutoff value, this function is based on the algorithm presented by Manichaikul and collaborators (2010), were the inference criteria goes from 0 to 0.5 (Monozygotic twin: 0.5; Parent–offspring: 0.25; Full sib: 0.25; 2nd Degree: 0.125; 3rd Degree:0.0625 Unrelated: 0).

Population Structure and Genetic Diversity

After filtering, to assess population structure two different approaches were used: the first one, Admixture was used in Plink 1.9 (Purcell, 2007) and the second one the *SNMF*-function of the LEA (v2.0) package (Frichot & François, 2015; Frichot, Mathieu, Trouillon, Bouchard, & François, 2014). Linkage disequilibrium (LD) in bumblebees breaks down very quickly (Maebe et al., 2016), so, we randomly sampled one variant from each RADtag to ensure independence of loci prior to running SNMF. The number ancestral populations *K* was allowed to vary between 1 and 10, keeping in mind the locations sampled, with 10 replicate runs for each K-value, and the best K was chosen based on cross-entropy and cross-validation errors (Frichot et al., 2014). Full R scripts of *SNMF* can be found in the LEA website (http://members-timic.imag.fr/Olivier.francois/LEA/index.htm). Then, to visualize genomic similarity among individuals, data was prepared for R using vcfR (Knaus & Grünwald 2017) and the adegenet package (Jombart, 2008) was used to tested for isolation by distance (IBD) using a Mantel test between a matrix of genetic distances (Euclidian distance) and a matrix of geographic distances. Because the correlation between genetic and geographical distances can occur under various biological scenarios, such as gradual change due to environmental conditions; or differentiation by comparison of distant populations; a scatterplot was performed showing the consistency between previously created matrices.

Landscape Genetic Analyses

In order to assess the influence of landscape on genetic variation, we obtained the following high resolution rasters: 1) A continuous cultivated and managed Vegetation cover map; all the data layers from the cover maps contain unsigned 8-bit values and the valid values range from 0-100, representing the consensus prevalence in percentage; 2) A categorical first order range global land cover map; 3) A categorical high resolution second order (Simpson and Shannon) homogeneity map; this two based on the textural features of Enhanced Vegetation Index (EVI), texture measures are statistics describing the frequency distribution of EVI values and measuring compositional variability within an area. The second-order texture measures are statistics of the occurrence probabilities of different EVI combinations among pixel pairs within an area and thus also reflect spatial arrangement and dependency of the EVI values (JetzLab: http://www.earthenv.org, Tuanmu & Jetz, 2014). 4) A continuous high-resolution digital elevation map (DEM) for the whole study area (WorldClim: http://www.worldclim.org/), where every pixel contained an elevation value expressed in meters. This last ones, since genetic differentiation has been found to be influenced by elevation in bumblebees (Lozier et al. 2011). With the purpose to accomplish our goal of comparing the genetic diversity with our sample data, we extracted the values of the rasters at the locations were each bumblebee was captured. Taking into account the distance of foraging and dispersion of *B. atratus,* boundary regions (called a buffer) were created representing the spatial extent of each sample. Then, we extracted all values pixels that fall within the buffer for each individual and used to estimate the environmental information.

Finally, we computed percentage of landscape variation, as previously described, and genetic diversity (H_O, H_E, F) for each sample, we used linear models (LM) with the package lmer, to account for genetic variation (H_E, F) as response of the habitat variables predictors described before. After, Akaike information criterion (AICc) was used to compare models with different correlation structures, fitted with restricted maximum likelihood. All models were validated checking for residual autocorrelation and plotting residual versus fitted values.

Results

Genotyping and Filtering

Our variants were filtered to produce a total of 30505 SNPs from 74483 for *B. atratus.* The relatedness analysis resulted in the removal of 20 samples (Table 1). The final set of data comprised 70 bumblebees from 10 different localities, however, not all locations maintained the same numbers of bumblebees.

Table 1

Filter summaries for the data with vcf and plink commands; "minQ" includes only sites with quality value above the threshold; "min-max alleles" include only sites with a number of alleles less, greater or equal to the value; "max-missing" exclude sites on the basis of the proportion of missing data; "max-meanDp" includes only sites with mean depth values; "hwe" assesses sites for Hardy-Weinberg Equilibrium using an exact test, sites with a p-value below the threshold defined by this option are taken to be out of HWE, and therefore excluded; "rel-cutoff" excludes one member of each pair of samples with observed genomic relatedness greater than the given cutoff value

Total SNPs / Total Indv	**minQ (10)**	***Min-max Alleles (2)***	***max-missing (0.95)***	***max-meanDp (132)***	***hwe (0.001)***	**rel-cutoff (0.2)**
74483 / 90	74483 / 90	73410 / 90	70320 / 90	45827 / 90	30505 / 90	30505 / 70

Population Structure and Genetic Diversity

Patterns of genetic differentiation varied among samples and locations, and are likely associated with the distribution and niche across a landscape gradient. The heterozygosity observed and expected, did not have a significant difference, however the variation observed is greater than expected, the inbreeding factor F presented a negative value consistent with the heterozygosity observed, however there are samples with high values of inbreeding and low levels of heterozygosity (Table 2, supplementary Table 2). Average π per SNP in *B atratus* population ranged from 0.095 - 0.153. The index of Tajima's D presented a deviation from the neutral expectation, with a negative value, this may indicate that our bumblebee population may be passing for a bottleneck process or an event of variability removal.

Table 2.

Genetic diversity estimates for *Bombus atratus* population. Effective population size (*Ne*); Observed and expected heterozygocity respectively (Ho; He); Inbreedinf factor (F); Nucleotide diversity measured as the degree of differences per site between the DNA sequences (*π*). Tajimas´D measured as the number of pairwise differences within the number of segregating sites.

Total	*Ne*	Ho	He	F	*π*	Tajima's D
70	6.4 (SD±0.013)	0.11873 (SD±0.013)	0.1118 (SD±0.0002)	- 0.06095 (SD±0.1224)	0.011259 (SD±0.0036)	- 0.6187 (SD±0.020)

We detected significant genetic differentiation relative to geographic distance (IBD) across our populations of *B. atratus* (r^2=0.196, p-value << 0.01). Nevertheless, we did not detect genetic clusters using the two different clustering approaches. Significant genetic structuring across the locations of *B. atratus* were not found, though cross entropy (Δ*K*) statistic was greatest at K = 2, with less explanatory power gained by additional K (Supplementary Figure 1.A). However, even at K = 2, our bumblebee species lack population structuring, which is corroborated by the scatterplot correlation across geographic distance and genetic distances (Supplementary Figure 1.A). The distribution of genetic variation among genotyped individuals in relation to the neutral loci filtered can be visualized in the figure 2. In this analysis using 30505 informative SNPs, the ten locations just formed one distinct cluster, still seems that there would be a gradient between north and south where there are some SNPs fixed at highlands and others in the lowlands. The fairly weak clustering observed from structure suggested a potential ongoing gene flow among geographic regions (Figure 2.B and C)

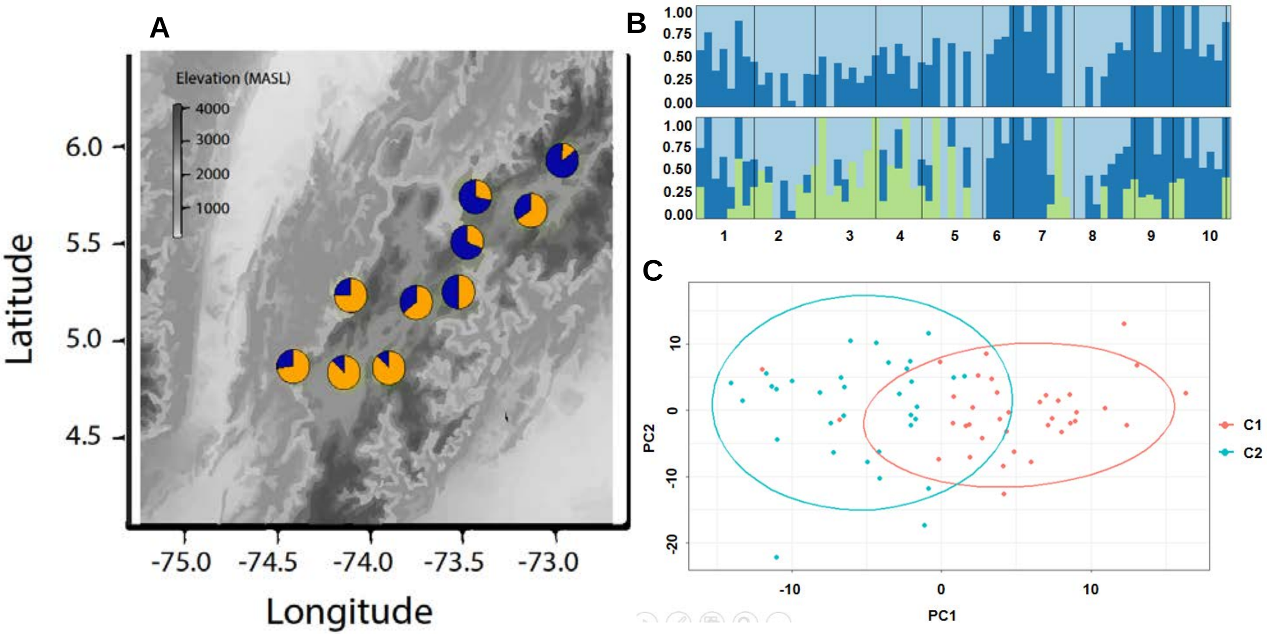

Figure 2. A. Genetic assignment of localities (sampling sites) to K=2 populations for the bumblebee *B. atratus* in the altiplano of the departments of Boyacá and Cundinamarca. The pie slice of each circle represent the average genetic assignment of all individuals in each location (10) to one of K populations. B. Admixture proportions of each individual from K = 2 and K=3, obtained in Structure for our data set, localities are assigned from 1 to 10 (1. Arcabuco 2. Choconta 3. Duitama 4. Facatativa 5. Guasca 6.

Morro 7. Pacho 8. Samaca 9. Tenjo 10. Villa Pinzon). C. Principal component analysis showing maximum explained variances (30%) for *B. atratus*.

Landscape genetics

Habitat use and altitude was found to be associated with heterozygosity an inbreeding (Figure 3). Predictors such as: heterogeneity; diversity and abundance of plants categorized by EVI turned out not to have a significant relationship with respect to genetic diversity and inbreeding. In spite of this, the interactions between the variables provided a better explanation for He and F, where the diversity trends were consistent across the full range of environmental variables. However, 50% of the variance was explained just by habitat use and altitude.

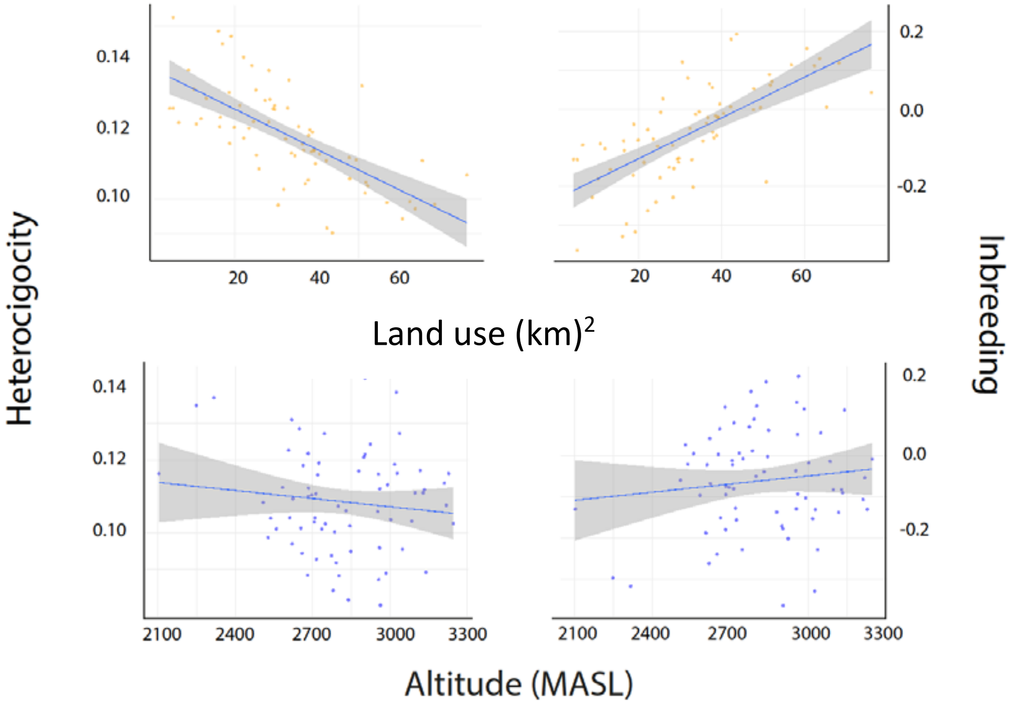

Figure 3. Individual average heterocigocity and inbreeding for *B. atratus* with respect to agricultural use km^2 and altitude. Solid lines are trend lines for each variable. (Table 4; 5)

Table 3

Summary statistics for the Linear regression models for Heterozygosity(LRM)

Predictor and Formula (Model)	*AIC*	*ΔAIC*	*Explained Variance*	*LogLik*	*P*
Heterozygosity ~ Land Use*** + Altitude **	-441.46	0	0.506	225.04	<< 0.001
Heterozygosity ~ Heterogeneity + Altitude	-436.93	4.53	0.015	233.2	0.6

Heterozygosity~(CF*EVIsim*EVIsha) + Altitude	-393.1	48.37	0.14	200.86	0.03
Heterozygosity ~ All predictors	-387.5	53.96	0.609	205.61	

Table 4

Summary statistics for the Linear regression models for Inbreeding(LRM)

Predictor and Formula (Model)	***AIC***	***ΔAIC***	***Explained Variance***	***LogLik***	***P***
Inbreeding ~ Land Use*** + Altitude **	-134.8	0	0.506	71.71	<< 0.001
Inbreeding ~ Heterogeneity + Altitude	-130.25	4.54	0.015	79.86	0.6
Inbreeding~(CF*EVIsim*EVIsha) + Altitude	-86.42	48.37	0.14	47.52	0.03
Inbreeding ~ All predictors	-161	53.96	0.609	52.27	

Final Considerations

We studied the process of differentiation of a bumblebee population at a fine geographic scale of 300 km, due to factors that affect its habitat such as an extensive agricultural use and landscape heterogeneity. Our study revealed a minimal gradual change in genetic structure across the sampled distribution of *B. atratus*, with no identifiable clusters. This low levels of genetic structure that were found for our bumblebee species and in congruence with the analysis of IBD most likely reflect the high dispersal abilities, in accordance with results from other continental bumblebee populations in Europe and North America (Spevak, Jepson & Williams, 2015) this is not surprising as reproductive bumblebees caste usually can move for a range of 3 km and a maximum of 15 km when there is a perfect condition for dispersion (Lepais et al., 2010).The lack of evidence for strongly isolated populations certainly raises questions regarding the factors that could be playing an essential role in the genetic variation of these bumblebees, it may thus be the case that substantial habitat heterogeneity is required to produce detectable genetic structure in bumble bees collected at local geographic scales.

Heterozygosity and inbreeding was influenced by land use of habitat and altitude (Figure. 3), but not for habitat heterogeneity; this agree with previous studies that found that heterogeneous landscapes in generalist and large distributed bumblebees, as *B. atratus*, may not have a broad effect on genetic diversity due to the behavior patterns of these bees (Laiolo et al., 2018; Rodriguez & Kouki, 2017; Kraus et al., 2009; Osborne et al., 2008). However, the land use is affecting the processes underlying genetic variation, we observed that in places where there is a greater use of the landscape the inbreeding factor per individual is greater, as well, the heterozygosity decreases in relation to the mean land used (Figure 4). As described by Cameron and Sadd (2019) and corroborated in this investigation, the extensive land use can cause irreparable damage to the resources necessary for the development and function of bumblebees (Colgan et al., 2019), making parasitic diseases more prevalent, decreasing the nesting sites and making floral resource deficient in quality. A clear example, is reflected in our data when we compared genetic diversity with respect to plant dominance (Table 3, 4), when there is a high dominance of a type of plant due to extensive agricultural use, genetic diversity tends to decline, despite that *B. atratus* is a generalist bumblebee, it has been shown that there are inflection points regarding the availability of resources that can shape the genetic stability of a population (Harmon-Threatt et al., 2017).

The other important factor with significance in the genetic diversity was the altitude, although our samples did not vary dramatically, there is a clear pattern which may be related to physiological responses to environmental pressures in the highlands as low temperature, low oxygen and low air density (Dillon & Lozier, 2019); Cold temperatures at high altitudes may shut down bumblebees for large parts of the day and night, also they rely in large part on aerobic respiration to supply energy demands (Dillion et al., 2006), this makes daily forages and flight patterns much shorter compared to lowland individuals (Oyen et al., 2016). Because of these challenges, we could be watching reductions in abundance and diversity of this bumblebees, and the patters of SNPs fixation founded in this study (Figure 2). The movement of mountain bumblebees in search of more temperate places (Sirois-Delisle, 2018), has been mostly reflected in bumblebees that do not have a wide altitudinal range, in contrast with our bumblebee species (Rasmont et al., 2015). Although the imposition of climate change on bumblebee populations with wide ranges cannot be ruled out (Kerr et al., 2015), a detailed study is necessary to determine if climate change in the last decade may be affecting this group of bees.

Although extended bottleneck processes usually show values of Tajima’s D normally positives (Tajima, 1989), we observe mean D = -0.06, this may be indicating the beginning of a process of loss of genetic variability in a short period of time, corresponding to changes in the landscape due to agricultural growth (Shmack et al., 2019; Zayed, 2004) In general, habitat loss, is found to be associated with changes in foraging processes (greater effort) and the prevalence of parasites, which in turn leads to the disruption of metabolic functions related to immunity genes (Barribeau & Schmid-Hempelp, 2017; Barribea et al., 2011). Although the strength of responses varies in ways that may reflect interesting evolutionary dynamics, and in our case is showing the loss genetic diversity, the potential for adaptation to habitat loss, or local adaptation to existing abiotic variation, requires further study for *B. atratus*. However, it is clear that these patterns are important to understand that local adaptation processes are unique (Lowe, & Hoffmann, 2011), and that the genetic diversity of these bumblebees should be preserved to maintain evolutionary potential of this species (Hoffmann & Sgrò, 2011).

Together, our results suggest that the processes that are affecting genetic variation may be related to local factors and possible physiological restrictions, as the use of land and altitude respectively. Our findings shed important light on the life history of *B. atratus* and highlight the role of land use as a phenomenon of change regarding the genetic diversity of these bumblebees. Likewise, our research reveals for the first time patterns of local inbreeding and loss of genetic diversity in this species in Colombia. This knowledge could help guide future conservation actions such as avoiding future schemes of bumblebee's importation, and better emphasize resources in conserving and restoring mountain ecosystems that may be supporting a genetic diversity still unknown. Considering the high biological and economic importance of this native pollinator, future work should test whether and how loci variants are of functional relevance for adaptation to life in a constantly changing ecosystem due to anthropogenic growth.

Acknowledgments

We thank to the Rufford Foundation by the supporting grant (26114-1), to the to the research fund of the science faculty of Universidad de los Andes (Convocatoria 2018-1 Financiación de proyectos de Investigación y presentación de resultados en eventos académicos). To the Ph.D Jeffrey Lozier for the advice throughout the investigation, and to all the families that allowed us to sample in their lands.

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Supplementary Tables

Sup. Table 1

Name of the samples with location and coordinates

Sample_ID	Location	Lat	Long	Providence
V1__sorted	VillaPinzon	5.2015545	-73.53361	Cun
V2__sorted	VillaPinzon	5.2463423	-73.51799	Cun
V3__sorted	VillaPinzon	5.2420756	-73.54682	Cun
V4__sorted	VillaPinzon	5.2205323	-73.56484	Cun
V5__sorted	VillaPinzon	5.2624143	-73.48536	Cun
V6__sorted	VillaPinzon	5.199523	-73.48587	Cun
V7__sorted	VillaPinzon	5.2297623	-73.50047	Cun
V8__sorted	VillaPinzon	5.2319342	-73.47255	Cun
G8A2__sorted	Guasca	4.8851234	-73.86743	Cun
G2__sorted	Guasca	4.8319142	-73.91326	Cun
G3__sorted	Guasca	4.8690243	-73.90228	Cun
G4__sorted	Guasca	4.8437143	-73.88992	Cun
G5__sorted	Guasca	4.8435443	-73.86674	Cun
G6__sorted	Guasca	4.8089243	-73.92064	Cun
G7__sorted	Guasca	4.8148234	-73.89343	Cun
G8H2__sorted	Guasca	4.81522	-73.8625	Cun
F1__sorted	Facatativa	4.8794323	-74.42136	Cun
F2__sorted	Facatativa	4.8498365	-74.41742	Cun
F3__sorted	Facatativa	4.8325565	-74.41693	Cun
F4__sorted	Facatativa	4.8496646	-74.39202	Cun
F5__sorted	Facatativa	4.8212623	-74.39459	Cun
T1__sorted	Tenjo	4.8203465	-74.12419	Cun

T2__sorted	Tenjo	4.8203443	-74.1242	Cun
T3__sorted	Tenjo	4.8394239	-74.1114	Cun
U1__sorted	Choconta	5.158352	-73.78243	Cun
U2__sorted	Choconta	5.1624732	-73.75596	Cun
U3__sorted	Choconta	5.1844645	-73.76869	Cun
U4__sorted	Choconta	5.1967823	-73.73877	Cun
U5__sorted	Choconta	5.180145	-73.73659	Cun
U6__sorted	Choconta	5.1604623	-73.72371	Cun
U7__sorted	Choconta	5.1717452	-73.69587	Cun
U8__sorted	Choconta	5.1907323	-73.69194	Cun
B1__sorted	Morro	5.6674935	-73.10643	Boy
B2__sorted	Morro	5.6520353	-73.13149	Boy
B3__sorted	Morro	5.639735	-73.11313	Boy
B4__sorted	Morro	5.6347753	-73.1539	Boy
T4__sorted	Tenjo	4.7932734	-74.14437	Cun
T5__sorted	Tenjo	4.7999467	-74.16135	Cun
D1__sorted	Duitama	5.9191164	-72.97925	Boy
D2__sorted	Duitama	5.8938466	-72.95865	Boy
D3__sorted	Duitama	5.8692535	-72.93668	Boy
D4__sorted	Duitama	5.883634	-73.01293	Boy
D5__sorted	Duitama	5.9423334	-72.87282	Boy
D6__sorted	Duitama	5.9771635	-72.96483	Boy
D7__sorted	Duitama	5.9464335	-72.92432	Boy
D8__sorted	Duitama	5.8337375	-72.97445	Boy
P1__sorted	Pacho	5.1500136	-74.11499	Cun
P2__sorted	Pacho	5.1876235	-74.11533	Cun
P3__sorted	Pacho	5.1807934	-74.08066	Cun
P4__sorted	Pacho	5.2081435	-74.13078	Cun

P5__sorted	Pacho	5.2153234	-74.09928	Cun
P6__sorted	Pacho	5.2495134	-74.09714	Cun
P7__sorted	Pacho	5.2546434	-74.04222	Cun
P8__sorted	Pacho	5.2163435	-74.06212	Cun
M1__sorted	Arcabuco	5.7484775	-73.51881	Boy
M2__sorted	Arcabuco	5.7758335	-73.42935	Boy
M3__sorted	Arcabuco	5.7239135	-73.31742	Boy
M4__sorted	Arcabuco	5.6848835	-73.36137	Boy
M5__sorted	Arcabuco	5.707513	-73.39227	Boy
M6__sorted	Arcabuco	5.6685753	-73.41561	Boy
M7__sorted	Arcabuco	5.7478235	-73.47124	Boy
M8__sorted	Arcabuco	5.7143534	-73.48084	Boy
S1__sorted	Sotaquira	5.5225853	-73.41875	Boy
S2__sorted	Sotaquira	5.4702935	-73.39025	Boy
S3__sorted	Sotaquira	5.4822653	-73.43866	Boy
S4__sorted	Sotaquira	5.511353	-73.48088	Boy
S5__sorted	Sotaquira	5.5147653	-73.51556	Boy
S6__sorted	Sotaquira	5.436853	-73.45961	Boy
S7__sorted	Sotaquira	5.4702935	-73.49702	Boy
S8__sorted	Sotaquira	5.4593635	-73.55264	Boy

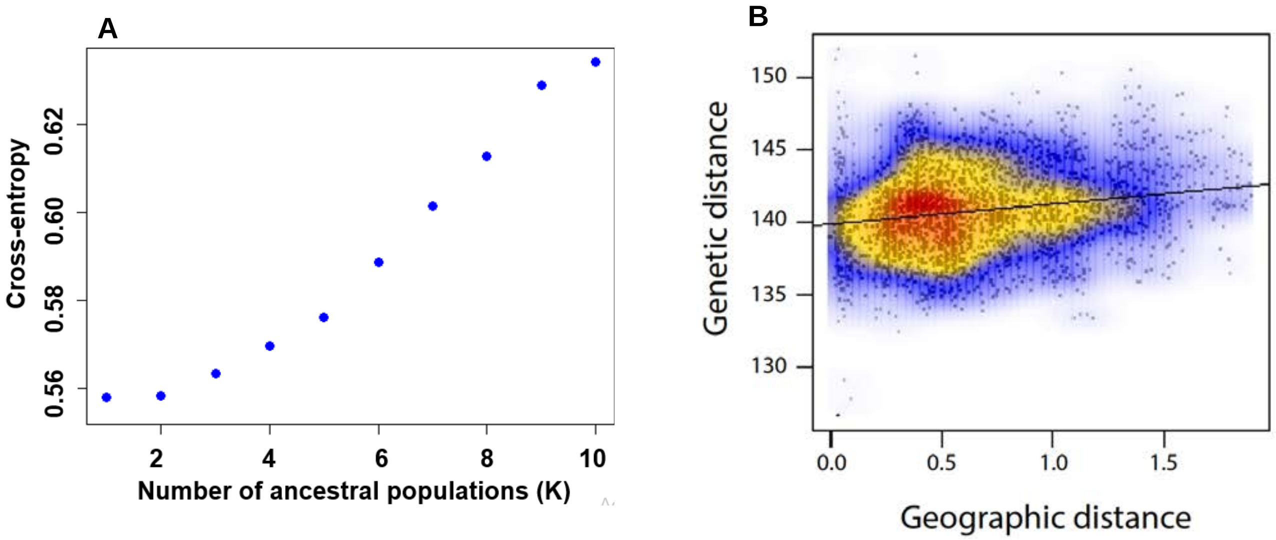
Sup. Table 2

Samples with values of inbreeding and observed heterosygocity

Sample_ID	F	Ho
V1__sorted	-0.13021	0.12631182
V2__sorted	-0.05293	0.11765074
V3__sorted	-0.08227	0.12096623
V4__sorted	-0.13071	0.12634358
V5__sorted	0.18086	0.09152921
V6__sorted	0.08051	0.10275161
V7__sorted	0.05579	0.1054889
V8__sorted	0.0127	0.11028084
G8A2__sorted	0.00367	0.11132497
G2__sorted	-0.08017	0.12075992
G3__sorted	-0.17993	0.13182332
G4__sorted	0.11921	0.09841762
G5__sorted	0.15696	0.09419931
G6__sorted	0.12137	0.09817663
G7__sorted	0.08946	0.1017498
G8H2__sorted	-0.0172	0.11367309
F1__sorted	-0.05936	0.11840952
F2__sorted	-0.01129	0.11300879
F3__sorted	-0.07743	0.12042444
F4__sorted	-0.02139	0.1141328
F5__sorted	-0.12735	0.12592872
T1__sorted	-0.07428	0.12004966

T2__sorted	-0.09409	0.12229093
T3__sorted	-0.15067	0.12856519
U1__sorted	-0.12901	0.12617173
U2__sorted	-0.22839	0.13734755
U3__sorted	-0.17049	0.13082291
U4__sorted	-0.36477	0.15260807
U5__sorted	-0.09278	0.12210974
U6__sorted	-0.03872	0.11609253
U7__sorted	0.06197	0.10484773
U8__sorted	0.19372	0.09009121
B1__sorted	0.00658	0.11106272
B2__sorted	0.0712	0.103797
B3__sorted	-0.13722	0.12703868
B4__sorted	-0.15619	0.12917847
T4__sorted	-0.18753	0.13274392
T5__sorted	0.00563	0.11111111
D1__sorted	-0.13773	0.12713461
D2__sorted	-0.32959	0.14865126
D3__sorted	-0.02175	0.11416122
D4__sorted	0.11437	0.09895833
D5__sorted	-0.08957	0.12175436
D6__sorted	-0.10256	0.12323484
D7__sorted	-0.20186	0.13440213
D8__sorted	0.04275	0.10696317
P1__sorted	-0.12951	0.12627922
P2__sorted	-0.29694	0.14496036
P3__sorted	-0.26232	0.14110199
P4__sorted	-0.31735	0.14717819

P5__sorted	-0.23993	0.13864587
P6__sorted	-0.22833	0.13724008
P7__sorted	0.05245	0.10587139
P8__sorted	0.11191	0.09923096
M1__sorted	-0.09588	0.12248227
M2__sorted	0.06562	0.10441094
M3__sorted	-0.0362	0.11585939
M4__sorted	-0.01343	0.1132476
M5__sorted	-0.1065	0.12367314
M6__sorted	-0.15311	0.12885136
M7__sorted	-0.02044	0.11403618
M8__sorted	0.02669	0.10873218
S1__sorted	-0.08246	0.12098038
S2__sorted	-0.04968	0.11737795
S3__sorted	-0.17691	0.13154253
S4__sorted	-0.0678	0.11932563
S5__sorted	-0.00685	0.11251091
S6__sorted	0.13054	0.09716096
S7__sorted	-0.00153	0.1119403
S8__sorted	-0.008	0.11262392



Supplementary Figure 1. A. Cross-entropy plot for the number of cluster K = 1-10. The retained value of K is K = 2. B. Scatterplot clearly showing one single consistent cloud of points between genetic and geographic distances